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Evaluation of different pretreatments for water hyacinth (*Eichhornia crassipes*) with subsequent anaerobic digestion

Autor: D. Enrique Regidor Alfageme
Tutor: D. Raúl Muñoz Torre

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Table of contents

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1. Introduction

1.1. Overgrowth of *Eichhornia crassipes*

Water hyacinth (*Eichhornia crassipes*) is a tropical-native free floating aquatic plant which is currently spread all over the world (Table 1) because of its adaptability and rapid growth rate (Abbasi and Ramasami, 1999; Center, 2001; Ferrer et al., 2010). Water hyacinth can tolerate a large temperature, nutrients and pH variation (Olivares and Colonnello, 2000; Wilson et al., 2005) Another fact that makes it difficult to control water hyacinth's overgrowth is that the seed stays active for up to 6 years and that the plant can regenerate from fragments of stems (Lee, 1979; Gunnarsson and Petersen, 2007). These characteristics recently affected human activities (interference with fishing, navigation and power generation) and caused environmental issues (block of light penetration, water stagnation and reduction of dissolved oxygen levels (Gunnarsson and Petersen, 2007; Malik, 2007; Ferrer et al., 2010). Because of acting as a breeding platform for various disease vectors the health risk has to also be accounted (Epstein, 1998). A feasible solution for harvested biomass is needed as soon as possible in order to reduce the costs derived from overgrowth and the impact caused on both human and environment.

1.2. Different applications for water hyacinth

Water hyacinth's moisture content is about 90% (Sivasankari and Ravindran, 2016) and, due to its high cellulose, hemicellulose, its moderate lignin composition (lignocellulose structure) and high protein content, it can be considered as a potential substrate for the following three biological treatments:

1.2.1. Ethanol production

This process consists on a succession of various steps (pre-hydrolysis, hydrolysis, fermentation and distillation) to obtain sugars and transform them into ethanol as a second generation biofuel (Jambo et al., 2016; Rezania et al., 2017). The hemicellulose content suggests water hyacinth as a possible substrate for bioethanol production (Nigam, 2002) but many of the substances generated during the hydrolysis step may inhibit the subsequent fermentation. A pretreatment is needed for bioethanol production which implies additional costs (Malik, 2007). Also, due to the energy balance, bioethanol production is only feasible if a lot of liquid ethanol is needed (Thomas and Eden, 1990).

1.2.2. Composting

A cheap alternative that does not require specific or expensive technology is always of big interest. Compost consists on degrading the organic matter aerobically to obtain a compost that can be used as a long-term nutrient supplement for fields as long as the pathogens are properly sterilized and there is no active plant seeds (Haug, 1993; Gunnarsson and Petersen, 2007). Because of being a cellulose and lignin rich substrate the time needed for performing a proper composting is long being these two compounds non-degraded after 185 operation days (Elserafy et al., 1980).

1.2.3. Anaerobic Digestion

The AD process can be divided into 4 different steps in the following order; Hydrolysis, acidogenesis, acetogenesis and methanogenesis (Meegoda et al., 2018). The methanogenesis step is easy to inhibit while the hydrolysis is usually the limiting step. Some authors even mention the "anaerobic hydrolysis" as a separated step when dealing with lignocellulose material (Hendriks and Zeeman, 2009). How does the lignocellulose matrix affect the hydrolysis process is explained in the section below.

Anaerobic digestion (AD) consists on the assimilation of organic matter under anaerobic conditions by microorganisms which produce CH_4 (Ferry, 2010; Meegoda et al., 2018). The process is usually operated under a moderately low temperature (Bendixen, 1994; Lier, 1995; Smith et al., 2005; Hartmann and Ahring, 2006; Mao et al., 2015; Meegoda et al., 2018) which means that operation costs are usually low compared with other processes e.g. aerobic digestion. Additionally, the methane produced can be utilized for producing energy by combusting it which can sometimes even be translated into income generation rather than just costs. AD is also defined as a strong method once it reaches a stable condition which can be operated with high organic matter loading rates (Ferguson et al., 2016; Meegoda et al., 2018) and produces less sludge as compared with an aerobic process.

As a result of the above mentioned AD is considered as a suitable treatment for water hyacinth and investigated during this study. However, due to the presence of the lignocellulose matrix on the plant structure, special considerations must be taken before performing AD. The section below will explain what the presence of the lignocellulose matrix implies.

1.3. Lignocellulose biomass

The lignocellulose matrix which is found in the cell wall of lignocellulose biomass consists on a tridimensional structure of cellulose, hemicellulose and lignin where the lignin surrounds both cellulose and hemicellulose acting as a glue obstructing hydrolysis (Liu, 2010; Shrestha et al., 2017). Cellulose and hemicellulose are both polysaccharides that constitute the main carbon source during the AD process. Lignin, which surrounds cellulose and hemicellulose, is a polymer that anaerobic microorganisms cannot degrade (Benner et al., 1984; Appels et al., 2008). Therefore, the access to cellulose and hemicellulose (by the microorganisms) is obstructed, making hydrolysis a limiting

step during AD of lignocellulose biomass (Shrestha et al., 2017). A negative correlation of lignin content and CH_4 production was reported by several authors as shown on Figure 1 (Triolo et al., 2011; Koyama et al., 2014). In the case of water hyacinth different chemical compositions were reported with a remarkable variation of the lignin content from 3.2 to 26% in dry matter basis (Gunnarsson and Petersen, 2007; Rezania et al., 2017). Even if the lignin content is not too high compared with other lignocellulose substrates (Gunnarsson and Petersen, 2007; Liu, 2010; Puligundla et al., 2016; Rezania et al., 2017; Wang et al., 2017; Ponnusamy et al., 2019) the lignocellulose structure will limit hydrolysis. An effective pretreatment for either breaking or separating the lignocellulose matrix is necessary to optimize AD operation when using water hyacinth as substrate. Based on the above mentioned, the main objective of this research was to find a suitable pretreatment for lignocellulose substrate.

1.4. Pretreatments for enhancing CH_4 production from lignocellulose biomass

Recently lignocellulose biomass pretreatment not only prior to AD but also for other treatments has been widely researched. Pretreatment methods are divided into three different groups (physical, chemical and biological pretreatments) being always possible to apply a combination between pretreatments from the same or different groups. Table 2 summarizes the above mentioned types of pretreatments with some examples for each group. Physical pretreatments consist on a mechanical break of the substrate which decreases the particle size increasing the specific surface and decreases the crystallinity and degree of polymerization of the cellulose matrix (Palmowski and Müller, 1999; Taherzadeh and Karimi, 2008). Physical pretreatments are short but sometimes expensive due to the high energy demand (Taherzadeh and Karimi, 2008; Carrere et al., 2016). Chemical pretreatments' mechanisms are completely different depending on the kind of chemical employed (strong or diluted acid or base, oxidizing compounds or CO_2) but the common objective pursued is to

solubilize the hemicellulose permitting this way microorganisms to reach cellulose (Hendriks and Zeeman, 2009). Even if a high temperature is not always needed for chemical pretreatments the addition of reagents makes it an expensive alternative (Carrere et al., 2016; Rodriguez et al., 2017). During biological pretreatments, cellulose, hemicellulose and lignin are degraded by fungi and bacteria usually at mild temperatures and without adding chemicals. On the other hand, the time needed for a proper operation is usually long enough not to be viable in a large scale process (Sun and Cheng, 2002; Taherzadeh and Karimi, 2008; Carrere et al., 2016; Andlar et al., 2018). Thermal pretreatments show a different response depending on how the thermal energy is applied (hot air, steam and infrared radiation). Also the exposure time affects both the hydrolysis efficiency and the volatile fatty acids (Barua and Kalamdhad, 2017) which may vary the response during a posterior AD process. The high temperature usually needed for producing a thermal hydrolysis and the possibility of generating inhibitors are two problems of this kind of pretreatment.

Out of all the pretreatments mentioned on Table 2 the selection for this study was based on the simplicity and technology needed. Also the efficiency of the pretreatment and operation cost were important when making a choice. After considering all these points, the four pretreatments chosen were; Compression, sonication, steam explosion and thermal hydrolysis.

