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Abstract

As a result of increasing demand for alternatives to fossil energy, the agricultural biogas sector is in expansion and lignocellulosic biomass (LCB) represents an interesting renewable feedstock. Nevertheless, due to the recalcitrance and complexity of its structure, deconstructive pretreatments are necessary to render possible biochemical conversions and efficient biomass exploitation. In this work, chemical-free, mild microwave pretreatment was evaluated (through BMP tests) as a method to improve anaerobic biodegradability of two grass biomass of industrial relevance and contrasted parietal content: corn stalks (low parietal content, high soluble content) and miscanthus (high parietal content, low soluble content). BMP tests carried out on raw biomass before pretreatment highlighted the negative correlation of BMP value to lignin and cellulose contents and the positive correlation to soluble and hemicellulose contents.

Efficiency of microwave pretreatment under two conditions, open vessel and high pressure (4 bars), with water as unique solvent was tested for tackling recalcitrance and results were compared to conventional heating pretreatment and a control treatment. Solid and liquid phases were separated after pretreatment with the aim to elucidate if microwave treatment had an impact on organic matter solubilisation and/or on the residual solid phase, which could improve the biodegradability of the pretreated solid fraction. To the authors' knowledge, this is the first study to dissociate methane production of the solid phase from that of the liquid phase after microwave pretreatment.

Observed BMP values in mesophilic conditions of raw biomass samples were 286 $NLCH_4/kgVS$ for corn stalks and 228 $NLCH_4/kgVS$ for miscanthus respectively (in agreement with literature). No significant improvement in BMP value nor in CH_4 production kinetics were observed following microwave pretreatment, while a harsh chemical pretreatment (10h soaking in 10% w/w NaOH) allowed 30% increase in BMP value. These results highlight the significant chemical effect -compared to thermal- on the biomass deconstruction and fibers breakdown during chemical-free microwave pretreatment. A synergy microwave effect with could allow to allow higher impact on biomass recalcitrance using lower NaOH amounts than chemical treatment alone.

Keywords

microwave technology, anaerobic digestion, corn stalk, miscanthus, lignocellulosic biomass

1. Introduction

In September 2015, the 193 UN Member States adopted the Sustainable Development Agenda 2030, which encourages countries to "mobilize efforts to end all forms of poverty, fight inequalities and tackle climate change", with a vision of transforming our world by eradicating poverty while ensuring transition to sustainable development (United Nations, 2020). Following the objectives for 2020 focused on energy aspects, the European objectives for 2030 target GHG emissions reduction by 55% compared to 1990, 32% of renewable energy in the overall energy mix, and at least 32.5% improvement in energy efficiency (European Commission, 2020). Biogas is one of the renewable energy sources that can contribute to attain these objectives (Bhatia et al., 2019) and LCB from agricultural residues and energy crops represents a promising sourcing because of their energetic potential per hectare (Rechberger et al., 2019). The ambitious objectives fixed by EU policies have greatly aroused interest in LCB utilization for bioenergy and green chemistry applications, but solutions are required in order to render biorefinery installations economically viable. Among other issues, it is necessary to solve the problems of land-use conflict, ineffective biomass supply and upstream transformation processes (ADEME, 2017).

World biogas production is still low with respect to the significant untapped potential that represent the available sustainable feedstocks (EBA, 2019): according to the International Energy Agency, biogas (plus biomethane) production in 2018 was around 35 Mtoe, while the estimated overall sustainable potential is estimated to 570 Mtoe for biogas and 730 Mto for biogas plus biomethane (IEA, 2020). In Europe for example, biogas contribution to bioenergy was 7.8% in 2015 (Scarlat et al., 2018), but it is expected that this production increase in the next years because of the implementation of national policies to develop the energy production from renewable resources (García and Daboussi, 2016; IEA, 2020). Nevertheless, this sector requires solutions to improve the yields of anaerobic digestion installations in order to render them profitable. Among other issues, it is necessary to solve the problem of resistance to degradation (or recalcitrance) of lignocellulosic by-products from agriculture and food industries, considered as cheap substrates, but which pretreatment can require large investment costs (Kampman et al., 2017).

Biomass cell wall, composed of cellulose, hemicelluloses and lignin, is organized as a physical barrier limiting biological degradation thus pretreatments are necessary to deconstruct the LCB network in order to allow biomass transformation processes (Bichot et al, 2018; Zhao et al., 2012b). Indeed, development of efficient but sustainable pretreatments is one of the main technico-economical challenges that limit the expansion of biogas installations, as pretreatment has been considered the second most expensive step in the biomass-to-energy transformation process (Den et al, 2018). LCB pre-treatments have thus been extensively addressed and numerous works have been published in the last years. Multiple technologies (thermal, biochemical, mechanical and enzymatic, or a combination of them) have been tested in order to improve anaerobic biodegradability of a wide diversity of LCB substrates, as oil palm empty fruit bunches pretreated by wet oxidation (Lee et al. (2020); combined thermal-chemical treatment of rice straw (Kim et al., 2018); sonication of maize straw and dairy manure (Zou et al., 2016) or municipal solid waste (Rasapoor et al., 2016), hydrothermal treatment of grass (Phuttaro et al, 2019), among many others. A recent review by Kumar and Sharma (2017) provide an update on different methods of pretreatment for lignocellulosic biomass.

Mild microwave treatment was chosen in this study because of its potential as low-environmental impact pretreatment: rapid heating in bulk biomass and the possibility of using less water and less chemical reactants than other thermal treatments (Kostas et al., 2017). Indeed, since the early 2000s, studies dealing with microwave pretreatment aiming at deconstructing LCB have been carried out, but to a considerably lesser extent than other physico-chemical pretreatments. According to literature review (Table S1), microwave pretreatment studies concern mainly 3 applications: 1) Polysaccharides release (the greatest proportion), for ethanol production mostly; applied to wheat straw (Saha et al., 2008; Xu et al., 2011; Aguilar-Reynosa et al., 2017; Tsegaye et al., 2019), rice straw (Sakdaronnarong et al., 2017), rapeseed straw (Lu et al., 2011), brewers' spent grain (Ravindran et al., 2018) and sugar cane bagasse (Zhu et al, 2016; Moodley and Kana, 2017), among other substrates; 2) Phenolic molecules release, applied to rice bran (Wataniyakul et al., 2012), bulrush (Oussaid et al., 2018), spent grain (Moreira et al., 2012), among others; 3) Energy (H_2 or CH_4) production, applied to wheat straw (Jackowiak et al., 2011a; Sapci, 2013; Nordmann et al., 2014), rice straw (Kainthola et al., 2019), switchgrass and/or miscanthus (Jackowiak et al., 2011b; Irmak et al., 2018), among others.

Always according to this literature review, the effect of microwaves on recovery of polysaccharides or phenolic molecules is improved, while the effect on anaerobic biodegradability, assessed by BMP tests (box 1) is less clear: methane production kinetics increase by 68% using microwave pretreated switchgrass was observed by Jackowiak et al. (2011b), while BMP value was not modified using microwave pretreated wheat straw (Nordmann et al., 2014) or increased by 28% (Jackowiak et al., 2011a). Moreover, Sapci (2013) did not observe any improvement in BMP value of different straws (wheat, oat or barley straws) even using harsh microwave conditions (between 200°C and 300°C for 15min); while Kan et al (2018) reported 52% increase of BMP value of brewer’s spent grain after microwave pretreatment. So far, it can be said that the effect of microwave on biogas production is not clear-cut: it can be positive or neutral.

