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► To cite this version:

Florian Monlau, Prasad Kaparaju, Eric Trably, Jean-Philippe Steyer, H el ene Carr ere. Alkaline pretreatment to enhance one-stage CH₄ and two-stage H₂/CH₄ production from sunflower stalks: Mass, energy and economical balances. *Chemical Engineering Journal*, 2015, 260, pp.377-385. 10.1016/j.cej.2014.08.108 . hal-01165743

HAL Id: hal-01165743

<https://hal.science/hal-01165743v1>

Submitted on 7 Aug 2023

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Alkaline pretreatment to enhance one-stage CH₄ and two-stage H₂/CH₄ production from sunflower stalks: mass, energy and economical balances.

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Abstract

Biological production of second generation biofuels such as biohydrogen (H₂) or methane (CH₄) represents a promising alternative to fossil fuels. Alkaline pretreatments of lignocellulosic biomass are known to enhance the accessibility and the bioconversion of hollocelluloses during anaerobic digestion and dark fermentation processes. In the present study, four different configurations were investigated: one-stage CH₄ continuous and two-stage H₂ batch / CH₄ continuous process with and without alkaline pretreatment of sunflower stalks (55 °C, 24 h, 4 gNaOH/100 gTS). The results showed that two stage H₂/CH₄ (150±3.5 mL CH₄ g⁻¹ VS) did not improve methane yields compared to one stage CH₄ (152±4 mL CH₄ g⁻¹ VS). Although alkaline pretreatment was shown to be inefficient in improving the H₂ yields in the two-stage H₂/CH₄ process, an increase in methane yields by 26 % and 29 % were observed with one-stage CH₄ and two-stage H₂/CH₄ production compared to one-stage CH₄ process without alkaline pretreatment, respectively. Chemical analysis of the solid digestate showed that hemicelluloses were the most preferred substrates compared to cellulose whereas lignin remained undegraded in all four studied configurations. Finally, energy balance showed that a positive energy balance and economic sustainability can be achieved when the alkaline pretreatment is applied at a

1 high substrate concentration and/or when heat is recovered at a maximum efficiency during the
2 pretreatment step.
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6 **Keywords**

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8 Anaerobic digestion; biohydrogen; dark fermentation; lignocellulosic biomass; methane; energy assessment.
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10 11 12 13 14 15 **1. INTRODUCTION** 16

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20 The use of the agricultural residues such as sunflower stalks, with a world cultivated area of 26 million
21 hectares in 2011, is considered as one of the most interesting renewable resources for bioenergy
22 production [1]. Sunflower stalk is a prominent example of agricultural residues lacking of alternative
23 reuse and that are left in the fields after seed harvesting, and must be burned to keep the fields cleared
24 and prevent the propagation of vegetal diseases [2]. Among the renewable bioenergies, methane and
25 biohydrogen represents promising future energy carriers. Their production from lignocellulosic
26 residues will generate renewable energy, reduce the greenhouse gas emissions by avoiding the on-field
27 burning and/or decomposition and by substituting fossil energies, provide renewable biomass without
28 competing with food supplies and close the energy and nutrient cycles.
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40 Production of hydrogen and methane has been recently investigated in two-stage biological processes
41 using various lignocellulosic substrates: olive pulp [3], reed canary grass [4], grass silage [5], sweet
42 sorghum [6], maize [7], cornstalks [8], wheat straw [9], thin stillage [10] and rapeseed stillage [11].
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47 The two-stage H₂/CH₄ process presents several advantages compared to the conventional one stage
48 CH₄ process since it leads to the selection and enrichment of specific and different bacterial consortia
49 in each stage, resulting in a better overall process stability [11]. In the first stage, low pH and short
50 hydraulic retention times, (HRTs) are maintained in order to favour the fermentation of lignocellulosic
51 residues into hydrogen and volatile fatty acids (VFAs) by enhancing the growth of H₂-producing
52 bacteria. These bacteria are less sensitive to substrate fluctuations than methanogens. In the second
53 stage, neutral pH and longer HRTs are maintained to develop the growth of acetogens and
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methanogens for further conversion of VFAs to methane and carbon dioxide. This process is very sensitive to pH changes, VFA accumulation, and organic loading rates. Moreover, the two-stage H_2/CH_4 can have a positive effect on methane production compared to one-stage CH_4 by enhancing the hydrolysis of lignocellulosic residues in the acidogenic H_2 stage. For instance, Nasr et al. (2012) reported previously an increase in methane production by 27 % from thin stillage after two-stage H_2/CH_4 compared to one-stage CH_4 process [10]. As only about 10-20 % of the energy potential of an organic substrate is recovered during the dark H_2 fermentation, subsequent valorisation of the H_2 stage effluent in an anaerobic methane digester would represent a promising alternative to improve the overall energy balance [12].

One major parameter to be considered when lignocellulosic residues are used as feedstock in biological fermentative processes, *i.e.* dark H_2 fermentation and anaerobic digestion, is the structural and compositional features of the lignocellulosic matrix. This lignocellulosic matrix inherently provides the tensile strength and acts as barrier against pests and pathogens, and also confers resistance to its hydrolysis [13]. Thus, for successful utilization of lignocellulosic biomass, a pretreatment step is often required to improve the biomass biodegradability by increasing the accessibility of holocelluloses to enzymatic or bacterial attack [13]. Alkaline pretreatments are known to be efficient in delignification, fiber expansion and increase the pore size and accessible surface area. All these elements facilitate the diffusion of hydrolytic enzymes and represent a new and promising opportunity for efficient pretreatment [14, 15]. However, too drastic pretreatments conditions could lead to the production of potentials inhibitory of dark fermentation and anaerobic digestion process [16]. In a previous study, an optimal alkaline pretreatment (55 °C, 24 h, 4 g NaOH / 100 g TS) led to an increase in methane yields of stalks of four sunflower varieties by 29 to 44 % [17]. Moreover, alkaline pretreatment was also shown to be efficient in delignification with a reduction of lignin content by 23.3- 36.3 % [17]. The effect of pretreatments on methane potential has been widely investigated in batch assays over the past few years [13, 18, 19]. So far, only a few studies reported the impact of pretreatment methods on lignocellulosic residues for H_2/CH_4 production