1.4.1. Compression

Amongst the physical processes showed on Table 2 compression is the simplest and can probably be the cheapest pretreatment method. Compression pretreatment applies a specific pressure on the substrate for separating a liquid and solid fraction. The liquid extracted depends on the pressure applied to cassava' paste and mash (Akely et al., 2010; Kolawole, 2011) which may be due to cell breaking. Since the lignocellulose matrix is in the cell wall (Barua and Kalamdhad, 2018), a liquid-solid separation by means of compression would separate the easily biodegradable

compounds (liquid phase) and the high molecular mass compounds such as lignin, cellulose and hemicellulose (solid phase). The solid-liquid separation enables performing AD for just the easily and fast biodegradable compounds (liquid phase) while using the solid phase for a different purpose more suitable for its characteristics. There is barely no publications about compression of macrophytes and, since water hyacinth has around 90% moisture content (Sivasankari and Ravindran, 2016), it is important to find out which components remain on the solid phase in order to confirm whether compression is a suitable pretreatment or not.

1.4.2. Sonication

Sonication consists on applying ultrasonic waves typically within the range 20-1000 kHz to the objective solution generating physical and chemical processes which help altering or hydrolyzing a heterogeneous sample.

The physical wave transmitted through the liquid has high and low pressure sections which generate cavitation zones. When these cavitation zones collapse, specific points are generated briefly reaching temperatures around 5000 K and pressures of 1000 atm which affects the substrate on both physical and chemical properties (Bussemaker and Zhang, 2013). Additional physical mechanisms such as shockwave, micro-jets and micro-convection occur in the solid-liquid interface (Bussemaker and Zhang, 2013; Luo et al., 2013).

While the pressure wave is transmitted through the liquid mixing is enhanced and, if the solution is heterogeneous, because of cell wall break and mass transfer improvement the accessibility of biomass is also increased. On the other hand, sonication also affects chemical processes reducing the temperature and chemicals required. Temperature and chemicals reduction are produced due to breaking O-H bonds to release radicals that react afterwards being able to generate lots of other radicals depending on the sample. Higher frequency conditions have been

proven to promote chemical effects while, for low frequency ones, the main effect comes from the physical ones (Bussemaker and Zhang, 2013).

Sonication has been proven to improve CH_4 and H_2 production either applying it to pretreat the sludge which enhanced biogas production by 50% (Pilli et al., 2011) or different substrates; apple pomace's H_2 yield was improved up to 8% (Wang et al., 2010a), when applied to cassava wastewater, H_2 production increased by 29% (Leaño and Babel, 2012) and, when applied to cattle manure, CH_4 production was increased by 121% (Castrillón et al., 2011).

Sonication pretreatment is well studied and widely spread but barely used for evaluating macrophytes for AD (Kist et al., 2018a) which is the aim of this study. Special consideration with power, frequency and concentration must be taken before performing this pretreatment.

1.4.3. Steam explosion

Out of the physical treatments displayed on Table 2 steam explosion (SE) is proved to be effective improving CH_4 production during AD probably because of solubilization and destruction of substrate (Converse et al., 1989; Laser et al., 2002) without needing chemicals addition or a long operation time. This method exposes a mix of substrate and water to a high temperature and pressure condition, leading to hemicellulose's hydrolysis to form acids which catalyze the hydrolysis process (Gregg and Saddler, 1996). After the desired retention time is elapsed, the pressure in the reactor is suddenly resealed displacing the treated mixture to a flash tank (Figure 2).

For quantifying the intensity of this pretreatment usually severity factor (SF) is used which only depends on the temperature (T) and the retention time (t) but there is no term associated to the sudden pressure release even though it is supposed to disrupt the structure. According to Liu, (2010)

the general SF equation (under assumptions as a first order hydrolytic equation) is expressed as follows [1]:

$$SF = \log\left[t \cdot \exp\left(\frac{T\text{-}100}{14.75}\right)\right] \quad [1]$$

As shown in Figure 3 CH_4 production is affected by the SF value so it is important to find the optimum pretreatment condition (T and t) at which the CH_4 production is maximized. The trend showed in Figure 3 is not lineal so it is important not to operate under too high SF values because CH_4 will decrease as the behavior follows a parabolic trend (Lizasoain et al., 2016). The CH_4 production decrease at high SF values partially caused by the generation of inhibitory compounds as furfural, hydroxymethylfurfural (HMF) and phenolic compounds during the thermal step (Ferreira et al., 2013; Vivekanand et al., 2014). If the concentration of the different inhibitors produced is low, anaerobic microorganisms can acclimatize and even degrade part of the inhibitors produced before (Benjamin et al., 1984; Noike and Eng, 2001; Fox et al., 2003). Additionally, every substrate has different specific properties varying from one to another the optimum SF condition as shown in Figure 4.

On the other hand, it requires specific and strong equipment capable of handling sudden pressure changes (which produce sudden volume changes that damage the equipment). These pressure and volume changes disrupt substrate's structure affecting the CH_4 production depending on the pretreatment conditions. In conclusion, it is important to evaluate for the different substrates the best steam explosion condition not only paying attention to the hydrolysis improvement but also to the inhibitory compounds generation and its effects on AD.

1.4.4. Thermal hydrolysis

Thermal hydrolysis (TH) includes, just like SE, a high pressure and temperature period that disrupts the substrate. The difference consists on not applying a sudden pressure release at the end of the pretreatment (Kist et al., 2018b). Because of not performing a sudden pressure change, the specifications required for the machine are not the same which reduces equipment specifications.

If both SE and TH are performed in the same reactor, the influence of the pressure release condition can be studied to suggest if it is necessary or not for pretreating lignocellulose biomass before an AD process.

1.5. Objectives

The objectives of this study are the following; (1) To evaluate the performance of four pretreatment methods (Compression, sonication, TH and SE) on water hyacinth and (2) To assess how the different pretreatments affect the AD of water hyacinth. For the experiment, the conditions for performing AD were mainly selected based on the SCOD obtained to evaluate how the mechanisms of the different pretreatments affect AD's outcome.

2. Materials and Methods

2.1. Substrate

Water hyacinth (*E. crassipes*) was bought from Charm Company (Japan) was used as a substrate. Water hyacinth was cut into 3-5cm pieces and stored at -20ºC. Unfroze water hyacinth was used for pretreatments and AD experiment within 2 days.

2.2. Pretreatments

2.2.1. Compression

The machine utilized for performing the compression pretreatment was a manual standard juicer (Dancer, Figure 5.). The compression step was performed 3 times in a row per sample batch and always applied manually. The liquid fraction separated during the operation contained small solid particles which were not filtered afterwards. After performing the compression, solid and liquid were separately frozen at -20ºC.

2.2.2. Sonication

2.2.2.1.Substrate preparation

Due to applying sonication waves using a probe-sonication homogenizer (UH-50, SMT Company; 50W; 20 kHz), it was necessary to mill the water hyacinth beforehand. Otherwise, the substrate would float and block the sonication-probe not allowing a proper irradiation. After milling, the sample was properly mixed with the liquid faction obtained to homogenize the substrate before pretreating.

2.2.2.2.Experiment design

The pretreatment design was based on the specific energy (SpE) applied to the substrate's total solids (TS) concentration. SpE was considered as below [2] (Kist et al., 2018a):

$$SpE\left({}^{J}/_{g_{TS}}\right) = \frac{Power\ (W)\cdot t(s)}{Volume(L)\cdot TS\ conc(g/L)} \quad [2]$$

For performing sonication the TS concentration was fixed to 2g-TS/L (Dilution rate 1:5 substrate:milliQ). The power was always 50W with a constant wavelength (20 kHz) while different exposure times (9, 19 and 28min) and volumes (50, 100 and 200) were tested operating this way

SpE within the range 70-855 MJ/kg-TS (Table 3). Since the pretreated volumes were fixed, the necessary amount for preparing the anaerobic digestion batch experiments was produced right before starting up the experiment to avoid substrate spoiling.