The objective of the present study was thus to determine the effect of microwave pretreatment on anaerobic biodegradability of two LCB of industrial interest: corn stalks (CS) and miscanthus (MSC). Both have good energetic potential per hectare (189 GJ/ha for miscanthus and 170 GJ/ha for corn stalks, according to Somer et al. (2014)), but whose energetic yield could be improved with an adapted pretreatment. Two microwave conditions (open vessel and pressurized vessel) were tested and results were compared to conventional heating pretreatment and a control. Pretreatment effect on biomass was evaluated in terms of methane production (BMP and kinetics) by gDM separately on solid and liquid phases with the aim to elucidate if microwave treatment had an impact on organic matter solubilisation and on the lignocellulosic network of the residual solid phase which could improve the biodegradability of the pretreated solid fraction. To the authors' knowledge, this is the first study to dissociate methane production of the solid phase from that of the liquid phase after pretreatment.

Box 1 BMP (Biochemical Methane Potential) is the "maximum amount of methane that can be recovered from a substrate per mass of substrate organic matter as volatile solids (VS) or chemical oxygen demand (COD)" according to Koch et al, 2020. BMP test is a protocol widely used to assess the methane potential/anaerobic biodegradability of a substrate (Filer et al, 2019). It is performed by monitoring the biogas produced per specific amount of substrate (S_0) by a specific amount of inoculum (X_0), i.e. anaerobic sludge, in a closed reactor.

2. Materials and Methods

The following section describes the different materials and methods involved in this study and particularly develops pretreatments setting up and BMP tests performing.

2.1. Raw biomass and inoculum

Two corn stalks (CS) genotypes were involved in this study: F 98902 noted CS1 and F 7025 noted CS2. Both were harvested in September 2016 by INRAE IJPB (Versailles-Grignon unit, Versailles Cedex, 78026, France). Three miscanthus clones (MSC) were studied and noted MSCB for *M. x giganteus* Britannique, MSCF for *M. x giganteus* Floridulus and MSCR for *M. sinensis* Rotsilber. They were harvested in February 2017 by INRAE AgroImpact (Estrées

Mons experimental unit, Péronne, 80203, France). Samples were grounded to 1mm using two successive crushers (Viking, model GE 220, STIHL, Stuttgart, Germany and Fritsch Pulverisette 19), sieved to retain only particles between 0.2mm and 1mm and kept in closed boxes at ambient temperature before usage. Biomass composition were compared between 2016/2017 and 2018 (date of the study) in order to determine whether storage had an impact on the biomass. No significant differences in biomass composition were detected before and after storage (results not shown) and biomass were considered to be of identical composition between 2018 and the harvest date.

The inoculum used in the study was provided by EMIN LEYDIER paper mill (573 Route des Ortis, 26240 Laveyron, FRANCE). It consisted of anaerobic sludge, stored at 35°C before usage.

2.2. Chemicals and biomass composition analysis

All treatments and analysis were performed using chemicals from Merck and High purity water (Merck Millipore Quantum TEX).

Before any treatment, dry matter rate (DM) and volatile solid rate (VS) were determined. DM corresponds to a sample dry residue after total evaporation of water at 105°C (NREL, 2008). Volatile solid (VS) is the mass of organic matter contained in a dry residue, obtained after, at least 2 hours, carbonization at 550°C (NREL, 2008).

Biomass composition was determined using Van Soest protocol (Van Soest and Goering, 1970) which is based on mass sequential partitioning of cell walls, from most extractible to less extractible, with successive extractions using different solvents (water, neutral detergent solution, acid detergent solution and sulfuric acid 72%). Van Soest protocol permitted to determine alterations in the amount of parietal polymers, consisting of hemicelluloses, cellulose and lignin, between the raw biomass and the pretreated biomass.

COD (Chemical Oxygen Demand) measurement was carried out on solid biomass and liquid phase after pretreatment using kits (AQUALYTIC 420721 Küvettentest CSB Vario MR-COD Vario). COD of liquid phase was expressed in mgO_2/L by diluting 0.2mL of the liquid sample in 1.8mL of high purity water in the kit. COD of solid sample was expressed in mgO_2/gDM. 1g of the sample was first soaked in 5mL H_2SO_4 for 12 hours, with stirring. After this time, the sample was considered fully diluted in the acid and high purity water was added to reach a volume 250mL. 0.5mL was collected and mixed in the kit with 1.5mL of high purity water. In both case COD was determined by reading absorbance at 610nm.

2.3. Pretreatments

Various pretreatments have been tested in this study including microwave pretreatment, compared to conventional heating pretreatment and a control pretreatment with no heating. The operating conditions are described below.

2.3.1. Microwave pretreatment

Microwave pretreatments were performed using a Minilabotron 2000 (SAIREM, FRANCE) lab pilot, operating at 2.45GHz with a maximum power of 2kW. This equipment was used to perform two pretreatments types: microwave pretreatment heating at atmospheric pressure (open vessel) named MWH and pressurized microwave pretreatment heating, named PMWH. These two pretreatments were chosen to evaluate the impact of two very different microwave conditions on the BMP. At atmospheric pressure, the microwave conditions have been optimized in a previous paper (Bichot et al., 2019a) to release phenolic acids and it would be interesting to see if these conditions also increased the BMP. Under pressure, the operating conditions demonstrated a more important impact on the biomass structure than at atmospheric pressure, especially concerning hemicelluloses solubilisation, which could allow to increase the BMP (Bichot et al., 2020). In both cases, the operating conditions were thought out upstream and adapted to the microwave pilot used in order to be as adequate as possible. All treatments were performed in duplicate and at constant incident power. As the objective of this study was to develop green physico-chemical pretreatment, water without chemical reactants was used as solvent.

The microwave pretreatment at atmospheric pressure, named MWH for microwave heating, was performed using a glass reactor in the following conditions: 14g of raw material were mixed with 285g of water, corresponding to 4.7%DM (dry matter). These conditions have been determined as optimal ones with the microwave pilot and the biomass used and were determined in a previous study (Bichot et al., 2019a). After one hour of soaking in water at ambient temperature, the reactor was closed with a glass cover connected to a refrigerant for avoiding water evaporation during treatment. The treatment lasted 800s at 710W corresponding to an incident power density of 2.4W/g. The development of this pretreatment has been described by Bichot et al. (2019a).

After the treatment, the reactor was air-cooled for 15min before opening. Reaction mixture was filtered through a 200µm sieve. Solid was washed with 300mL of deionized water to remove by-products. The solid fraction was placed in an oven for 7 days at 40°C to dry in order to be stored without deterioration. Moreover, drying permitted to measure dry matter content to determine the amount of solubilised matter during processing. The amount of recovered solid ($g_{pretreated\ solid\ biomass}/g_{dry\ raw\ matter}$) was an indicator of the effectiveness of the treatment. The supernatant was filtered through cellulose filter (2.7µm) and stored at -20°C until BMP tests. The final volume was considered as the initial volume subtracted from the volume absorbed by the material, called swelling volume and equal to 1mL/gDM. No evaporation occurred in open vessel trials due to the refrigerant.