1 in two-stage processes [5, 7, 8]. Pakarinen et al. (2011) studied two types of pretreatments (water
2 extraction and HCl) to enhance hydrogen and methane production from maize in a two-stage H₂/CH₄
3 process. In the above study, methane yields from water extracted and HCl-treated maize were
4 improved by 9 % and 27 % respectively, in a two-stage process (H₂/CH₄) compared to one-stage
5 process [7]. Nonetheless, the effect of lignocellulosic biomass pretreatment to enhance one-stage CH₄
6 and two-stage H₂/CH₄ has been poorly investigated in continuous mode. Such data are essential to
7 assess long-term productivity and feasibility prior to industrial scale-up, and provide basis for further
8 energy and economical assessments of the whole process.

9 The objectives of this study were to compare the energy yields and process performances (i.e. energy
10 production and volatile solids removal) of one-stage CH₄ (continuous) and two-stage H₂ (batch)/ CH₄ (continuous)
11 process coupled with or without alkaline pretreatment and to evaluate the energy balance and
12 economical assessment of the alkaline pretreatment in the above studied configuration. However, it
13 should be noted that in the present study H₂ (batch)/ CH₄ (continuous) configuration was used instead of a H₂
14 (continuous)/ CH₄ (continuous) system for several reasons. The main reason is the lack of process stability of
15 continuous H₂ reactors when fed with complex substrates such as lignocellulosic biomass. The second
16 reason is the difference in HRTs in continuous digesters, i.e. 6 to 24 hours in H₂ digesters and 20-40
17 days CH₄ digesters. Thus, H₂ digesters were operated in batch mode and can also be considered as a
18 biological pretreatment (prehydrolysis) for the subsequent methane production, during which some
19 energy (hydrogen) can be recovered.

20 2. MATERIALS AND METHODS

21 2.1 Feedstock collection and preparation

22 Sunflower stalks of “Serin” variety were used as feedstock. The stalks were collected directly from the
23 field, two weeks after harvest, from a farm located in the Southern France. The stalks were milled to
24 obtain a particle size of 500 µm using a cutting mill (Ika Werke MF 10). Alkaline pretreatment (55 °C,
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24 h, 4 g NaOH / 100 g Total Solids (TS)) was carried out at a solid loading of 35 g TS L⁻¹ in glass bottles (600 mL) with a working volume of 100 mL. The pretreatment conditions (temperature, time, concentration, solid loading) were selected according to an earlier investigation [17, 20]. The bottles were continuously mixed by magnetic stirrer and incubated at 55 °C in a thermostatically controlled water bath. After pretreatment, the whole slurry was used as feedstock. In addition, pretreated slurry was filtered through 0.25 mm mesh filter to separate in to a solid and liquid fractions for further chemical analysis. The solid fraction was dried at 60 °C for 24 h prior to analysis.

2.2 Batch experiment for hydrogen production

Hydrogen production from raw and pretreated sunflower stalks was performed at 37 °C in batch reactors with a working volume of 4.5 L. Heat shock pretreated anaerobic granular sludge (90 °C for 15 min) was used as inoculum. The substrate/inoculum ratio of 20 g TS substrate g⁻¹ Volatile Solids (VS) inoculum was used. The initial pH was adjusted to 6 by using 37 % HCl. Biogas volume was measured by using an acidified water displacement method and biogas composition was analysed according to the protocol described in section 2.4. Biohydrogen production was expressed in standard conditions (0°C, 1013 hPa). Once the cumulative hydrogen production reached plateau, reactors were stopped and sampled for metabolite analysis. Reactor digestate was stored at -20 °C and was defrosted just before further use as feed for continuous methane production. In this way, the composition of methane reactors feed was constant throughout the experimental period.

2.3 Continuous reactor experiment for methane production

Four identical semi-continuous anaerobic continuously stirred tank reactors (CSTR) with a working volume of 1.5 L were operated for methane production. Reactor configurations are presented in Figure 1. The reactors were initially inoculated with granular sludge from a mesophilic anaerobic digester of a sugar factory, at an initial concentration of 50 g VS L⁻¹. Reactors were fed manually on weekdays

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(Monday through Friday) at $2.1 \text{ g VS L}^{-1} \text{ d}^{-1}$ corresponding to an organic loading rate (OLR) of $1.5 \text{ g VS L}^{-1} \text{ d}^{-1}$. HRT was 21 days and each experiment was carried out for at least 3 HRT. Reactors were continuously mixed with a magnetic stirrer and maintained at $35 \text{ }^\circ\text{C}$ by external water recirculation system. Biogas was collected in a 10 L gas bags (Environmental Sampil Supply[®]). Biogas volume was measured three times a week by liquid displacement method and results were expressed in standard conditions (0°C , 1013 hPa). The liquid used was 10 g/L of NaCl solution and acidified (pH of 2) by adding HCl to avoid CO_2 absorption. After two HRTs, 10 mL of NH_4Cl (30 g L^{-1}) was added each week in the digesters to counterbalance nitrogen exhaustion. At the end of the experiment, the solids in digestate were separated by centrifugation at 9000 g for 10 min. , dried at 105°C for 24 h , and stored at 4°C for further chemical analysis.