2.2.3. Steam explosion and Thermal hydrolysis

The Steam explosion reactor was rented from Nitou Kouatsu Company (Ibaraki, Tsukuba, Japan) to carry out the operations at Soka University, Japan (Figure 2). The reactor volume was 2.8L with an effective volume of 1.8L. An electrical heater controls the temperature inside of the reactor and the pressure release valve was manually operated.

500g of water hyacinth and 600mL of milliQ water (ratio 1:1.2 substrate:milliQ) were introduced from the top of the reactor. When inputting these amounts in the reactor special attention was paid to the upper liquid surface to ensure water hyacinth was completely soaked to prevent a possible incineration of the upper substrate while carrying out the pretreatment. The temperature is then increased until it reaches 120, 170 or 210ºC depending of the experimental conditions. When the desired retention time elapses, the valve is manually opened displacing the mixture of pretreated sample to a flash tank from where the sample was collected. After collection, the pretreated sample was mixed and frozen at -20ºC. In this study, steam explosion machine was operated using 2 different procedures in the final pressure release step; sudden pressure release (Steam explosion, SE) which enables the final physical expansion and gradual pressure release (Thermal hydrolysis, TH) which prevents the final physical step producing only the thermal step also occurred during the SE pretreatment. These two methods were investigated in order to clarify the influence of final pressure release step for the pretreatment and subsequent biological process.

A total of 18 conditions were performed (9 for TH and 9 for SE) being the same conditions for both pretreatments. The combination of three temperatures (120, 170and 210ºC) and retention times (10, 30 and 60 min) were tested (Table 4).

Intensity of the pretreatments is defined as severity factor (SF) which depends on the retention time and the temperature during the process. This variable is defined as follows [3] (Liu, 2010):

$$SF = log\left[t \cdot exp\left(\frac{T-100}{14.75}\right)\right] \quad [3]$$

Where *t* refers to the retention time (min) and *T* is the temperature (ºC) during the pretreatment.

2.2.4. Analytical parameters

For all the different pretreatments samples the total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total solids (TS), volatile solids (VS) and total organic carbon (TOC) were measured following standardized methods (American Public Health Association, 1998). Additional analyses were performed depending on the pretreatment's mechanism. Referring to steam explosion samples, pH was measured by a pH meter (HORIBA, B-212, Japan). For SCOD determination, samples were filtered through 0.45µm glass-fiber filters (Advantec, GC-50, 47mm, Japan). Also CHN (2400 CHNS/O Series II System (100V), Perkin Elmer) was measured for these conditions. Lignocellulose composition was measured using the detergent method (Van Soest et al., 2010) and a fiber analyzer (ANKOM Technology, A-200, USA). Due to the possibility of producing inhibitory compounds while pretreating using both steam explosion and sonication, phenolic compounds were measured according to Folin-Ciocalteau method (Singleton et al., 1998) preparing triplicates to evaluate the error outcome. Soluble lignin concentration was also measured following the same method explained at Koyama et al. (2017) by

acid precipitation of the soluble humic fraction and soluble lignin (both are accounted into the lignin fraction). For the compression samples, nutrients on the liquid phase were measured (HPLC; Column IC I-524A, Guard column IC IA-G from Shodex Company for anion analysis; Column IC YS-50, Guard column IC YS-G from Shodex Company for cation analysis).

2.3. AD batch experiments

2.3.1. Substrate and sludge

The substrates used were the untreated condition and the best conditions from all the different pretreatments tested during this study; from both the SE and TH pretreatments the 210ºC for 10min, 170ºC for 60min and 120ºC for 60min conditions, from sonication pretreatment all the 28min operations for the three different volumes tested (50, 100 and 200mL) and for the compression pretreatment both the liquid and solid samples were tested separately. The inoculum was a mesophilic anaerobic digestion sludge collected from a full-scale biogas plant, the Hokubu Sludge Treatment Center at Yokohama, Japan. The sludge obtained was kept at 37ºC for three days before starting up the batch AD experiments to replicate inoculums activity and organic content.

2.3.2. Batch experiments

Batch experiments were conducted using an automatic anaerobic digestion system II (AMPTS II, Bioprocess Control AB, Sweden). The batch experiments were designed based on the VS ratio between substrate and inoculum (ratio 1:2 substrate-VS:inoculum-VS). The operation volume was always fixed to 300mL. VS values were adjusted (always maintaining the ratio) depending on the pretreated sample's characteristics. The temperature was fixed to 37±1ºC by water thermal bath, agitation speed set as 100rpm operating semi-continuously every 10s for a 10s period. Before

starting the process' monitoring, all the flasks were flashed using Ar as an inert gas for 2 to 3 min to ensure anaerobic condition. For every batch experiment a blank condition was prepared adding only inoculum and milliQ water without any substrate to quantify the methane production from the inoculum itself. All the conditions and blanks were tested as triplicates to evaluate the error output. The biogas produced was guided through a CO_2 absorption unit willed with a 3M NaOH solution. The final gas volume was automatically monitored by a gas-volume quantifier.

2.3.3. Special considerations

Some liquid samples were tested (liquid fraction obtained from the compression pretreatment) so the VS values were low. Because of this sample's characteristics, the volume needed for preparing the batch experiments was adjusted to maintain the operation volume constant. This derived into reducing the VS input for the batch test but the amount was adjusted with the inoculum's VS to maintain the ratio used when designing all the other experiments (ratio 1:2 substrate:inoculum).

2.3.4. Statistical analysis

For simulating the AD processes, the modified Gompertz equation [4] (Donoso-Bravo et al., 2010) was applied to the different triplicates of all the AD batch experiment's conditions and adjusted by the minimum R^2 method:

$$f(x) = a \cdot \exp\left(-\exp\left(\frac{b \cdot \exp(1)}{a}\right) \cdot (c - x) + 1\right) \quad [4]$$

"a" represents the maximum value the simulation would reach if the system is stable, "b" refers to the kinetics of the simulation and "c" to the inhibitory effect during the initial phase.

3. Results & Discussion

3.1. Substrate characterization

Moisture content obtained from untreated water hyacinth was 95%. Hemicellulose, cellulose and lignin compositions were 31, 28 and 9%-TS, respectively (Table 5).

The moisture content of water hyacinth was slightly higher than usually but the chemical composition was similar to previous studies ensuring that the substrate is representative (Gunnarsson and Petersen, 2007; Rezania et al., 2017; Kist et al., 2018a; Zhang et al., 2018). Therefore, the water hyacinth used in this study is considered as suitable for investigating the effects of the four pretreatments selected and the subsequent AD operation.

3.2. Pretreatments

3.2.1. Compression

With the compression pretreatment two thirds of the initial mass was extracted as liquid fraction (Figure 6). However, as showed on Table 6, the final moisture of the solid fraction after performing compression is still high (89%). The TCOD and SCOD values obtained from both the solid and liquid fractions after performing the compression are shown on Table 7. The difference between TCOD and SCOD for the liquid fraction is due to the presence of small root particles which were not filtered after performing the pretreatment. VS and TCOD mass balances are displayed on Table 8 and Table 9, respectively.

The moisture of the solid fraction is high (89%), which means that compression can be improved with a proper machine applying a high enough pressure (Akely et al., 2010; Kolawole, 2011). High molecular weighted compounds stay in the solid (Barua and Kalamdhad, 2018) and the

water-soluble compounds go with the liquid fraction. If a higher pressure is applied on the initial substrate, cells may break releasing more compounds that could be used as substrate during the AD process. These compounds are easily biodegradable. In order to evaluate whether there is still easily biodegradable compounds in the solid fraction after performing compression, both the liquid and solid fractions were tested by AD separately.

3.2.2. Sonication

An improvement on SCOD values compared with only milled water hyacinth was observed after applying sonication (Figure 7).The SCOD concentration was not directly proportional with the SpE applied (Figure 8).

Figure 7 shows the improvement of hydrolysis due to sonication. SCOD content is increased which means that the pretreatment affects solubilization. Also in previous research, using sonication on aquatic weeds does not show a remarkable difference on SCOD values even if the SpE value is doubled (Kist et al., 2018a). During this study the SpE correlation with SCOD was found as logarithmic as shown in Figure 8 ($p < 0.01$). This relation means that for a low SpE value, the hydrolysis will be greatly improved but after reaching a certain number, it will reach a maximum.