The pressurized microwave pretreatment, named PMWH for pressurized microwave heating, was performed using a PTFE hydrolyzing digestion vessel (PTFE/TFM.BOLA (T18) with membranes (Cat. No. A250-08) resisting pressure up to 20bar. Following preliminary tests (Bichot et al., 2020), 2g biomass were added to 40mL water in the reactor corresponding to 4.7%DM. No magnetic nor mechanical stirring was implemented as the reaction mixture mixed itself during boiling. Samples underwent one-hour pre-soaking in water at ambient temperature before the microwave treatment, which lasted 180 seconds at 300W, corresponding to an incident power density of 7.03W/g. After treatments, samples were processed as described before. Energy consumption was not discussed here, but the energy balance was done and was presented elsewhere (Bichot et al., 2019b).

2.3.2. Conventional pretreatment

Conventional heating (CH) treatment was used to compare thermal effects on methane production from microwave heating and from conventional heating. 14g of raw biomass were mixed to 285g water in the glass reactor. After one-hour soaking, the glass cover connected to the refrigerant was immersed in an oil bath at 110°C for 800s. After treatments, samples were processed as described before.

2.3.3. Control treatment

A control treatment, with no heating (NoH), was also carried out: 14g of biomass were added to 285g water in a beaker. Liquid and solid phases were separated after one hour of soaking at room temperature, without any heating. After treatments, samples were processed as described before.

2.4. Biochemical methane potential tests

The BMP (Biochemical Methane Potential) tests were carried out according to the standard protocol of the laboratory in 569mL serum bottles covered with rubber stoppers. Each bottle contained 2gVS of inoculum and 1gVS of raw or pretreated solid or 1g of COD (liquid phase) in order to attain a S_0/X_0 ratio of 0.5. The bottles were N_2 flushed before being closed and incubated at 35°C with constant agitation for at least 60 days. For each pretreatment condition studied, four bottles were prepared: two for the solid phase and two for the liquid phase.

Two positive controls in which substrate was replaced by ethanol, easily biodegradable, were carried out to verify the good activity of the inoculum, which was always the case during this study. Moreover, two negative controls without substrate were also prepared to determine the residual methane potential of the inoculum. This endogenous production was then removed to each test production to calculate the net methane potential.

Biogas production was measured every two days for the two first weeks and subsequently every three or four days. Produced biogas was analysed with a gas chromatograph (Varian Micro GC CP 4900). Vector gas was nitrogen, injection volume was 200nL for an injection time of 40ms. The two columns used were: Molsieve 5Å for the separation and analysis of N_2/O_2,

CH_4, CO and NO, operating at 40°C and 21psi; and 0PoraPLOT Q for the separation and analysis of CO_2, SO_2, operating at 40°C and 21psi.

BMP results were expressed in $NLCH_4$/kgVS for solid phases or LCH_4/kgCOD for liquid phases. In order to evaluate the impact of pretreatment on the kinetics of methane production, the first-order kinetic constants were calculated using the least-squares fit of methane production vs. time (t) with the following equation:

$$V = V_{max} (1 - e^{-kt}),$$

with V the volume of methane in $NLCH_4$/kgVS, V_{max} the maximum producible methane volume in $NLCH_4$/kgVS, k the first-order kinetics constant in $days^{-1}$ and t the digestion time in days. The Microsoft Excel Solver function was used to determined V_{max} and k. The model had already been applied under the same operating conditions and with the same devices by Thomas et al. (2018), that demonstrated the relevance of the model to miscanthus raw biomass with $R^2>0.95$. This unique model was chosen as the objective of the study was not to determine the model that best matched the data but to highlight the impact of pretreatments on BMPs value.

3. Results and discussion

Results of the study are presented in the following: first the raw biomass compositions were analysed and BMP values determine. Then BMP tests were carried on two biomass of interest in order to produce more biogas.

3.1. Raw biomass composition and BMP results

As a first step, raw materials composition were analysed and an effort was made to understand the impact of biomass composition on BMP values. Then biomass were pretreated by various pretreatments (microwave heating MWH, pressurized microwave heating PMWH, conventional heating CH and no heating considered as control NoH) and BMP tests were carried out in order to determine the effect of the pretreatment on BMP values.

3.1.1. Raw biomass composition

In order to determine the biomass composition, biomass samples were analysed in triplicate by Van Soest method and results are summarized in Table 1.

Table 1: Composition of raw biomass used for this study

		CS1	CS2	MSCB	MSCF	MSCR
Dry matter	%	92	92	92	92	92
Van Soest soluble	%DM	37.30 ± 1.9	35.70 ±1.3	8.29 ± 0.2	5.77 ± 2.4	6.63 ± 4.5
Cell wall	%DM	62.70 ± 1.9	64.30 ± 1.3	91.71 ± 0.2	94.23 ± 2.4	93.37 ± 4.5
Distribution:						
Hemicelluloses	%DM	26.00 ± 0.9	30.09 ± 1.5	22.91 ± 3.6	25.86 ± 0.4	35.68 ± 1.2
Cellulose	%DM	28.51 ±0.9	27.36 ± 1.5	52.78 ± 3.5	51.78 ± 1.6	47.33 ± 2.4
Lignin	%DM	6.85 ± 1.5	5.30 ± 1.0	15.46 ± 0.4	16.21 ± 0.8	10.14 ± 2.1
Ash	%DM	1.18 ± 0.4	1.55 ± 0.6	0.56 ± 0.4	0.38 ± 0.38	0.22 ± 1.9
BMP	$NLCH_4/kgVS$	287 ± 23	285 ± 7	228 ± 8	250 ± 2	278 ± 5

According to Table 1, CS and MSC were very different in terms of composition. The proportion of cell wall (equivalent to parietal polymers) vs Van Soest soluble content in MSC is more than 10 times higher than in CS, respectively 13.5:1 and 1.7:1 ratio in average of the DM%. More precisely, lignin contents in MSC were on average twice as much as corn stalks content in lignin. Within MSC types, the highest BMP value observed was for MSCR, which had the lowest lignin content and the highest hemicelluloses content. The lowest BMP observed was related to the MSCB, which had the highest cellulose content and a high lignin content. These results concerning raw CS and MSC composition were consistent with those in literature. Van der Weijde et al. (2013) outlined proportions of 27-40% cellulose, 25-34% hemicelluloses and 9-15% lignin in corn stover and 28-49% cellulose, 24-32% hemicelluloses and 15-28% lignin in miscanthus.

3.1.2. BMP of raw biomass

The Biochemical Methane Potential (BMP) of the different raw biomass samples were first determined, with the aim of choosing the genotype for CS - or the clone for MSC- with the lowest methane potential (having the more room for improvement). Figure 1 presents methane production curves with respect to time for the raw biomass studied.