Figure 1. Four configurations of one-stage CH_4 and two-stage H_2/CH_4 with or without alkaline pretreatment (55°C , 24 h , $4 \text{ g NaOH} / 100\text{gTS}$) investigated

2.4 Chemical and gas analyses

TS and VS were analysed in triplicate according to the APHA standard methods [21]. Soluble sugars from starch, sucrose and inulin were extracted using a mild acid hydrolysis method [22] and quantified with the anthrone method. Structural carbohydrates (glucose, xylose and arabinose) as well as uronic acids, i.e. galacturonic and glucuronic acids coming from cellulose, hemicelluloses and pectins were quantified in triplicates using a strong acid hydrolysis protocol as described elsewhere [20]. VFA composition in the liquid phase, i.e. acetic (C2), propionic (C3), butyric and iso-butyric (C4 and iC4), valeric and iso-valeric (C5 and iC5) and caproic (C6) acids, was determined by using a gas chromatograph (GC-3900, Varian) equipped with a CP 8400 sampler, an Alltech FFAP ECTM 1000 column and with a flame ionization detector (FID). The column temperature was $80 \text{ }^\circ\text{C}$ for 1 minute and heated to $120 \text{ }^\circ\text{C}$ within 2 minutes and maintained at $120 \text{ }^\circ\text{C}$ for 9 minutes. The carrier

1 gas was nitrogen (25 kPa). Non-VFA metabolic end-products (lactate and ethanol) were analyzed
2 by High Performance Liquid Chromatography (HPLC) coupled to refractometric detection (Waters
3 R410). The components were separated with an Aminex HPX-87H column (Biorad). The eluting
4 solution corresponded to 0.005 M H₂SO₄, and the flow rate was 0.4 mL min⁻¹. The column
5 temperature was maintained at 35 °C. Finally, total phenols in liquid fractions were determined using a
6 microtube test (Spectroquant, Merck) followed by a 4-aminoantipyrine colorimetric measurement after
7 a two-hundred dilution.
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18 Biogas composition (CH₄, O₂, CO₂, H₂ and N₂) was analysed by using a gas chromatograph (Clarus
19 280, Perkin Elmer) equipped with HayeSep Q and molecular sieve (5 Å) columns, coupled to a
20 thermal conductivity detector (TCD). The operating conditions were as follows: the carrier gas was
21 argon under a pressure of 102 kPa and a flow rate of 4.5 mL min⁻¹; temperatures of the injector and the
22 detector were both fixed at 150°C and column temperature was 65 °C.
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31 ***2.5 Preliminary energy balance and economic assessment of the alkaline pretreatment***

32 A preliminary energy balance and economical assessment on the use of alkaline pretreatment was
33 carried out by comparing the extra costs (i.e. heating and chemical reagent) required for the alkaline
34 pretreatment with the extra energy gained by improving the methane yields after pretreatment.
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40 The heat energy requirement (HER) to treat 1 ton TS of sunflower stalks during the thermo-alkaline
41 pretreatment (55 °C) was evaluated according to equation 1.
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$$47 \text{ HER} = \left\{ \frac{[m \times C_p \times (T_{\text{final}} - T_{\text{initial}})]}{3600} \right\} \quad \text{(Equation 1)}$$

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50 Where HER is the heat energy requirement expressed in kWh t⁻¹ TS, m is the mass of water and
51 substrate in kg; C_p the water specific heat (4.18 kJ kg⁻¹°C⁻¹); T initial (°C) is the initial temperature of
52 the substrate suspension, assumed as 25 °C; T final (° C) is the final temperature of the substrate
53 suspension fixed at 55°C in our study. For assessing the total cost of chemical pretreatment, the
54 European cost of sodium hydroxide was used (412 €/ton) [23].
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3. RESULTS AND DISCUSSION

3.1 Effect of alkaline pretreatment on chemical composition of sunflower stalks

Chemical composition of sunflower stalks before and after alkaline pretreatment is presented in Table 1. As expected, alkaline pretreatment resulted in solubilization of organic matter. The increase in soluble VS was 22.5 % in alkaline pretreated sunflower stalks. Alkaline pretreatment also increased the solubilization of uronic acids, originating from pectins and hemicelluloses (64 %), hemicellulose (26 %) with subsequent solubilization and removal of lignin (22 %). This result is in agreement with Xie et al. (2011) who also reported that alkaline pretreatment (100 °C with a NaOH concentration of 1 g / 100g VS) resulted in the removal (of more than 21 %) of lignin from grass silage [24]. Our observations on chemical composition were also consistent with those of Zhu et al. (2010) who suggested that alkaline pretreatment is effective in delignification while preserving most of the carbohydrates, and in particularly cellulose [25]. In addition, the total phenolic compounds concentration of 25.7 mg.L⁻¹ noticed in the alkaline pretreated samples indicates that the solubilization or degradation of lignin into potential inhibitors i.e. vanillin, syringaldehyde (Table 1). These results are in accord to those reported previously during the alkaline pretreatment on lignocellulosic substrates [30].

Table 1. *Composition of sunflower stalks before and after alkaline pretreatment (average values of triplicates and standard deviation errors). Except for phenols content, all chemical contents were determined on the solid fraction obtained after the alkaline pretreatment.*