3.2.3. Steam explosion (SE) and thermal hydrolysis (TH)

3.2.3.1.Morphological differences

After performing all the different conditions for SE pretreatment (Table 4) morphological differences were easily perceived by mere observation of the pretreated mixture (Figure 9). For the different temperatures tested the differences were obvious but for different retention times and pressure release conditions, the appearance remained the same.

3.2.3.2.Lignocellulose composition

For the lignocellulose analysis the results from the SE operations are similar to the TH ones (Figure 10). While increasing temperature and retention time hemicellulose content decreases reaching really low values for the highest temperature (210ºC). Solubilization temperatures of hemicellulose (Wang et al., 2017) and the lignin (Bobleter, 1994) might affect the compositional differences.

3.2.3.3.Biomass solubilization

When increasing the exposure time for the 120 and 170ºC condition, SCOD concentration increased but it decreases with treatment time for the 210ºC condition. Comparing the SE and TH (Figure 11) operations, the solubilization was barely affected.

The increasing SCOD trend when increasing the temperature (Figure 11) suggests temperature as the main parameter when performing SE probably followed by the retention time (both included in the SF variable) and lastly by the pressure release condition that shows a minor effect compared to temperature. Comparing results from the SE and TH performed during this study (Figure 11) the SE operation tends to achieve a slightly higher SCOD value but nothing remarkable. Some small SCOD variations were also obtained during previous research when performing a SE and a TH operations (Kist et al., 2018b).

When plotting the SF of the different SE conditions against the SCOD obtained (Figure 12) the trend shows that the optimum SF value is obtained at SF = 4.2 when using SCOD as the selection criteria. As showed on Table 4, this value corresponds to the 210ºC for 10min retention time. After surpassing this optimum SF value, the SCOD decreases as demonstrated in previous studies (Kobayashi et al., 2004).

The selection of the best conditions for performing anaerobic digestion was mainly based on SCOD values but, in order to compare the influence of temperature (which, as mentioned above, seems to be really important for pretreating water hyacinth) the conditions selected were the following; 210ºC 10min, 170ºC 60min and 120ºC 60min. For all the conditions mentioned both the SE and TH pretreated substrates were tested by AD (total of 6 conditions) in order to find out if the pressure release condition affected the digestibility of the sample.

3.2.3.4.Mass balances

On every SE operation around a 15% of the initial mass is lost due to either volatilization or remaining matter inside the reactor after collecting the sample. The loss value (15%) did not change when different SE conditions were tested.

Nutrients (C, H and N) concentration (mg/g-TS) obtained after analyzing SE and TH operations do not show big differences or trends. The initial carbon, nitrogen and hydrogen masses for the raw substrate are always higher than the pretreated ones (after correcting the dilution factor) excepting a few of them probably due to measurement errors (Figure 13).

Total mass of the different elements (C, H and N) correcting the dilution rate and TS values to the %TS obtained from CHN analysis in order to be able to directly compare values are shown on Figure 13. The fact that no trend is shown means that the total amount of C, H and N remains constant not depending on the SE condition operated. The compounds present on the sample may vary but the total amount barely changes.

3.2.3.5.Inhibitors generation

Soluble lignin, which starts solubilizing at around 180ºC (Bobleter, 1994), showed a decreasing trend when decreasing the SE temperature when analyzed for the TH operation (Figure

14). On the other hand, there is no clear trend for the SE one (Figure 14). Also, the maximum concentrations obtained were 500 mg-lignin/L for both the TH and SE operations which were not high enough to strongly inhibit the AD process. Phenolic compounds are clearly dependent on the condition, being higher its production when the temperature increases in both the SE and TH (Figure 15) operations. More precisely, phenolic compounds show an exponential correlation with T and a linear correlation with the SF (Figure 16). The maximum concentration obtained during this study was about 700mg/L which is much lower than the 4g/L mentioned as inhibitory concentration during previous research (Bajaj et al., 2009). Inhibitors production is correlated with the pretreatment's temperature and time (SF) (Patwardhan et al., 2011a,b; Wang et al., 2017) which is also proven during this study.

3.3. Anaerobic digestion batch experiments

AD of untreated and pretreated water hyacinth was conducted to clarify the effect to methane productivity. Then, for the statistical analysis, the modified Gompertz equation was adjusted to the experimental results (Table 10).

3.3.1. Compression

Compression's CH_4 yield results show a fast degradation of the liquid fraction finishing the digestion within approximately 7 days which is much shorter than the other conditions tested (Figure 17). 350 NmL/g-VS CH_4 yield was obtained from this condition. On the other hand, the solid fraction was slowly degraded during a long time reaching 167 NmL/g-VS CH_4 yield within 19 days. The fast and efficient result obtained for the liquid fraction suggests that the easily biodegradable compounds were successfully collected with the liquid fraction. CHN for the solid

phase separated during the pretreatment show a lower concentration of nitrogen compared with the raw water hyacinth analyzed (Table 11).

The main purpose of performing AD for the solid phase was to assess which kind of organic compounds remain there and, as shown in Figure 17, there is no lag phase. From this fact the presence of more easily biodegradable compounds can be inferred. This suggests that using a higher pressure or maintaining the pressure application for a longer time may improve the pretreatment method (Akely et al., 2010; Kolawole, 2011). After performing an optimized compression pretreatment, because of extracting liquid, the hemicellulose and cellulose composition of the solid fraction remaining increases. These compounds are the main substrate for ethanol production processes (Malik, 2007) which could be a good alternative for this fraction since CH_4 production of the solid fraction did not obtain a good result in this study and the operation should be even worse if more liquid is extracted with the previous compression.

3.3.2. Sonication

Sonication obtained 174, 159 and 200 NmL-CH_4/g-VS for the 50, 100 and 200mL conditions, respectively. Compared with the untreated substrate (264 NmL-CH_4/g-VS), results show a negative effect of sonication on AD (Figure 18) showing a lower CH4 yield and also a slower response.

Sonication improves both physical and chemical processes (Bussemaker and Zhang, 2013) and, because of not improving the hydrolysis as much as initially expected (Figure 7), the extra energy provided may have affected the molecular structure of the substrate. Luo et al., (2013) mentions that due to lignocellulose being a non-volatile structure, cavitation zones are not the main source of structure alteration but the shockwave, micro-jets and micro-convection mentioned on the introduction section. When it comes to parameters for the sonication, the power usually used for delignification varies from 1 to 3 W/mL (Shirsath et al., 2012) and the concentration varies from 2

to 5%wwt since for a higher concentration, sonication efficiency is decreased (Montalbo-lomboy et al., 2010; Nitayavardhana et al., 2010; Yunus et al., 2010). During this study the initial design pursued a different approach, some of these criteria were followed (power applied varied from 0.25 to 1 W/mL; concentration was fixed to 2%wwt) but not all of them, which could be a reason for the results obtained.

Chemical reactions are more likely to occur on a high frequency sonication condition (Bussemaker and Zhang, 2013) which is just the opposite than the applied on this study but, as a possibility during some applications (Bizzi et al., 2019), inhibitors generated from lignocellulose structures were also measured for this pretreatment. Both soluble lignin (Figure 19) and phenolic compounds (Figure 20) did not show any trend or high values. Soluble nutrients remained constant (Figure 21) when comparing with the milled sample before sonication. This result means both that the heat produced during the sonication pretreatment did not volatilize nutrients and no nutrients were hydrolyzed from the solid phase.

Sonication pretreatment not only did not improve AD but also inhibited it which contradicts most of the previous research (Sangave and Pandit, 2006; Wang et al., 2010a; Wu-Haan, 2010; Castrillón et al., 2011; Pilli et al., 2011; Leaño and Babel, 2012). Probably the problem for not working was the power applied. Other authors suggested to supply powers from 1 to 3 W/mL (Shirsath et al., 2012). Also some other non-measured inhibitors such as furfural compounds (Bizzi et al., 2019). After testing SpE in this study, it should not probably be used as the main design parameter for this sonication pretreatment. The reason was not found during this study so further research is needed.