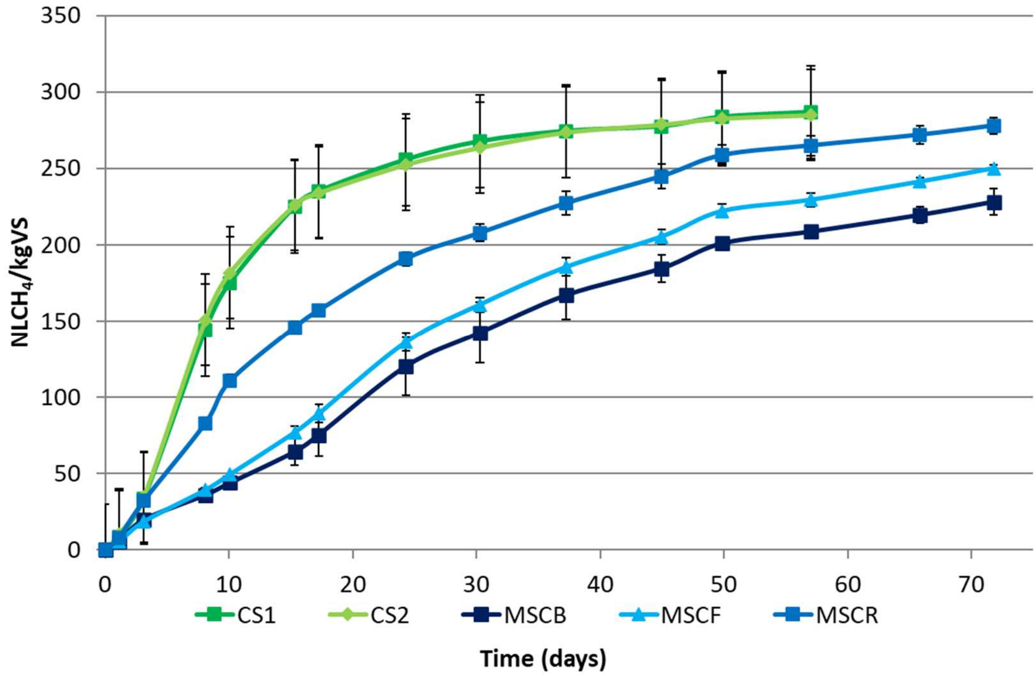

Figure 1: Methane production curve vs. time for raw biomass (CS1, CS2, MSCB, MSCF and MSCR)

From Figure 1, it can be observed that anaerobic biodegradation pattern is not the same for CS and MSC. For both CS1 and CS2, methane production led to a BMP value of 286 $NLCH_4$/kgVS on day 57, with significant error bars between the duplicates, certainly due to samples heterogeneity. Sun et al. (2015) and Sawatdeenarunat et al. (2015) respectively registered 256 $NLCH_4$/kgVS and 291 $NLCH_4$/kgVS for corn stalks methane potential and thus it can be considered that BMP values observed in the present study were consistent with literature analysis.

Concerning MSC, differences were observed in BMP final values for the different clones, with lower error bars than those observed with corn samples, certainly due to lower samples heterogeneity. Indeed, BMP value of MSCB and MSCF were significantly lower than MSCR (respectively -17% and -10%) (p value = 0.00463). This result was in agreement with Thomas et al. (2019) who observed that miscanthus BMP was largely dependent on the clone considered. Thus, in the present study, the initial biomass had a significant impact on BMP (p = 0.01688). For MSC, biodegradation was two times slower for MSCR, 3 times slower for MSCF and 4 times slower for MSCB than for CS samples; and led to a BMP value respectively

2.8%, 12.5% and 19.5% lower than CS BMP value, but biodegradation was not completed when the experience was stopped on day 75; actually, the stationary phase had not yet been reached (Figure 1).

No lag phase was observed, neither for CS nor for MSC and the methane production increased in an exponential way since the start-up of the tests, especially in the case of CS (Figure 1). The fast methane production at the early stages of the reaction corresponded to the biodegradation of molecules easily degradable by microorganisms, such as soluble sugars. It could also be explained by the very small particle size, as Filer et al. (2019) recommended to crush the particles at less than 10mm, which was largely the case in this study. Then, the methane production slowed down, this phase corresponding to the hydrolysis of less accessible molecules such as parietal sugars (Phuttaro et al., 2019). As soluble content, predominantly sugars, in CS was higher than in MSC, methane production rate was higher. On the contrary, MSC had a higher parietal content, that had to be first hydrolysed by microorganisms explaining the slower kinetics in the case of MSC than CS. (Phuttaro et al., 2019).

Using the first order kinetic model, V_{max} and k could be determined for each raw biomass with a reliable approximation ($R^2 > 0.98$) and values are summarized in Table 2. For a good fit of a model, Joglekar and May (1987) suggested that the R^2 should be superior to 0.8, which was the case in the present study Table 2. The kinetic constant k was equal to 0.09 for CS and was more than 4 times inferior (0.02) for MSC, confirming the previous predictions that MSC kinetic was slower than CS. Moreover, according to Table 2, the maximum theoretical volumes were 289 $NLCH_4$/kgVS and 286 $NLCH_4$/kgVS for CS1 and CS2 respectively. These values were close to the actual volumes produced (Figure 1), meaning that the BMP tests were finished and permitted to reach the maximum volumes. On the contrary, the maximum theoretical volumes were 350 $NLCH_4$/kgVS, 341 $NLCH_4$/kgVS and 286 $NLCH_4$/kgVS for MSCB, MSCF and MSCR respectively. Except for MSCR, these values were more than 100 $NLCH_4$/kgVS higher than those actually measured, implying that BMP tests were not finished and by running the tests longer, a higher biogas volume would be produced. As they had already been in progress for two and a half months and the production increase was minimal, it was decided to stop the MSC tests.

Table 2: V_{max} and k determined on raw biomasses (CS1, CS2, MSCB, MSCF and MSCR) using the first order model

	V_{max} mod ($NLCH_4$/kgVS)	k (day^{-1})	R^2
CS1	289 ± 29	0.0872 ± 0.005	0.99
CS2	286 ± 8	0.0905 ± 0.000	0.99
MSCB	350 ± 93	0.0177 ± 0.008	0.98
MSCF	341 ± 9	0.0197 ± 0.002	0.99
MSCR	286 ± 6	0.0445 ± 0.001	0.99

3.1.3. Correlation between raw biomass composition and BMP values

In order to understand the link between raw biomass composition and BMP values, a correlation matrix was constructed (Figure 2) using as variables BMP ($NLCH_4$/kgVS), soluble content from Van Soest analysis (%DM), hemicellulose content (%DM), cellulose content (%DM), lignin (%DM) and ash content (%DM). In the matrix (Figure 2), the larger the number (from -1 to +1) the more positive the correlation between two variables. This correlation was also represented by the colour of the box at the intersection of the variables: blue corresponded to a positive correlation, red to a negative correlation and white to no correlation.