3.2 Performances of the anaerobic bioprocesses

3.2.1 Dark H₂ production in batch experiments

1 Biogas production started immediately in all dark fermentation assays and was free of methane
2 indicating that heat shock pretreatment for the inoculum was effective in suppressing the
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4 methanogenic activity. Hydrogen yield of 7.1 mL H₂ g⁻¹ VS was obtained from untreated sunflower
5 stalks. This yield was similar to the H₂ yield of 8 mL H₂ g⁻¹ VS reported for wheat straw inoculated
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7 with a similar anaerobic mixed culture [37]. The low H₂ yield in the present study can be attributed to
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9 the low amount of soluble sugars in the untreated sunflower stalks (1.9 g / 100 gVS), as shown in
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11 Table 1. Indeed, recent studies have also shown that the H₂ production by mixed cultures could be
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13 directly correlated to the content of soluble sugars [14].
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20 As a result, alkaline pretreatment did not enhance the H₂ yield of sunflower stalks. A maximum H₂
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22 yield of 6 mL H₂ g⁻¹ VS was obtained from the pretreated biomass (Table 2). The low H₂ yield in the
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24 present study was attributed to the low solubilization yield of hemicelluloses (24 %), leading to
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26 soluble sugars representing 5.6 % of initial VS. However, considering that H₂ production is directly
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28 linked to soluble sugars content [14], a higher H₂ yields should have been obtained with pretreated
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30 than raw sunflower stalks. But this was not observed because of the release of phenolic compounds
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32 during the alkaline pretreatment [26] and these phenolic compounds are known to have some
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34 inhibitory effects on dark H₂ fermentation micro-organisms [28-30]. These results are in accordance
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36 with the observations made by Fangkung and Reungsang [31] who reported a reduction in hydrogen
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38 production with increasing alkaline concentration. The above authors also showed that complete
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40 inhibition of biohydrogen production was noticed when soda dosage was higher than 1% (w/v). The
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42 decrease in H₂ yields in the above study was attributed to the process inhibition by the release of by-
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44 products especially phenolic compounds from lignin degradation [31]. Chang et al. [33] showed that
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46 removal of by-products generated during pretreatments by lime and activated carbon treatment can
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48 improve the biohydrogen production compared to control, which was completely inhibited. However,
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50 the specific and negative effect on anaerobic digestion of the by-products released during pretreatment
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52 is difficult to ascertain as the beneficial effect of opening the plant cell structure and liberating the
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54 sugars can overcome the chemical inhibitory effect of released by-products [32].
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1 As shown in both hydrogen assays with and without alkaline pretreatment, the slight decrease in pH
2 noticed at the end of experiment was attributed to the accumulation of microbial metabolites.
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4 Interestingly, only acetate and butyrate were accumulated indicating that hydrogen was produced
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6 exclusively through these acetate-butyrate pathways (Table 2). The production of butyrate as well as
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8 acetate as end-products is typical for dark H₂ fermentation from carbohydrates rich-substrates.
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10 Quéméneur et al. [27] reported that acetate and butyrate were the dominant intermediate metabolites
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12 produced during mesophilic H₂ production from wheat straw using heat pretreated (90 °C, 10 min)
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14 mesophilic anaerobically-digested sludge as inoculum. The high concentration of acetate (1.5 g/L) and
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16 the low hydrogen yield in the alkaline pretreated sunflower stalks assays suggests that partial
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18 solubilization of acetyl groups of hemicelluloses into acetate might have probably occurred during
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20 alkaline pretreatment [26].
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26 *Table 2. Feedstocks parameters, biohydrogen and methane performances for the four configurations. Data for*
27 *continuous anaerobic digester are presented during the third hydraulic retention time (measured over two days*
28 *for methane production and each week for the pH).*
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34 **3.2.2 Methane production in continuous anaerobic digesters**

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37 In this section methane production performance will be compared, first the impact of soda
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39 pretreatment on one-stage methane process will be discussed, second one-stage and two-stage
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41 processes will be compared and finally the impact of soda pretreatment on two-stage process
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47 After two HRTs, the anaerobic digesters were stable and considered at steady state (Table 2). Process
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49 performance of the anaerobic digesters with and without alkaline pretreatment was compared during
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51 the third HRT for the four configurations (see Table 2). Neither feed pH nor digester pH was adjusted
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53 in methane digester of configuration 2 fed with NaOH pretreated sunflower stalks. The pH in the
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55 methane digester was nearly neutral (pH=6.9) and close to that of pH in configuration 1 methane
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57 digester (pH= 6.6). Good buffer capacity of the digester and dilution of feed may have enabled to
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59 operate the digester with a high pH (pH 11) feed. Moreover, the relatively high sodium concentration
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in the feed (0.8 g L^{-1}) did not have any detrimental effect on the process. Alkaline pretreatment was found to be effective in increasing the methane yield of the one-stage CH_4 process (26 %) compared to the control. A methane yield of $191 \pm 3 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$ (configuration 2) was obtained compared to $152 \pm 4 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$ (configuration 1). Only a few studies dealt with the effect of pretreatment to enhance one-stage CH_4 process operated in continuous mode. Sambusiti et al. [33] reported alkaline pretreatment (40°C , 24 h, 10g NaOH / 100gTS) of ensiled sorghum and further use of pretreated biomass as feedstock for biogas production in CSTR reactors. The above authors reported an increase of 25 % in methane yields from pretreated sorghum biomass compared to control. In addition to increase in methane yields, alkaline pretreatment was also found to provide stability to the process by maintaining a high alkalinity in the system and limiting pH drop.

On comparison of two-stage process H_2/CH_4 (configuration 3) with one-stage CH_4 process (configuration 1), the results showed that two-stage H_2/CH_4 ($152 \pm 4 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$) did not improve the methane production compared to one-stage CH_4 ($150 (\pm 3.5) \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$). Freezing and defrosting of digestate from H_2 fermentation can be considered as a pretreatment before anaerobic digestion and might be expected to enhance methane production by damaging sunflower stalks cells. However, as methane yields after freezing (configurations 3 and 4) were the same as the yields obtained in configurations 1 and 2, we can assume that freezing/defrosting step had no impact on the methane production results. The similar methane yields obtained from two-stage H_2/CH_4 compared to one-stage CH_4 can be explained by the low hydrolysis in the H_2 stage as shown by the low amount of VFAs produced (Table 1). Similar results were previously observed by Pakarinen et al. [7] who compared a two-stage H_2/CH_4 with a one-stage H_2 or CH_4 from maize, and did not observed any significant increase in methane yields. In contrast, Pakarinen et al. [5] showed that a higher CH_4 yield was obtained from grass silage in a two-stage process ($467 \text{ mL g}^{-1} \text{ VS}_{\text{original}}$) compared to a one-stage process ($431 \text{ mL g}^{-1} \text{ VS}_{\text{original}}$). Nasr et al. [11] also reported a maximum methane yield of $333 \text{ mL g}^{-1} \text{ COD}$ (Chemical Oxygen Demand) in a two-stage H_2/CH_4 process, that was 24 % higher than the yield obtained from one-stage CH_4 process. In a different study, Luo et al. [11] compared one-stage CH_4 with two-stage H_2/CH_4 processes fed with a mixture of effluents (i.e. cake, glycerol, stillage) produced