3.3.3. Steam explosion and thermal hydrolysis

The results obtained from the AD batch experiments of the untreated and SE samples are shown in Figure 22. For comparing the SE and the TH conditions, error bars must be accounted. The difference between the 170ºC and 210ºC conditions are important because of their respective error bars but the 120ºC conditions have big error bars not being able to ensure if the results are different or the same.

3.3.3.1.Methane production rate

The kinetics parameter of the simulation ("b" from Table 10) shows that the 170ºC and the 120ºC conditions were initially faster than the untreated condition. Therefore, hydrolysis of water hyacinth was successfully enhanced by means of the pretreatments. However, the 210ºC conditions were slower than the untreated. This may be because of inhibitors or recalcitrant substances produced (Taherzadeh and Karimi, 2008) or because of the degradation of easily biodegradable compounds during the pretreatment since the temperature is high (potentially being the reason for the low productivity for 210ºC samples). These recalcitrant compounds are produced by the condensation of substances between polymers not affected by the pretreatment, creating a bond (Taherzadeh and Karimi, 2008). Also, the soluble lignin concentration was not high enough for a complete inhibition but it could help, even more during the initial days, because of the microorganisms needing to adapt (Koyama et al., 2017a). Measured soluble lignin and phenolic compounds concentrations are shown on Figure 23 for the different AD batch experiments and comparing them with the final CH_4 yield achieved.

3.3.3.2.Final CH_4 yield achieved

Methane yield for the SE and TH operations from 120ºC are 179 and 192 NmL-CH_4/g-VS, respectively (Figure 22). The difference is not big because of being a low temperature that does not produce a big overpressure during the pretreatment. It also ended up producing less CH_4 yield than

the untreated condition. It is possible that, as mentioned by other authors (Wang et al., 2017), hemicellulose monosaccharides easily cracked producing 1-hydroxy-2-propanone, furanone and other small compounds possibly inhibiting the AD.

On the other hand, cumulative methane yield of the 170ºC (347 and 295 NmL-CH_4/g-VS for SE and TH conditions, respectively) and the 210ºC conditions (304 and 283 NmL-CH_4/g-VS for SE and TH, respectively) show a remarkable difference between the SE and the TH operations being unexpectedly bigger for the 170ºC instead of the 210ºC. As showed in Figure 9 the remaining solid fraction for the 170ºC is more than the present on the 210ºC which might have helped enlarging this CH_4 yield difference. Another factor enhancing the 170ºC results is that, due to the high water content, longer retention times are more effective for SE and TH pretreatments (Brownell et al., 1986; Hendriks and Zeeman, 2009). About reasons for a possible reduction on CH_4 yield for the 210ºC condition is that, when surpassing the 200ºC threshold, cellulose suffers disruption of intra- and intermolecular hydrogen bonds (Wang et al., 2017), ether-linkages on the lignin molecules are broken producing extra phenolic compounds (Chu S., 2013; Collard and Blin, 2014). Furfural compounds production is also increased after surpassing the 190ºC threshold (Thomsen et al., 2009; Ferreira et al., 2013). Additionally, the crystallinity of the cellulose and lignin structure affects the pyrolysis mechanisms involved (Wang et al., 2017).

A final comparison between the different AD performed is displayed (Tables 12, 13 and 14) showing ratios of general interest. In terms of CH_4 yield the best performances were for the liquid fraction obtained from compression pretreatment (350 NmL/g-VS) and the 170ºC 60 min SE condition (347 NmL/g-VS). Both obtained a 39.1 and 37.9% CH_4 yield improvement, respectively. Amongst them, the fastest is the compression condition (8 days against 17).

On the other hand, because of the low COD composition of the liquid compressed fraction, the best conditions for total cumulative CH_4 production would be both the 170ºC 60 min SE and TH conditions. An interesting fact is that the best conditions for the mL-CH_4/g-raw substrate are the 170ºC 60min SE operation but also the untreated one with a 10.7 and 10.2 mL CH_4/g-raw, respectively.

4. Conclusion

- Compression: separated a big amount of liquid with a low SCOD concentration improving CH_4 yield a 39% with AD of the liquid fraction. The pretreatment can still be optimized and an alternative for the solid fraction is necessary.
- Sonication: improved SCOD values until reaching a maximum value but inhibited the AD. A different approach for designing the pretreatment is necessary.
- Thermal hydrolysis (TH): the optimum SCOD value was obtained from 210ºC 10 min but the highest CH_4 yield was 170ºC 60 min. CH_4 yield was improved a 16%.
- Steam explosion (SE): the optimum SCOD value was obtained from 210ºC 10 min but the highest CH_4 yield was also 170ºC 60 min. CH_4 yield was improved a 38%.
- Differences between TH and SE were not found during the pretreatments assessment. For AD application, inhibitors and pressure release method played a determinant role.

The best pretreatments were SE (170ºC 60 min) and compression (liquid fraction). SE uses the whole substrate therefore it's a better option when the aim is to generate CH_4. On the other hand, AD of compression pretreatment was faster. If there is an alternative use for the solid fraction, compression may be better.

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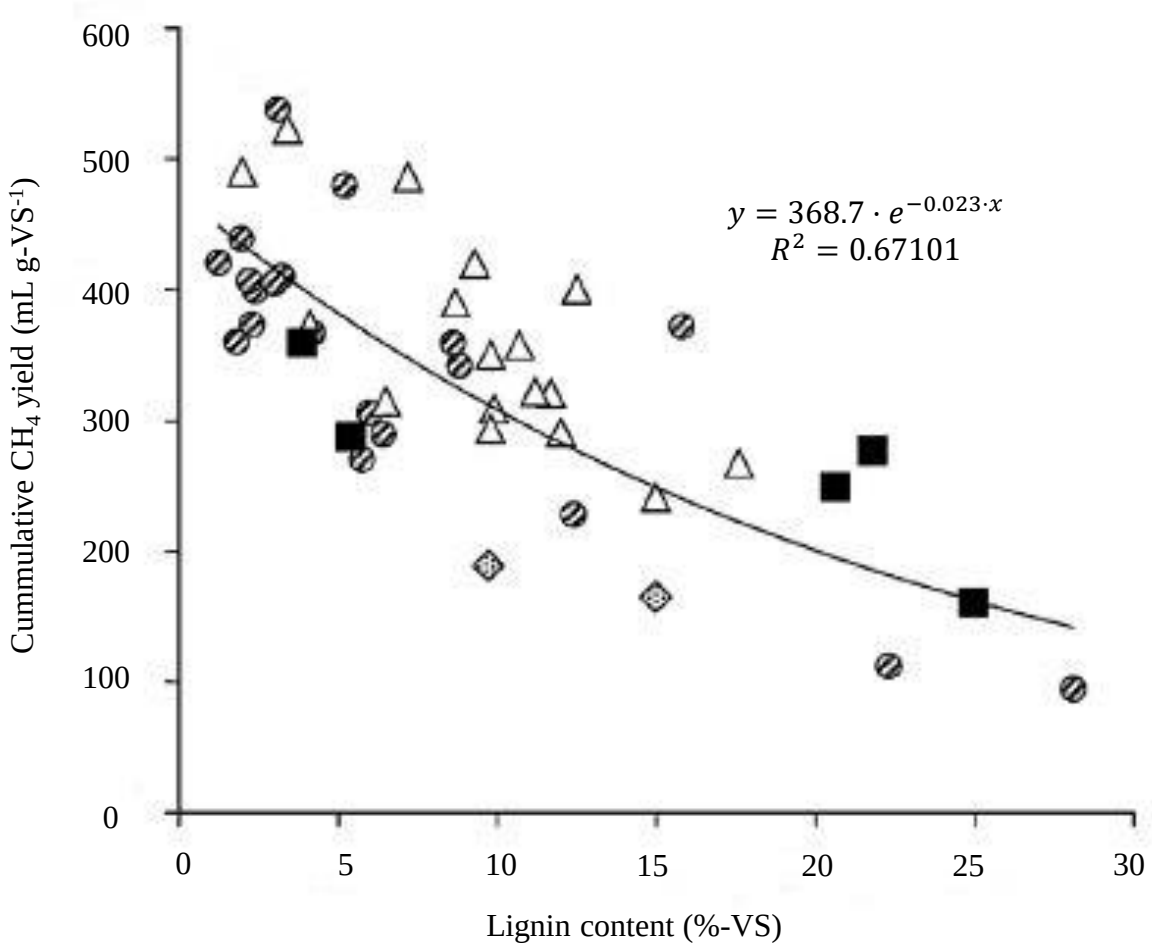