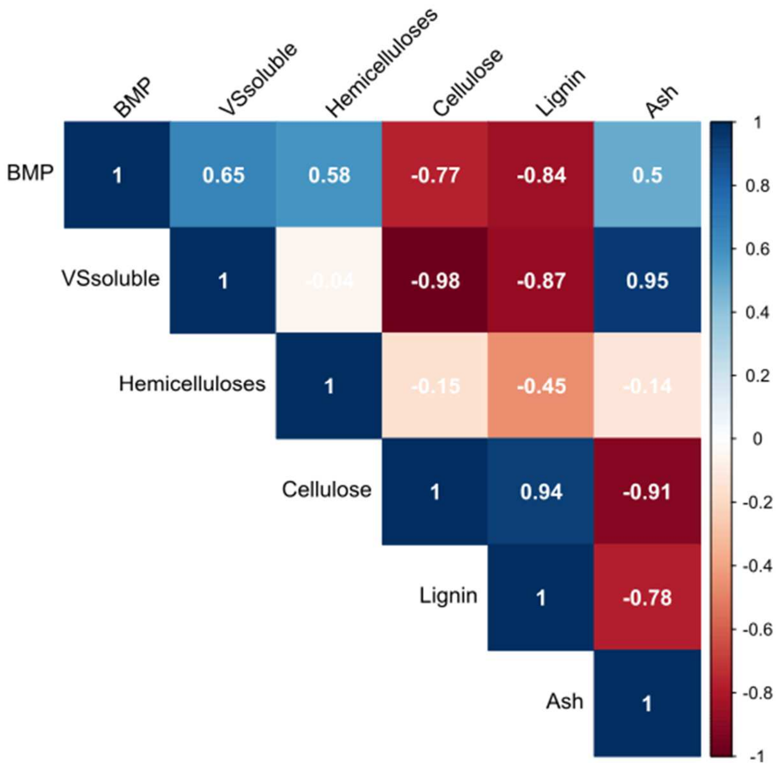

Figure 2: Correlation matrix of BMP and raw biomass composition

In this study and for both tested biomass, BMP value was negatively correlated to lignin and cellulose contents (-0.84 and -0.77 respectively) and positively correlated to soluble and hemicellulose contents (0.65 and 0.58 respectively). The higher the lignin and cellulose contents in the biomass, the lower the BMP. This result was consistent with Monlau et al. (2012) study that showed the negative correlation between the BMP value and lignin content and between the BMP value and crystalline cellulose content on 20 lignocellulosic materials including rice straw, sorghum, maize stalks and sunflower stalks. Various studies highlight the critical role of lignin and cellulose in parietal protection against external attacks (Miedes et al., 2014) and their negative correlation within BMP value (Triolo et al., 2011; Kobayashi et al., 2004). Indeed, crystalline cellulose reduces biodegradability because of the highly resistant hydrogen bonds network forming a recalcitrant wall to enzymes and microbial attacks, compared to amorphous cellulose (Zhao et al., 2012a). Concerning lignin, it acts as a physical barrier, limiting the access of enzymes to cellulose and adsorbing enzymes during enzymatic hydrolysis due to its hydrophobic structural features (Zoghlami and Paës, 2019). The lower BMP value observed for miscanthus compared to corn stalks can thus be understood, as miscanthus is richer in lignin and cellulose than corn stalks (Table 2). Moreover, the slower methane production rate observed during the first period of the BMP test for miscanthus compared to corn stalks can also be explained by the smaller amount of soluble content (sugars, proteins…), corresponding to the more easily degradable material content, in average 6.9%DM and 36.5%DM respectively for MSC and CS (Phuttaro et al., 2019).

3.2. BMP of pretreated biomass

From the results obtained in the previous section, MSCB and CS1 were selected for the microwave pretreatment study; the first one because its BMP value was the lowest observed between the three MSC (more room for biodegradability improvement) and the second because there was not substantial difference between CS BMP.

MSCB and CS1 were pretreated by conventional heating (CH), classic microwave heating (MWH, 710W, 800s), pressurized microwave heating (PMWH, 300W, 180s) and control treatment (NoH). The different pretreatments were performed with the aim to compare microwave heating pretreatment with conventional heating pretreatment. After treatment and phase separation, COD and Volatile Solid analysis were performed on solid and liquid phases in order to determine the dry matter solubilisation obtained after the pretreatments tested. Results are summarized in Table 3.

Table 3: COD and VS analysis on solid and liquid phases after treatments. MWH classic microwave heating, CH conventional heating treatment, NoH control treatment, PMWH pressurized microwave heating treatment

	Solid phase		**Liquid phase**	
	COD (gO_2/gDM)	**VS (%) (g/gDM)**	**COD_{sol} (gO_2/L)**	**DM (%) (g/g)**
CS1 Raw	1.25	88.87%		
CS1 MWH	1.25	91.74%	12.4	1.12%
CS1 CH	1.26	91.57%	12.0	1.08%
CS1 NoH	1.25	92.05%	10.1	0.90%
CS1 PMWH	1.26	91.51%	14.2	1.26%
MSCB Raw	1.30	92.20%		
MSCB MWH	1.32	96.09%	2.31	0.18%
MSCB CH	1.30	95.64%	1.68	0.13%
MSCB NoH	1.29	95.52%	1.12	0.09%
MSCB PMWH	1.49	94.74%	2.04	0.16%

From Table 3, it can be seen that no significant effect on organic matter solubilisation was observed after microwave pretreatment (MWH or PMWH); as measured soluble organic matter content in the liquid phase of pretreated (MWH, PMWH and CH) and no pretreated samples (NoH) were similar. DM and COD content in the liquid phases of CS samples, whatever the treatment, was higher than in MSC liquid phase, which could be explained by the higher parietal content of miscanthus, as discussed previously (Table 1), with no link to the pretreatment performed.

Consequently from previous results, it was physically impossible to add 1gVS of the liquid samples into the BMP flasks in order to keep the 0.5 S_0/X_0 ratio (Initial substrate VS/Initial Inoculum VS) in agreement with standard laboratory protocol. As a consequence of the low S_0/X_0 ratio applied (0.045), it was expected that methane production due to the organic load would be difficult to dissociate from the methane production due to the endogenous production. BMP tests were performed in duplicate and results were expressed in $NLCH_4/kgVS$ for solid phase and in $NLCH_4/kgCOD$ for liquid phase.

3.2.1. BMP of solid phase of pretreated samples

The methane production curves of the solid phases of pretreated samples (CS1 and MSCB) are presented in Figure 3.