1 from rapeseed biodiesel and bioethanol processes. The authors in the above study reported that, under
2 similar process conditions, one-stage CH₄ process failed due to a decrease in pH associated with the
3 VFAs build-up whereas the two-stage H₂/CH₄ remained stable [11]. Nevertheless, alkaline
4 pretreatment in the present study was found to be effective and improved the methane yields in the
5 two-stage H₂/CH₄ (Table 2). This was evident from the higher methane yield of 196 (±3) mL CH₄ g⁻¹
6 VS obtained from two-stage H₂/CH₄ process fed with alkaline pretreated sunflower stalks compared to
7 methane yields of 150 (±3.5) mL CH₄ g⁻¹ VS obtained for the same configuration without alkaline
8 pretreatment. However, the similar increase in methane yields from the alkaline pretreated sunflower
9 stalks in one-stage CH₄ and two-stage H₂/CH₄ suggests that the increase in methane production was
10 mainly due to the effect of alkaline pretreatment rather than hydrolysis in the H₂ stage.
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25 **3.3 Overall mass balance and digestate composition for the four configurations**

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27 Mass balances of the four configurations were assessed and are presented in Table 3. VS balance was
28 computed for all 4 reactor configurations by considering “Sunflower stalks” as the input variable and
29 “methane”, “carbon dioxide”, “hydrogen” and “digestate” as output variables.
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36 *Table 3. Organic matter distribution for one ton of initial sunflower stalks converted through the four*
37 *configurations*
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42 VS conversion to biogas in reactor configurations with alkaline pretreatment were higher in one-stage
43 CH₄ (49.6 %) and two-stage H₂/CH₄ (50.6 %) than the corresponding conversion efficiencies of the
44 same configurations without alkaline pretreatment. Higher VS removal with alkaline pretreatment
45 suggests that the pretreatment probably increased the accessibility of anaerobic microorganisms for a
46 better conversion of the organic matter into biogas. Cellulose, hemicellulose and lignin contents in the
47 digestate from the four configurations are shown in Figure 2. The results provide an interesting data on
48 which component of the lignocellulosic matrix of sunflower stalks was preferentially degraded during
49 the anaerobic digestion process. Interestingly, in all four configurations, hemicelluloses were almost
50 completely degraded. In addition, anaerobic digestion was found to be less efficient in degrading the
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1 cellulose polymers than hemicelluloses especially in the absence of alkaline pretreatment. The
2 alkaline pretreatment may have likely increased the accessibility of cellulosic compounds and
3 therefore, their biodegradation. Nevertheless, a part of cellulose still remained undegraded after the
4 anaerobic process. The undegraded cellulose might correspond to the crystalline part of cellulose
5 which has been already shown as more recalcitrant to degradation in anaerobic digestion [14, 17]. The
6 decrease in the Klason lignin in configurations 2 and 4 was not attributed to the biodegradation during
7 the anaerobic process but to a partial solubilization of lignin during alkaline pretreatment. Such lignin-
8 based compounds are known to be recalcitrant and poorly degraded during anaerobic digestion [34].
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23 *Figure 2. Total solids removals and digestate composition after anaerobic fermentative process for the four*
24 *configurations investigated. Chemical composition (i.e. cellulose, hemicelluloses, Klason lignin and ash) are*
25 *provided on the raw samples and on the digestate solid remaining part after anaerobic digestion process*
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31 **3.4 Energy balance and economic assessments of alkaline pretreatment**

32 Results on biohydrogen and methane production showed no significant difference between the total
33 energy produced in the one-stage CH₄ (1910±30 kWh t⁻¹ TS) and two-stage H₂/CH₄ (1974±38 kWh t⁻¹
34 TS) process when coupled to alkaline pretreatment. As two-stage H₂/CH₄ process requires
35 supplementary costs for the construction of the hydrogen-producing reactor, the energy balance and
36 economic assessments of alkaline pretreatment were considered only for one-stage CH₄ process. The
37 results are presented in Table 4.
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46 Conversion of biogas into heat and electricity using combined heat and power (CHP) system was
47 considered with a thermal efficiency of 50 % and an electricity efficiency of 35 %. A part of the heat
48 produced can be further used to cover the thermal energy requirement of alkaline pretreatment (Table
49 4). Moreover, this point is consistent with the present development of farm anaerobic digestion in
50 European countries where biogas is converted by CHP in most of the cases. The energy balance and
51 economic assessment of alkaline pretreatment was achieved to verify if the surplus energy (thermal
52 and electrical) and economic gain (through sale of surplus electricity) obtained by the application of a
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1 pretreatment, prior to anaerobic digestion step, can cover the inputs *i.e.* thermal energy for heating the
2 pretreatment tank, electrical energy for mixing the pretreatment tank and also cost of the chemical
3 reagent.
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6 Thermal requirement for alkaline pretreatment was assessed by considering the energy needs to raise
7 the temperature of sunflower stalks mixture (35 g TS L⁻¹) from 25 °C to 55 °C assuming that specific
8 heat of the substrate suspension in water can be evaluated by the water specific heat (4.18 kJ kg⁻¹ °C⁻¹).
9 Heat losses during pretreatment were not considered as in the case of insulated digesters, they were
10 shown to be negligible (2-8 %) [42]. To assess the interest of using pretreatment in terms of thermal
11 energy, the surplus heat (185 kWh t⁻¹ TS) was calculated as the difference between the heat produced
12 by the single one-stage CH₄ process with alkaline pretreatment (configuration 2) compared to the
13 single one-stage CH₄ process without alkaline pretreatment (configuration 1). The surplus heat was
14 further compared to the thermal energy requirement for alkaline pretreatment. At a solid loading of
15 35g TS L⁻¹, the surplus heat (184 kWh t⁻¹ TS) was far not sufficient to cover the heat requirement for
16 alkaline pretreatment (1034 kWh t⁻¹ TS).
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31 Table 5 summarizes literature data on the heat balance of various thermal and thermo-chemical
32 pretreatments applied on biomasses *i.e.* sludge, lignocellulosic residues, algae to enhance methane
33 production. Solid loading and heat recovery from the pretreatment step were identified as the main
34 factors for reducing the heat requirement of thermal and thermo-alkaline pretreatment. Besides
35 operational temperature, the energy required for thermal pretreatment of 1 ton of dry solids is highly
36 dependent on the solid loading. Low solid loadings have been previously identified as a critical point
37 on the energy assessment when using thermal or thermo-chemical pretreatments [36-38]. Several
38 studies were investigated to evaluate the feasibility of applying pretreatments at higher solid loading
39 (≥ 15 % solids, w/w) [39, 40]. Schell et al. [40] demonstrated the feasibility of a pilot scale plant (1
40 ton day⁻¹) capable of performing continuous acid pretreatment of corn stover at a solid loading of 200
41 kg TS m⁻³. When compared at this solid loading, net heat energy of alkaline pretreatment was slightly
42 negative (-25 KWh t⁻¹ TS).
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In addition, thermal energy integration has to be carried out in a full scale implementation of thermal or thermo-chemical pretreatment [36, 41]. Moreover, the heat energy of pretreated substrate suspension can be recovered by heat exchanger to heat the digester [42] or to preheat raw substrate suspension [43]. Dhar et al. [44] reported that up to 80 % of heat energy can be recovered from thermally pretreated sludge. In the present study, assuming a heat energy recovery of 80 % from the pretreatment step would lead to a negative net heat energy of $-22 \text{ kWh t}^{-1} \text{ TS}$ when solids loaded at 35 g TS L^{-1} and a positive net heat energy of $38 \text{ kWh t}^{-1} \text{ TS}$ for a solid loading of 50 g TS L^{-1} . In the most favorable case with a solid loading of 200 g TS L^{-1} , net heat production was $143 \text{ kWh t}^{-1} \text{ TS}$.