Figure 1. CH_4 yield and lignin content correlation (Koyama et al., 2014)

CH_4 yield of different plant biomass during anaerobic digestion batch experiments . ■ = (Koyama et al., 2014) ◈ = aquatic weeds (Cheng et al., 2010; Wang et al., 2010) ⊘ = herbaceous plants (Gunaseelan, 2007; Triolo et al., 2011; Xie et al., 2011; Frigon et al., 2012) △ = fruits and vegetable waste (Gunaseelan, 2007)

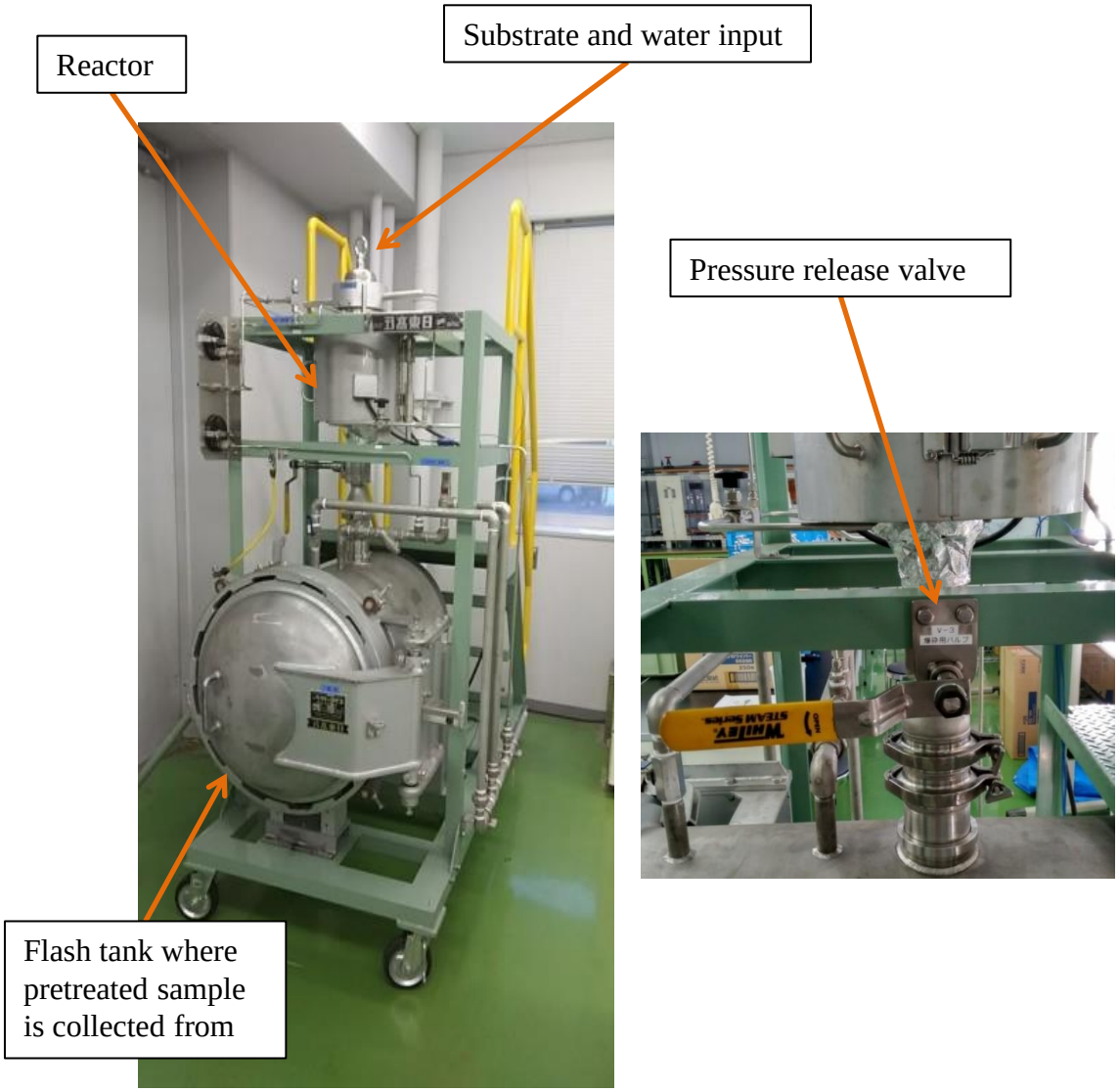


Figure 2. Equipment used for SE and TH pretreatment of water hyacinth in the present study

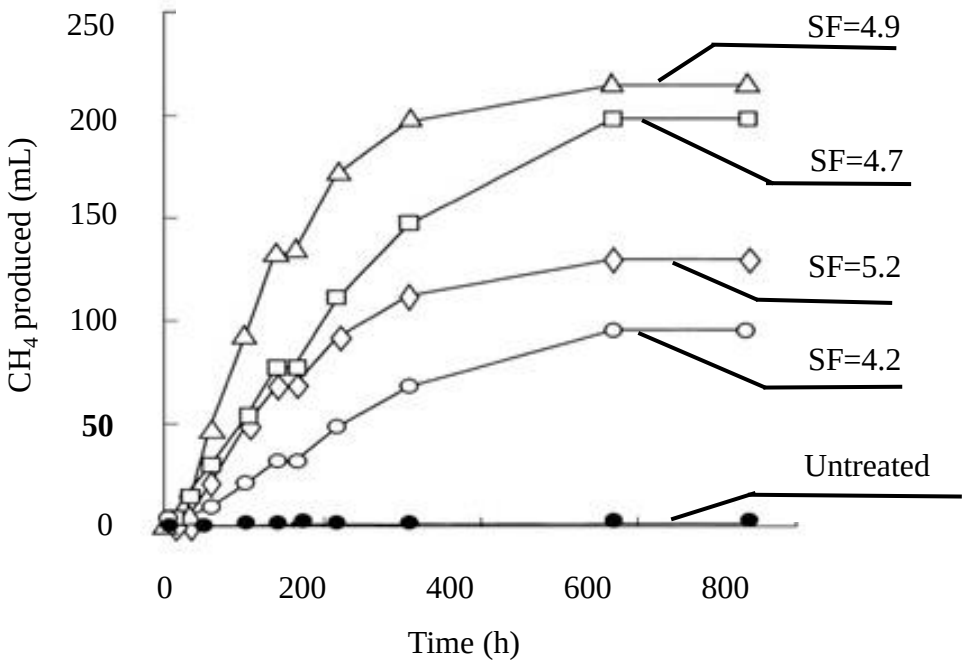


Figure 3. CH_4 production from different SF conditions using bamboo stem as substrate (Kobayashi et al., 2004)

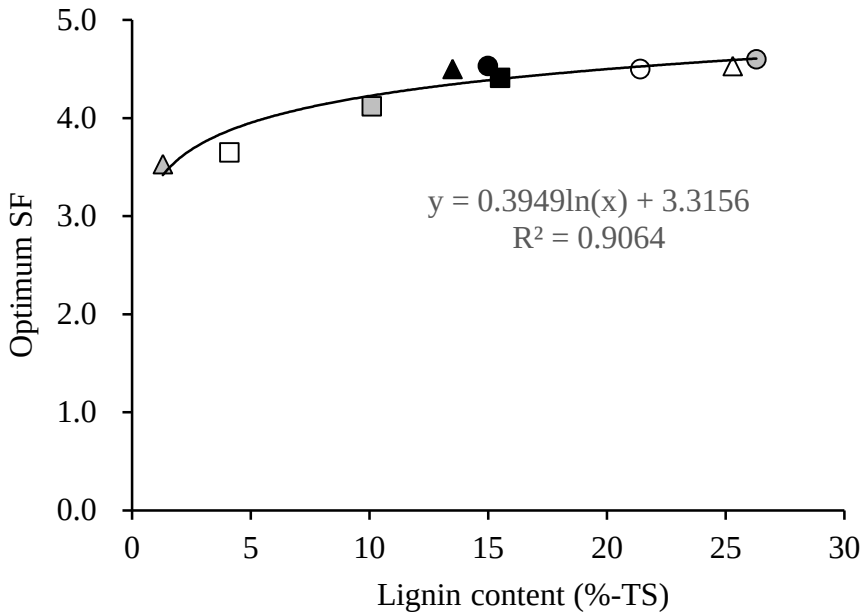


Figure 4. Relationship between lignin content and optimum SF.