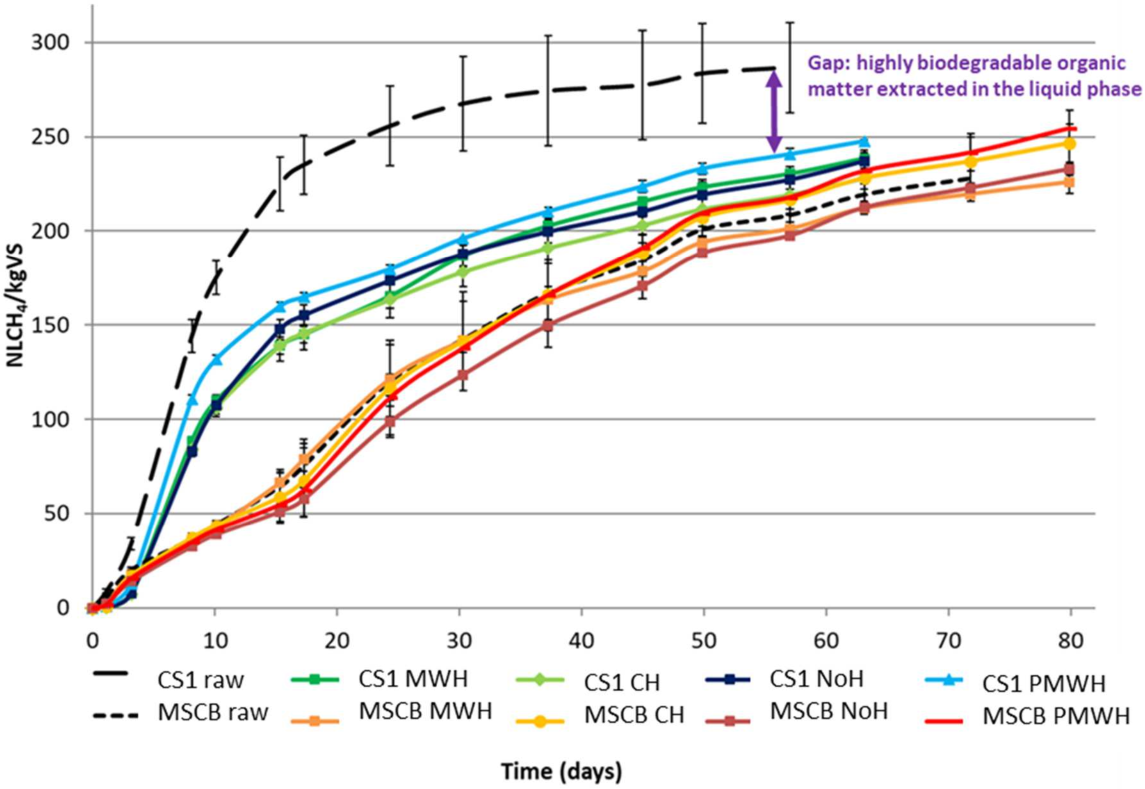

Figure 3: Methane production vs. time from solid phase after various treatments for CS1 and MSCB. MWH classic microwave heating, CH conventional heating treatment, NoH control treatment, PMWH pressurized microwave heating treatment

According to Figure 3, whatever the treatment, the methane production rate was similar for all CS1 and MSCB pretreated samples. Nevertheless, production rate was slower for MSCB samples than for CS1, which can certainly be explained by the more lignified structure of the miscanthus. As discussed in the previous section with Figure 2, lignin content being negatively correlated to BMP (-0.84), this could also affect the methane production rate. In Figure 3, the observed BMP value of pretreated CS1 solid phase, 250 $NLCH_4$/kgVS, was lower than BMP of the raw CS1, which was 286 $NLCH_4$/kgVS. This was due to the easy solubilisation of soluble organic matter observed during the pretreatment, as soluble content represented 37.3 % of the CS1 DM, whereas MSCB soluble content was only 8.29 %DM. The gap in BMP final value between raw and pretreated CS1 equals to the biodegradable soluble COD fraction that was removed following solubilisation and Liquid:Solid phase separation of pretreated CS1 samples. Biodegradability of released soluble compounds is discussed later.

In the case of MSCB, the BMP of raw and solid phase pretreated biomass were most probably similar (at 5% risk, according to student test, *p* value was largely superior to 0.05), meaning that a non-significant amount of organic matter was solubilised during the heating treatment or during soaking. These hypotheses are discussed in the following section. At 5% risk, methane production rate and BMP values obtained for pretreated MSC were not significantly lower than raw MSC BMP values (with p values equal to 0.6367). Pretreatments did not allow organic matter solubilisation neither weakening of the lignocellulosic network, which could improve the samples biodegradability of the pretreated solid fraction, in terms of methane production kinetics and BMP.

Most studies on the effects of LCB pretreatment - not necessarily thermal pretreatments - on anaerobic biodegradability show an increase in the BMP after pretreatment. For example, in the study ofThomas et al. (2019), BMP value of NaOH treated miscanthus at atmospheric pressure and ambient temperature, increased by 55% and in the case of Siddhu et al. (2016) BMP of steam-exploded corn stover, increased by 56%, demonstrating the positive pretreatment effect on methane production. Nevertheless, in the case of microwave pretreatment, results are more unclear. In this way, Jackowiak et al. (2011b) demonstrated that the BMP produced from switchgrass pretreated by high-pressure microwave (260°C and 33bars) was not improved when compared to raw switchgrass but the reaction rate was improved: a reduction of 4.5 days to reach 80% of the methane volume was observed. In comparison, the maximum methane volume increased by 28% using wheat straw pretreated in the same microwave conditions (Jackowiak et al., 2011a). Similarly, Kainthola et al. (2019) demonstrated an increase of more than 100 NmLCH$_4$/gVS after treating rice straw with microwave for 4min at 190°C, reaching 325 NmL/gVS. On the contrary, Sapci (2013) pretreated by microwave, under temperatures between 200° and 300°C, different LCB (barley, oat, spring and winter wheat) and demonstrated that the microwave treatment did not improve the anaerobic digestion and that the increase in temperature led to a lower methane production. The microwave conditions described above were more severe than those used in the present study, but prove that the microwave pretreatment is not always effective in increasing the BMP value of a lignocellulosic biomass. Studies tried to understand microwave effects on biomass organization using both Field Emission Scanning Electron Microscopy and Fourier Transform Infrared. When BMP increased after microwave pretreatment, this could be explained by a breakdown in polysaccharides parietal polymers (Kainthola et al., 2019).

In this study, the first order kinetic model was implemented on methane production data for CS1 and MSCB to better understand the link between biomass pretreatment and anaerobic digestion kinetic. Results are summarized in Table 4.

Table 4: Methane production during BMP tests, V_{max} and k determined on pretreated biomasses solid phases using the first order model. MWH classic microwave heating, CH conventional heating treatment, NoH control treatment, PMWH pressurized microwave heating treatment

		V_{max} mod ($NLCH_4$/kgVS)	**k (day^{-1})**	**R^2**
CS1	NoH	236.4 ± 4.5	0.0552 ± 0.002	0.98
	CH	227.7 ± 5.7	0.0544 ± 0.002	0.98
	MWH	243.0 ± 3.5	0.0509 ± 0.004	0.98
	PMWH	242.0 ± 4.4	0.0639 ± 0.004	0.97
MSCB	NoH	398.6 ± 56	0.0120 ± 0.003	0.99
	CH	386.1 ± 73	0.0130 ± 0.009	0.98
	MWH	290.8 ± 23	0.0206 ± 0.003	0.99
	PMWH	415.4 ± 40	0.0128 ± 0.001	0.98

For both pretreated biomass with the fourth treatments, the model fitted well with the experimental kinetic with R^2 superior to 0.97 (Table 4). Concerning CS1, the predicted volume production was the same as the experimental volume production, whatever the treatment, demonstrating that CS1 digestion was complete at the end of the 60 days of digestion and this was also reflected in the methane production curve (Figure 3) which tends to a plateau from day 50. Concerning MSCB, the predicted maximal volume was higher than the experimental maximal volume, up to 165 $NLCH_4$/kgVS in the case of PMWH MSCB. Moreover, the standard deviations were high, between 23 $NLCH_4$/kgVS and 73 $NLCH_4$/kgVS. The difference between the two values can be explained by the uncomplete biodegradation of the samples at the time the BMP tests were stopped: the model predicted that production could continue and thus no plateau was observed on the MSCB methane production curves (Figure 3). There was no difference for MSCB methane production kinetics between raw and pretreated solid phase, with a value of 0.02 day^{-1} because of the low organic matter solubilised in the liquid phase.