With respect to electrical energy requirements, electricity required for the mixing of the alkaline pretreatment tank was only considered. In the present study, the electrical energy consumption by the bale breaker and shredder used to grind the feedstock was not considered as it can be assumed that these machines will be in use even when untreated sunflower stalks are anaerobically digested. Menardo et al. [45] reported that the energy required for straw bale breaker and shredder was $15 \text{ kWh t}^{-1} \text{ TS}$. On the other hand, the electrical energy required for mixing was reported to be $10.5 \text{ kWh t}^{-1} \text{ TS}$ during thermo-alkaline pretreatment of wheat straw at a solid loading of 50 g TS L^{-1} for 24 h duration [46]. Thus, the estimated net electrical energy required for alkaline pretreatment of sunflower stalks at a solid loading of 50 g TS L^{-1} was $118.5 \text{ kWh t}^{-1} \text{ TS}$.

In addition, sale of surplus of electricity ($119 \text{ kWh t}^{-1} \text{ TS}$) to the public grid at a fixed rate would provide some economical benefits. It was considered here that the methane produced in configuration with alkaline pretreatment would provide enough heat energy to cover both energy requirements for the pretreatment and heating anaerobic digester. Thus, thermal pretreatment cost was not considered. For the economical assessment, as first assumption, three government incentive policies for biogas energy were considered in three European countries: France ($0.18 \text{ € kWh}^{-1}_{\text{el}}$), Germany ($0.25 \text{ € kWh}^{-1}_{\text{el}}$), and Italy ($0.28 \text{ € kWh}^{-1}_{\text{el}}$). As second assumption, the European cost of the NaOH was used (412 € ton^{-1}). Results are presented in Table 4 for three European countries. Net gains of 4, 13 and $16 \text{ € t}^{-1} \text{ TS}$ were respectively obtained for France, Germany and Italy.

1
2 **Table 4.** Energy and economical assessment analysis for the two configurations: one-stage CH₄ and
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4 NaOH one-stage CH₄.
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10 **Table 5.** Comparison of heat balances of thermal and thermo-chemical pretreatment to enhance
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12 methane potentials from biomasses according literature data.
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17 **4. CONCLUSION**

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19 In the present study, four different configurations one-stage CH₄ continuous and two-stage H₂ batch / CH₄
20 continuous with and without alkaline pretreatment were compared in terms of biohydrogen and methane
21 yield. Total energy produced from two-stage H₂/CH₄ was found equivalent to one-stage CH₄. On the
22 contrary, alkaline pretreatment improved the total energy of both one-stage CH₄ and two-stage
23 H₂/CH₄. The increase in methane yields in two-stage H₂/CH₄ was due to the effect of alkaline
24 pretreatment rather than hydrolysis in the H₂ stage. Chemical analysis of the solid digestate for the
25 four configurations have clearly shown that hemicelluloses were preferred components compared to
26 cellulose whereas lignin remained undegraded. Energy balance results showed a negative thermal
27 balance for alkaline pretreatment of sunflower stalks at a solid loading of 35 g TS L⁻¹. However, a
28 positive thermal balance can be achieved if high substrate concentration and/or heat recovery from the
29 pretreatment step are incorporated in the process, which requires further investigations.
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44 **Acknowledgements**

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47 The authors are grateful to ADEME, the French Environment and Energy Management Agency, for financial
48 support in the form of F. Monlau's PhD grant.
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2 **List of table and figure captions**
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6 **Table 1.** *Composition of sunflower stalks before and after alkaline pretreatment (average values of triplicates*
7 *and standard deviation errors). Except for phenols content, all chemical contents were determined on the solid fraction*
8 *obtained after the alkaline pretreatment*
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14 **Table 2.** *Feedstock parameters, biohydrogen and methane performances for the four configurations. Data for*
15 *continuous anaerobic digester are presented during the third hydraulic retention time (measured over two days*
16 *for methane production and each week for the pH).*
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24 **Table 3.** *Organic matter distribution for one ton of initial sunflower stalks converted through the four*
25 *configurations*
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31 **Table 4.** *Energy and economical assessment analysis for the two configurations: one-stage CH₄ and*
32 *NaOH one-stage CH₄.*
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37 **Table 5.** *Comparison of heat balances of thermal and thermo-chemical pretreatment to enhance*
38 *methane potentials from biomasses according literature data.*
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45 **Figure 1.** *Four configurations of one-stage CH₄ and two-stage H₂/CH₄ with or without alkaline pretreatment*
46 *(55°C, 24h, 4g NaOH / 100gTS) investigated*
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51 **Figure 2.** *Total solids removals and digestate composition after anaerobic fermentative process for the four*
52 *configurations investigated. Chemical composition (i.e. cellulose, hemicelluloses, Klason lignin and ash) are*
53 *provided on the raw samples and on the digestate solid remaining part after anaerobic digestion process*
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Table 1. *Composition of sunflower stalks before and after alkaline pretreatment (average values of triplicates and standard deviation errors). Except for phenols content, all chemical contents were determined on the solid fraction obtained after the alkaline pretreatment.*