● : Bulrush (Wang et al. 2010)　　△ : Salix (Horn et al. 2011)
▲ : Miscanthus (Menardo et al. 2013)　　○: Birch (Vivekanand et al. 2013)
◎ : Bagasse (Vivekanand et al. 2014)　　▲: Wheat straw (Theuretzbacher et al. 2015)
▣ : Reed (Lizasoain et al. 2016)
■ : Aquatic weed (*P. maackianus*) (Suzuki's Master thesis)
□ : Aquatic Weed (*E. nuttallii*) (Suzuki's Master thesis)

Figure 5. Manual juicer used for compressing water hyacinth during the present study

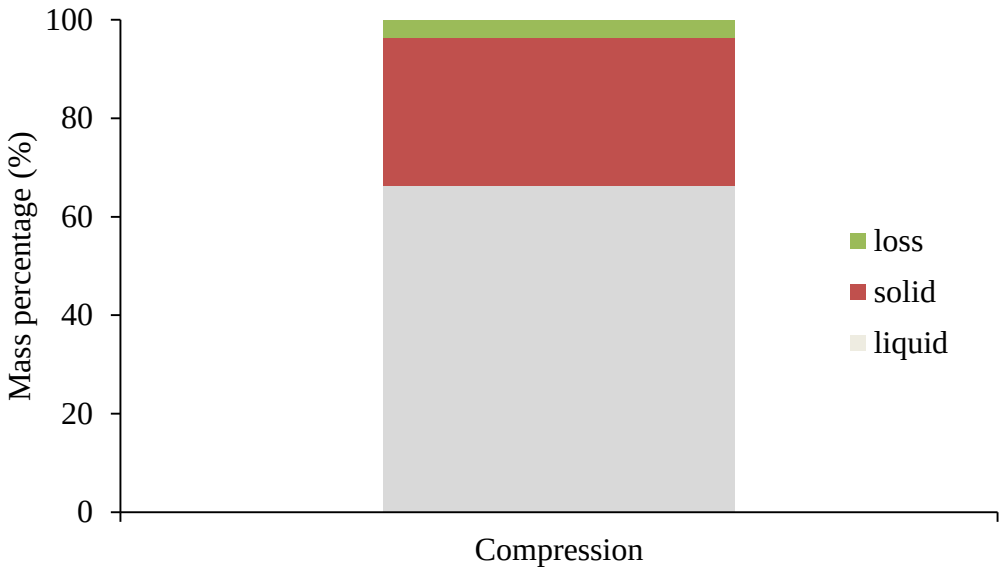


Figure 6. Mass balance for Compression pretreatment

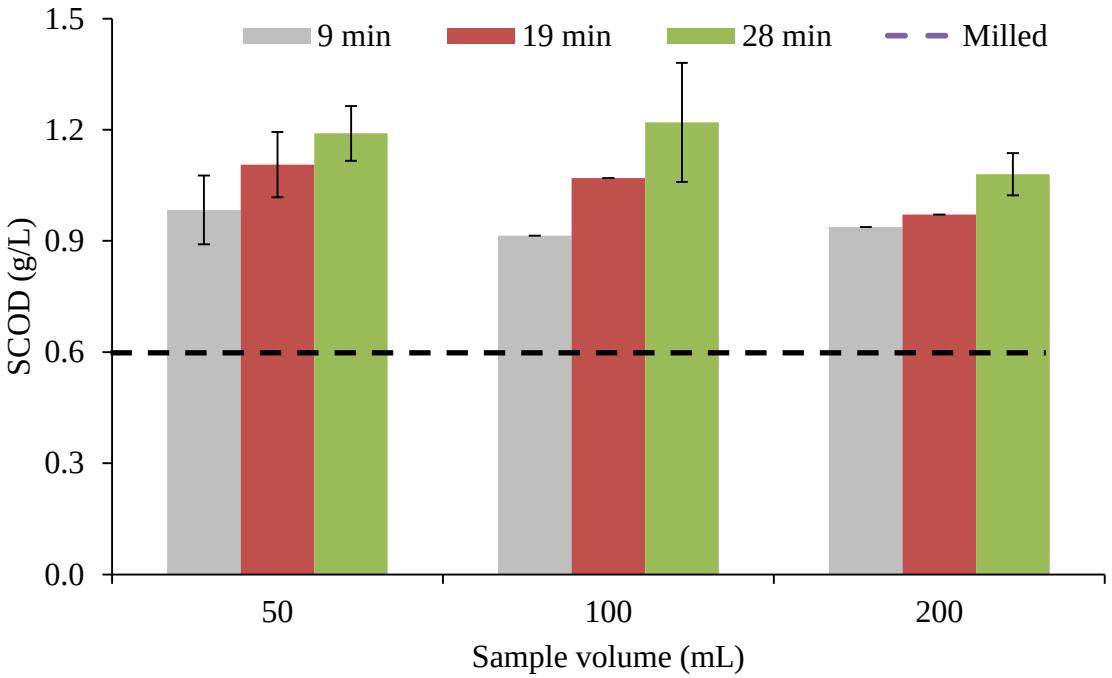


Figure 7. Sonication SCOD as compared with milled* substrate.
Standard deviation bars were calculated using the different monoplicates for each condition performed

*milled water hyacinth without applying any extra alteration to quantify the influence on SCOD of milling the substrate

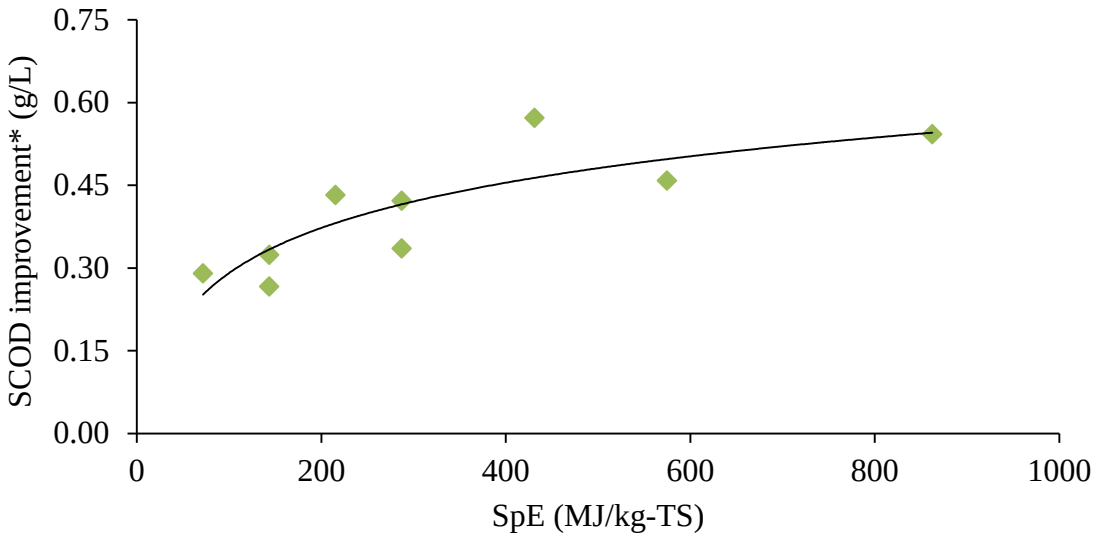


Figure 8. Correlation between SCOD improvement obtained by sonication pretreatment with the specific energy (SpE) applied