In addition, the observed methane production kinetics of the solid phase of the pretreated CS samples were slower than the raw sample, a difference of 0.04 day^{-1}, as a consequence of the L:S phase separation (soluble, easily biodegradable compounds were removed) (Figure 3). These observations suggested that microwave heating did not favour organic matter solubilisation neither weakening of the lignocellulosic network, which could improve the samples biodegradability of the pretreated solid fraction, in terms of methane production kinetics and BMP.

To compare results obtained with microwave pretreatment, a chemical NaOH pretreatment alloiwng to obtain an efficient breakdown of the lignocellulosic network (Monlau et al, 2012) was implemented. At ambient temperature and with the same operating conditions as NoH treatment, 10g CS1 were pretreated for 10 hours with 10%w/w NaOH before performing BMP tests on the mixture solid+ liquid phase, in duplicate. In these conditions, BMP reached 405.5 $NLCH_4$/kgVS representing an increase of more than 100 $NLCH_4$/kgVS compared to raw CS1 (equivalent to +30%). During alkaline pretreatment ester bonds between lignin and hemicelluloses were saponified resulting in biomass delignification (Zhao et al., 2012b) and allowing better action of microorganisms producing biogas. This was in agreement with Thomas et al. (2019) demonstrating an increase of 55% in miscanthus BMP results after 6 days of treatment with 10% NaOH. Chemical pretreatment, by subjecting biomass to difficult conditions, dislocated the cell wall structure and thus facilitated the production of biogas by microorganisms.

3.2.2. BMP of liquid phase of pretreated samples

BMP tests were implemented in duplicate with the liquid phases after the different pretreatments (MWH, PMWH, CH and NoH), in order to determine the biodegradability of the COD fraction solubilised by microwave pretreatments (MWH and PMWH) and to compare it to the COD released by the control treatment without heating (NoH). The methane production curves of CS1 and MSCB liquid phases are presented in Figure 4.

(a)

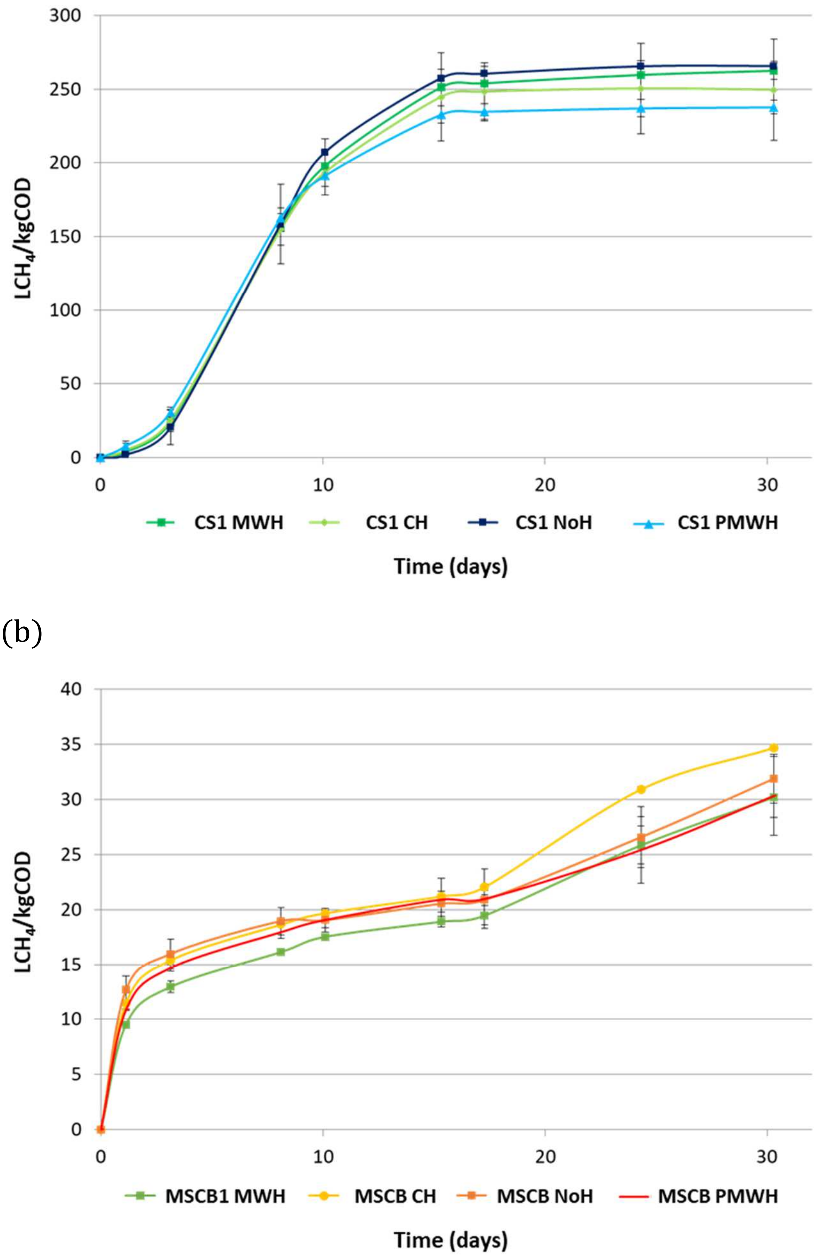

Figure 4: Methane production vs. time from liquid phases after various treatments for CS1 (a) and MSCB (b). MWH classic microwave heating, CH conventional heating treatment, NoH control treatment, PMWH pressurized microwave heating treatment

According to Figure 4a, biodegradability of the released COD in liquid phase for CS1 was not influenced by pretreatments. Whatever the pretreatment, final BMP values reached 265 LCH_4/kgCOD, which was close to the raw BMP value and meant that the solubilised COD was 71% biodegradable. Moreover, the soluble COD was fast biodegradable as in 10 days, the BMP value was already 200 LCH_4/kgCOD whereas it took 35 days for the solid fraction (Figure 3). This result was consistent with Table 1 demonstrating that raw CS1 was rich in soluble content easily biodegradable and with Figure 2, illustrating that soluble compounds were positively correlated with biogas production (0.65).

Concerning MSCB, the trend in Figure 4b was different than for CS1: the total volume produced was low, without exceeding 35 LCH_4/kgCOD, corresponding to a biodegradability of 10%. These results could partially be explained by the low soluble content in miscanthus (Table 1) and the hardly biodegradable nature of solubilised molecules. Nevertheless, these results must be taken with caution, given the COD values out of range in the liquid phase that could lead to misinterpretations.

To the authors' knowledge, this is the first study to dissociate methane production of the solid phase from that of the liquid phase after microwave pretreatment.

3.2.3. Total methane production from solid and liquid phase

A balance of the methane production from the pretreated solid and liquid phases was carried out for each pretreatment in order to determine whether or not pretreatment had an effect on anaerobic biodegradability and methane production. Results are reported in Table 5.