Parameters	Sunflower stalks	Pretreated sunflower stalks
pH	8	11.1
TS (% wet weight)	96.4 ± 0.3	100 ^a
VS (% wet weight)	89.4 ± 0.6	94.4 ± 0.6
VS / TS	92.7 ± 0.4	94.4 ± 0.6
Cellulose (% VS)	25.1 ± 1.7	24.2 ± 1.2
Hemicelluloses (% VS)	11,6 ± 1.2	8.8 ± 0.4
Klason Lignin (% VS)	32.5 ± 0.6	24.9 ± 1.0
Uronic acids (% VS)	2.2 ± 0.3	0.85 ± 0.2
Soluble sugars (% VS)	1.9 ± 0.2	5.6 ^b
Phenols (mg L ⁻¹)	-	25.7

^aThe solid fraction was dried after alkaline pretreatment at 60°C for 24h.

^bcalculated by the sum of initial soluble sugars and by the solubilization of cellulose and hemicelluloses.

Table 2. Feedstock parameters, biohydrogen and methane performances for the four configurations.

	Configuration 1 (one-stage CH ₄)	Configuration 2 (NaOH one-stage CH ₄)	Configuration 3 (two-stage H ₂ /CH ₄)	Configuration 4 (NaOH two-stage H ₂ /CH ₄)
Feedstock parameters				
TS (g.L ⁻¹)	35	36.4	35	36.4
VS (g.L ⁻¹)	32.4	32.4	32.4	32.4
pH feeding ^a	8	11.1	8	11.1
H₂ batch assay				
H ₂ production (mL H ₂ g ⁻¹ VS added)	--	--	7.1	6.3
Acetate ^b (g L ⁻¹)	--	--	0.55 (±0.02)	1.50 (±0.05)
Butyrate ^b (g L ⁻¹)	--	--	0.40 (±0.03)	0.20 (±0.02)
pH outlet	--	--	5.5	5.2
CH₄ continuous digesters				
pH outlet ^b	6.60 (±0.01)	6.90 (±0.05)	6.90 (±0.1)	7.00 (±0.04)
Biogas production (mL d ⁻¹) ^c	646 (±10)	782 (±14)	603 (±15)	752 (±6)
CH ₄ (%)	52.5	54.6	56	58
CH ₄ production (mL CH ₄ g ⁻¹ VS added) ^c	152 (±4)	191 (±3)	150 (±3.5)	196 (±3)
Increase (%) ^d		26%	No difference	29%
Total Energy (kWh t ⁻¹ VS) ^e	1520 (±40)	1910 (±30)	1506 (±38)	1974 (±38)

^a before dark fermentation stage, the pH was adjusted to 6

^b Values corresponds to the means of duplicates analysis

^c Data for biogas values are presented during the third hydraulic retention time (measured over two days for methane production and each week for the pH).

^d Increase is expressed as percentage over one-stage CH₄ alone.

^e Methane conversion was considered as 10 kWh m⁻³ and hydrogen conversion as 3 kWh m⁻³

Table 3. Organic matter distribution for one ton of initial sunflower stalks converted through the four configurations

	Configuration 1	Configuration 2	Configuration 3	Configuration 4
Input				
Sun flower stalks (kg)	1000	1000	1000	1000
VS (kg)	890	890	890	890
Output				
CH ₄ (kg)	95	120	95	122
CO ₂ (kg)	238	275	213	252
H ₂ (kg)	0	0	0.5	0.44
digestate (kgVS)	520	449	520	440
Sum (kg)	853	844	828	814
Mass balance				
(Input-Output)/Input (%)	4.2	5.2	6.9	8.5
VS removal (%)	41.6	49.6	41.6	50.6

Table 4. Energy and economical assessment analysis for the two configurations: one-stage CH₄ and NaOH one-stage CH₄.

	One-stage CH ₄	NaOH one-stage CH ₄		
<i>Energy produced (Heat and Electrical from CHP)</i>	1399	1769		
Thermal energy produced (KWh t ⁻¹ TS)	700	884		
Electrical energy produced (KWh t ⁻¹ TS)	490	619		
<i>Heat balance</i>				
		solid loading (g TS L ⁻¹)		
		35	50	200
Thermal energy gain (KWh t ⁻¹ TS) ^a		185	185	185
Heat Energy requirement (KWh t ⁻¹ TS)		1034	733	210
Heat Energy requirement with 80 % of heat recovery (KWh t ⁻¹ TS)		207	147	42
Net Heat energy (KWh t⁻¹ TS)^b		-849	-548	-25
Net Heat energy with 80 % of heat recovery (KWh t⁻¹ TS)		-22	38	143
<i>Electrical balance</i>				
Electrical energy increase (KWh t ⁻¹ TS) ^c		129	129	129
Mixing pretreatment tank (KWh t ⁻¹ TS)		-	10.5	-
Net electrical energy (KWh t⁻¹ TS)			118.5	
<i>Economic assessment</i>				
NaOH cost (€ t ⁻¹ TS)			17	
Extra net gain (€ t⁻¹ TS), France			4	
Extra net gain (€ t⁻¹ TS), Germany			13	
Extra net gain (€ t⁻¹ TS), Italy			16	

^aThermal energy gain corresponds to the difference of heat energies produced by NaOH one-stage CH₄ configuration minus the one-stage CH₄ configuration.

^bNet heat energy is the difference between the thermal energy increase and the heat energy requirement for the alkaline pretreatment.

^cElectrical energy gain corresponds to the difference of electricity energies produced by NaOH one-stage CH₄ configuration minus the one-stage CH₄ configuration.

Table 5. Comparison of heat balances of thermal and thermo-chemical pretreatment to enhance methane potentials from biomasses according literature data

Substrates	Pretreatments conditions (Assumptions)	Methane increase (m ³ t ⁻¹ TS) / mode	Biogas conversion conversion	Surplus thermal energy (KWh t ⁻¹ TS)	Thermal pretreatment requirements (KWh t ⁻¹ TS)	Net Heat energy (KWh t ⁻¹ TS)	Refs.
Primary sludge	Hydrolysis pretreatment, 70°C, HRT: 2 days Solid load: 10 g TS L ⁻¹ 85 % of heat recovery from pretreatment step	Continuous	CHP : 35 % electricity; 55% heat	3.01 ^a	2.34 ^a	0.67 ^a	[55]
Sunflower oil cakes	Thermal (170°C; 1h) Solid load: 50 g TS L ⁻¹	32 / batch	CHP : 35 % electricity; 50% heat	161	3536	-3375	[45]
	Thermal (170°C; 1h) Solid load: 200 g TS L ⁻¹	32 / batch	CHP : 35 % electricity; 50% heat	161	1010	-849	
	Thermal (170°C; 1h) Solid load: 200 g TS L ⁻¹ 80% of heat recovery from pretreatment step	32 / batch	CHP : 35 % electricity; 50% heat	161	152	9	
Microalgae	Thermal (75°C; 15 min) Solid load: 11.7 g TS L ⁻¹ 85% of heat recovery from pretreatment step	32 / batch	100 % heat conversion	316	458	-142	[46]
	Thermal (75°C; 15 min) Solid load: 20 g TS L ⁻¹ 85% of heat recovery from pretreatment step	32 / batch	100 % heat conversion	316	268	48	
	Thermal (75°C; 15 min) Solid load: 30 g TS L ⁻¹ 85% of heat recovery from pretreatment step	32 / batch	100 % heat conversion	316	173	143	
Ensiled sorghum forage	Thermo-alkaline (100°C; 30 min, 10% NaOH w/w) Solid load: 160 g TS L ⁻¹	92 / batch	CHP : 40 % electricity; 41% heat	378	547	-169	[56]

	Thermo-alkaline (100°C; 30 min, 10% NaOH w/w) Solid load: 160 g TS L ⁻¹ <i>80% of heat recovery from pretreatment step</i>	92 / batch	CHP : 40 % electricity; 41% heat	378	109	269	
	Thermo-alkaline (100°C; 30 min, 10% NaOH w/w) Solid load: 160 g TS L ⁻¹	137 / batch	CHP : 40 % electricity; 41% heat	577	547	30	
Wheat straw	Thermo-alkaline (100°C; 30 min, 10% NaOH w/w) Solid load: 160 g TS L ⁻¹ <i>80% of heat recovery from pretreatment step</i>	137 / batch	CHP : 40 % electricity; 41% heat	577	109	468	[56]
	Thermo-alkaline (55°C;4% NaOH (w/w); 24h) Solid load: 35 g TS L ⁻¹	36 / continuous	CHP : 35 % electricity; 50% heat	185	1034	-849	
	Thermo-alkaline (55°C;4% NaOH (w/w); 24h) <i>Solid load: 50 g TS L⁻¹</i>	36 / continuous	CHP : 35 % electricity; 50% heat	185	733	-548	
Sunflower stalks	Thermo-alkaline (55°C;4% NaOH (w/wTS); 24h) <i>Solid load: 200 g TS L⁻¹</i>	36 / continuous	CHP : 35 % electricity; 50% heat	185	210	-25	Our study
	Thermo-alkaline (55°C;4% NaOH (w/w); 24h) <i>Solid load: 50 g TS L⁻¹</i> <i>80% of heat recovery from pretreatment step</i>	36 / continuous	CHP : 35 % electricity; 50% heat	185	147	38	

^a Values are expressed in kJ d⁻¹

Figure 1

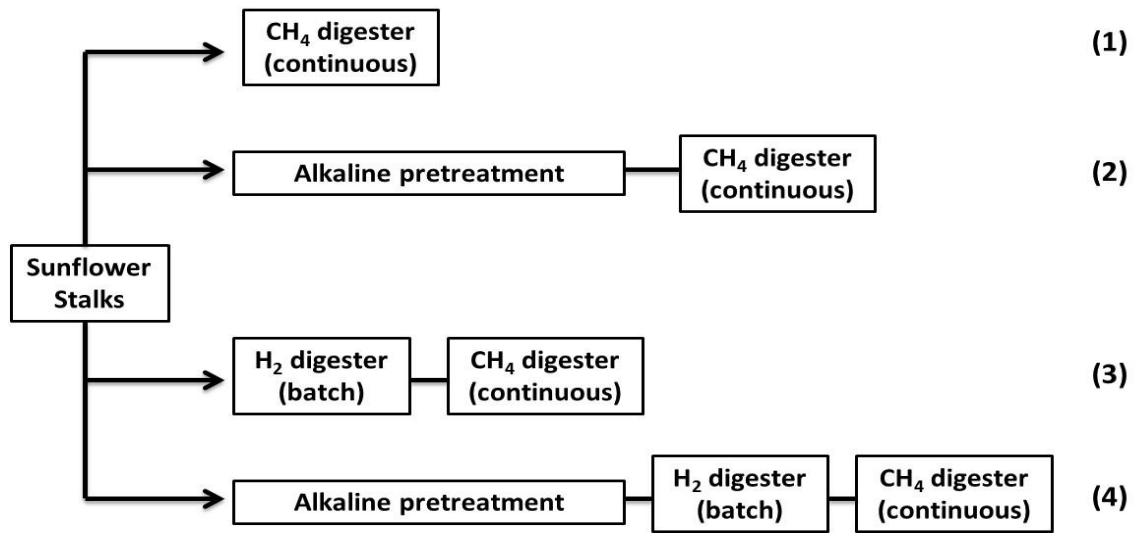


Figure 2