Table 5: Detailed methane production for 1g of biomass CS1 or MSCB (solid and liquid phase), equivalent to 0.92gDM. MWH classic microwave heating, CH conventional heating treatment, NoH control treatment, PMWH pressurized microwave heating treatment

			CS1				MSCB			
			NoH	CH	MWH	PMWH	NoH	CH	MWH	PMWH
Raw COD		gO_2/gDM	1.253				1.304			
Raw BMP		$mLCH_4$/gCOD	193.12				160.86			
		$mLCH_4$/gDM	**222.62**				**192.98**			
Solid phase	**COD**	gO_2/gDM	1.245	1.259	1.253	1.258	1.286	1.301	1.322	1.489
	BMP	$mLCH_4$/gCOD	174.4	166.8	174.6	180.2	172.9	144.0	164.3	161.9
	Mass recovered	gDM/gDM	0.7	0.66	0.7	0.68	0.93	0.93	0.91	0.84
	Methane produced	$mLCH_4$/ gDM	152.0	138.6	153.2	154.1	206.8	174.3	197.6	202.5
Liquid phase	**COD**	gO_2/L	10.12	11.97	12.36	14.15	1.12	1.68	2.322	2.04
	BMP	$mLCH_4$/gCOD	265.7	249.5	262.4	237.8	31.9	34.7	30.2	30.3
	Volume	mL/gDM	20	20	20	19	20	20	20	19
	Methane produced	mL/gDM	53.8	59.7	64.9	63.9	0.7	1.2	1.4	1.2
Total methane produced		**$mLCH_4$/gDM**	**205.7**	**198.3**	**218.0**	**218.1**	**207.6**	**175.4**	**199.0**	**203.7**

From Table 5, it can be seen that the sum of methane production from solid and liquid phases for each pretreatment was not significantly different from the raw biomass methane production. In the case of PMWH treated CS1, the maximum volume produced was 218 $mLCH_4$/g raw biomass, not significantly different from raw CS1 BMP, 222 $mLCH_4$/gDM. In the case of PMWH treated MSCB, the maximum volume produced was 204 $mLCH_4$/g raw biomass, close to raw MSCB BMP, 192 $mLCH_4$/gDM. In the case of MSCB, the methane production from the liquid phase was insignificant compared to that of the solid phase. In conformity with part 3.2.2., this result confirmed that no organic matter was solubilised in the liquid phase during the treatment and thus methane production was not observed in this phase. Results from Table 5 could be compared to those obtained in the same conditions without separating solid and liquid phase during BMP tests: another set of experiments were conducted on raw CS1, NoH CS1 and MWH CS1. Obtained results were 275 $mLCH_4$/gDM, 286 $mLCH_4$/gDM and 308 $mLCH_4$/gDM for raw, NoH and MWH respectively. The different inoculum used during these experiments could explain the higher values obtained compared to those from Table 5. Moreover, for the three conditions tested, high standard deviations (about 20 $mLCH_4$/gDM) were calculated and made it impossible to compare results with one another: NoH and MWH pretreatments seemed having no effects on BMP values, which was similar to the result obtained by separating the solid phase from the liquid phase.

This study demonstrates that the tested microwave pretreatments had no significant effect on methane production, certainly due to the very mild microwave conditions: even in the case of pretreatment under pressure, the temperature did not exceed 140°C and the pressure 4 bar. For example, Thomas et al. (2019) demonstrated an improvement in methane production up to 55% when miscanthus was pretreated with NaOH 10g/100 g_{TS}^{-1}NaOH (without microwave pretreatment), demonstrating the importance of chemical pretreatment and specially the significant effect of chemicals as NaOH in improving biodegradability. In another study, Kan et al. (2018) optimized brewers' spent grain microwave-assisted alkali pretreatment and demonstrated an increase in BMP value up to 52% under optimized conditions: microwave power 70.7W, treatment time 3.31min and alkali/biomass 0.25. Nevertheless, the most impacting term in the second-order polynomial model fitting to the BMP results remained the alkali loading, with a 2.8728 positive coefficient, meaning that under any microwave conditions, microwave are currently unable to compete with chemicals. Indeed, by doubling the pressure (8 bar), Phuttaro et al. (2019) increased the napier grass BMP by 35% by carrying out a hydrothermal pretreatment for 90min at 175°C. However, higher temperatures (200°C) were not recommended as they can cause the formation of anaerobic digestion inhibitors, such as 5-hydroxymethyl furfural resulting from the hemicelluloses degradation. As an example, Wang et al. (2018) observed a rice straw BMP value of only 200 $NmLCH_4/gTS$ following a thermal treatment at 210°C, whereas it reached 300 $NmLCH_4/gTS$ at 180°C. Chemical pretreatments have an important effect on the biomass structure and fibers breakdown. Thus, on olive pomace, alkaline pretreatment combined with microwave for a few minutes permitted to obtain similar BMP (an increase by 13%) to alkaline pretreatment alone during 2 days: pretreatment time was largely reduced using microwave (Elalami et al., 2019). In another study (Kumar Singh et al., 2019), it was the alkaline concentration that can be reduce from 6% to 4% when microwave were combined to chemical treatment for 30min to pretreat kitchen residues. But it is worth mentioning that these results were obtained on very different raw materials than grass biomass: olive pomace were still very rich in fatty acids and kitchen residues in proteins.

In this study, we focused on physical pretreatment with the objective to limit the use of chemicals as much as possible. Chemical-free microwave pretreatment having appeared to be ineffective to increase methane yield, the next step is to study combined microwave/chemical pretreatment at low chemical concentration (synergy effect). Our aim is to develop greener pretreatment technologies, with low chemical consumption.

4. Conclusions

Chemical-free microwave pretreatments (in open vessel and under pressure) were performed on two LCB of industrial interest (corn stalks and miscanthus) with the aim of evaluating microwave chemical-free pretreatment as a method of improving anaerobic biodegradability of biomass, by reducing its recalcitrance. BMP tests carried out on raw biomass before pretreatment highlighted the negative correlation of BMP value to lignin and cellulose contents and the positive correlation to soluble and hemicellulose contents, and made it possible to select the least "efficient" genotype and clone (with the more room for biodegradability improvement), on which pretreatments could be tested: corn stalk genotype F 98902 noted CS1 and miscanthus clone *M. x giganteus* Britannique, noted MSCB, respectively.

From biomass analysis, it appeared that depending on raw biomass, liquid phase could account for a significant percentage of total BMP, up to 25% in the case of corn stalks (cell wall rich in soluble content). On the contrary in the case of miscanthus, the liquid phase represented only 0.5% of the total BMP (cell wall rich in parietal elements for miscanthus). According to our experimental results, chemical-free microwave pretreatment (open vessel or under pression) did not allow to increase BMP value of miscanthus nor corn stalks samples, because these conditions were not harsh enough to affect the lignocellulosic network, as it was observed following 10 hours 10%w/w NaOH pretreatment (+30% increase of BMP value). To conclude, with the tested operating conditions, no improvements in BMP could be reached, but this work constitutes a basis for further microwave pretreatment investigations.

An interesting perspective would be combining microwave heating to low NaOH (or other chemicals) proved to be efficient for biomass deconstruction. A synergy microwave effect could allow to obtain higher impact on recalcitrance using lower NaOH amounts than chemical treatment alone. Finally, it is important to emphasize that the energy recovery from biomass must remain only the last step in a cascade process.

Credit statements

Conceptualization, JP.D., V.M. and D.GB.; methodology, A.B. and M.L.; formal analysis, A.B.; investigation, A.B. and D.GB.; writing—original draft preparation, A.B.; writing—review and editing, JP.D., D.GB., V.M., and N.B. All authors have read and agreed to the published version of the manuscript.

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