*The SCOD value is the difference between the SCOD of the milled substrate and the SCOD obtained after sonication pretreatment

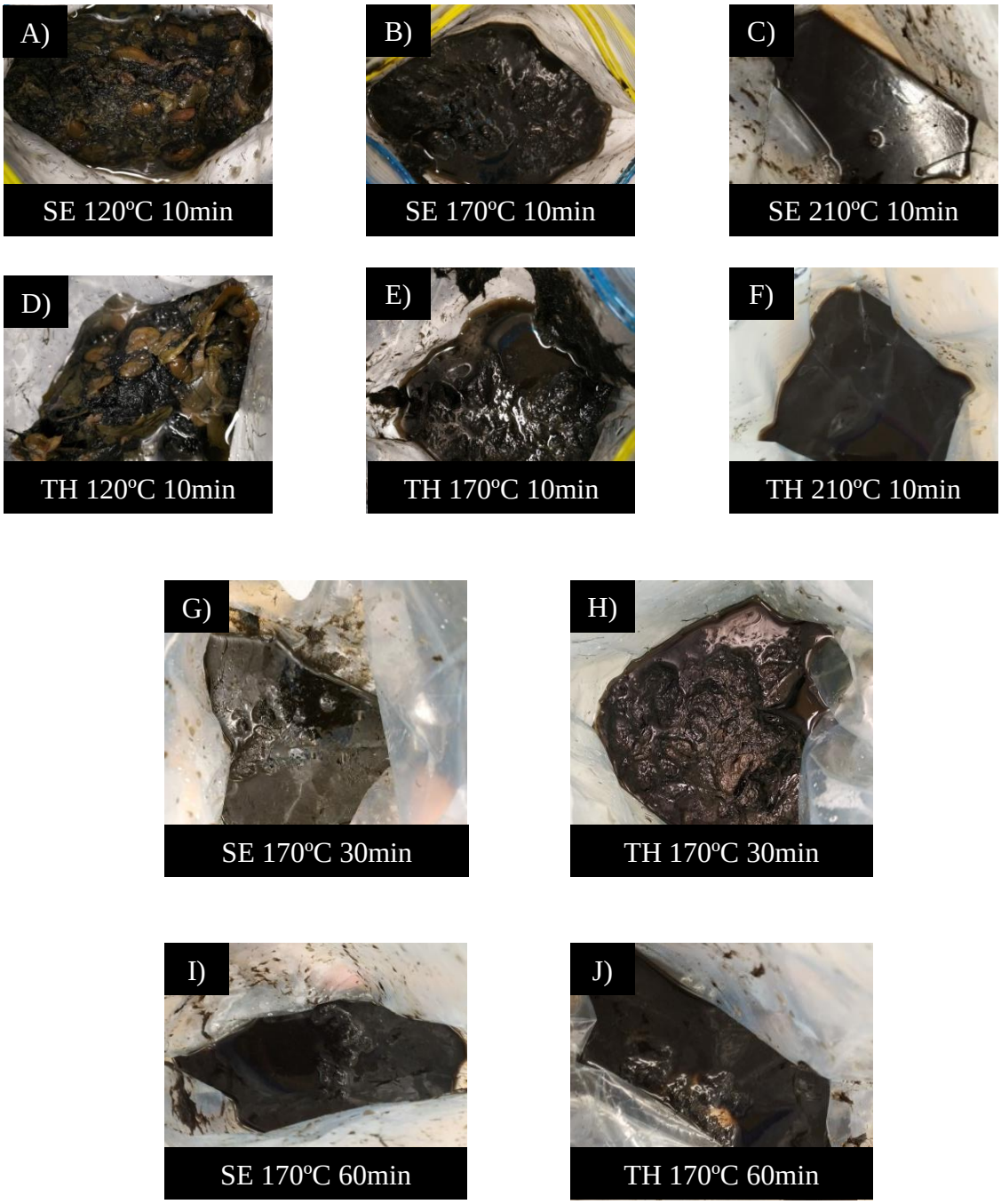


Figure 9. Pretreated steam explosion (SE) and thermal hydrolysis (TH) morphological differences

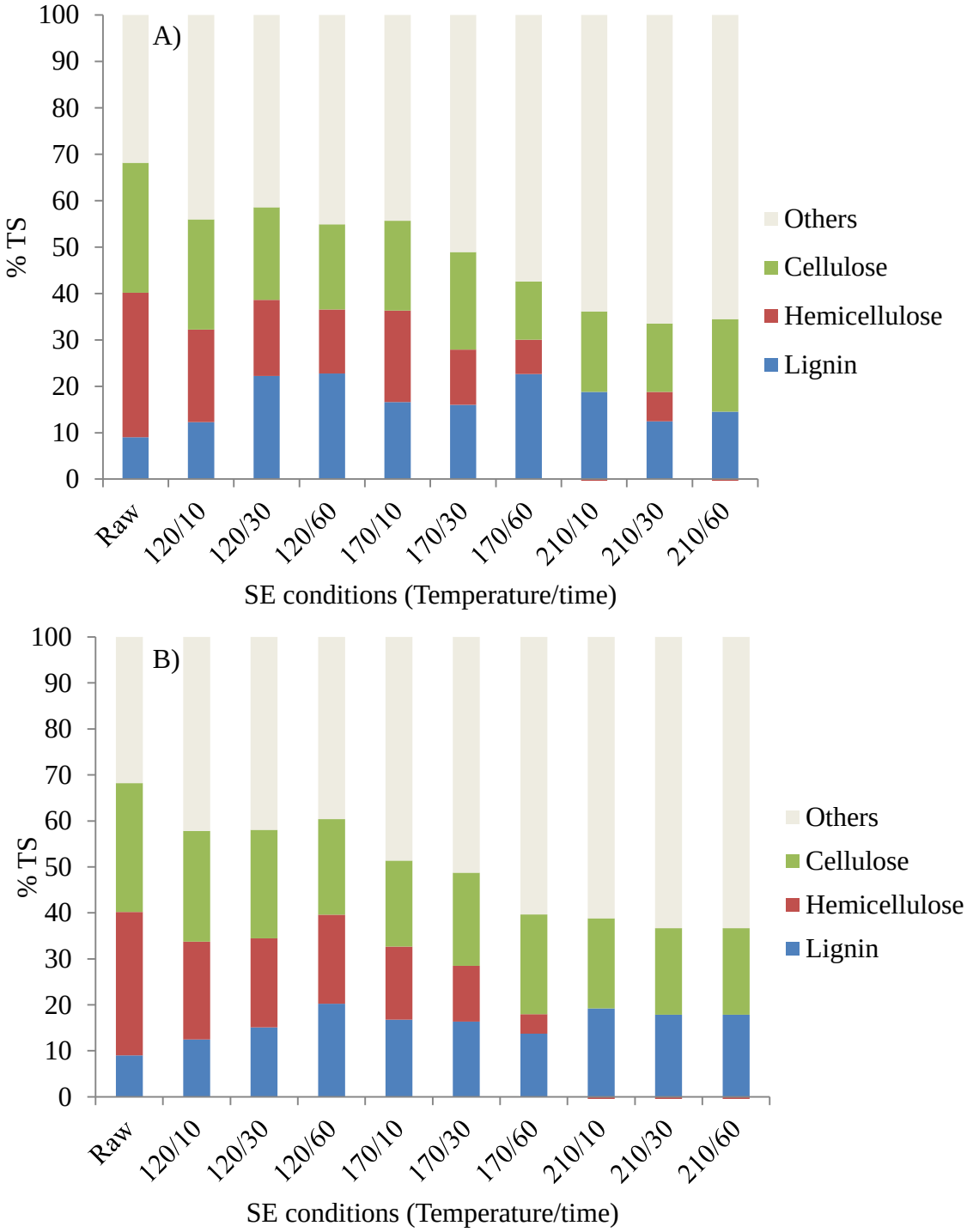


Figure 10. Lignocellulose composition from raw, steam explosion (SE; A) and thermal hydrolysis (TH; B) pretreated conditions in this study.

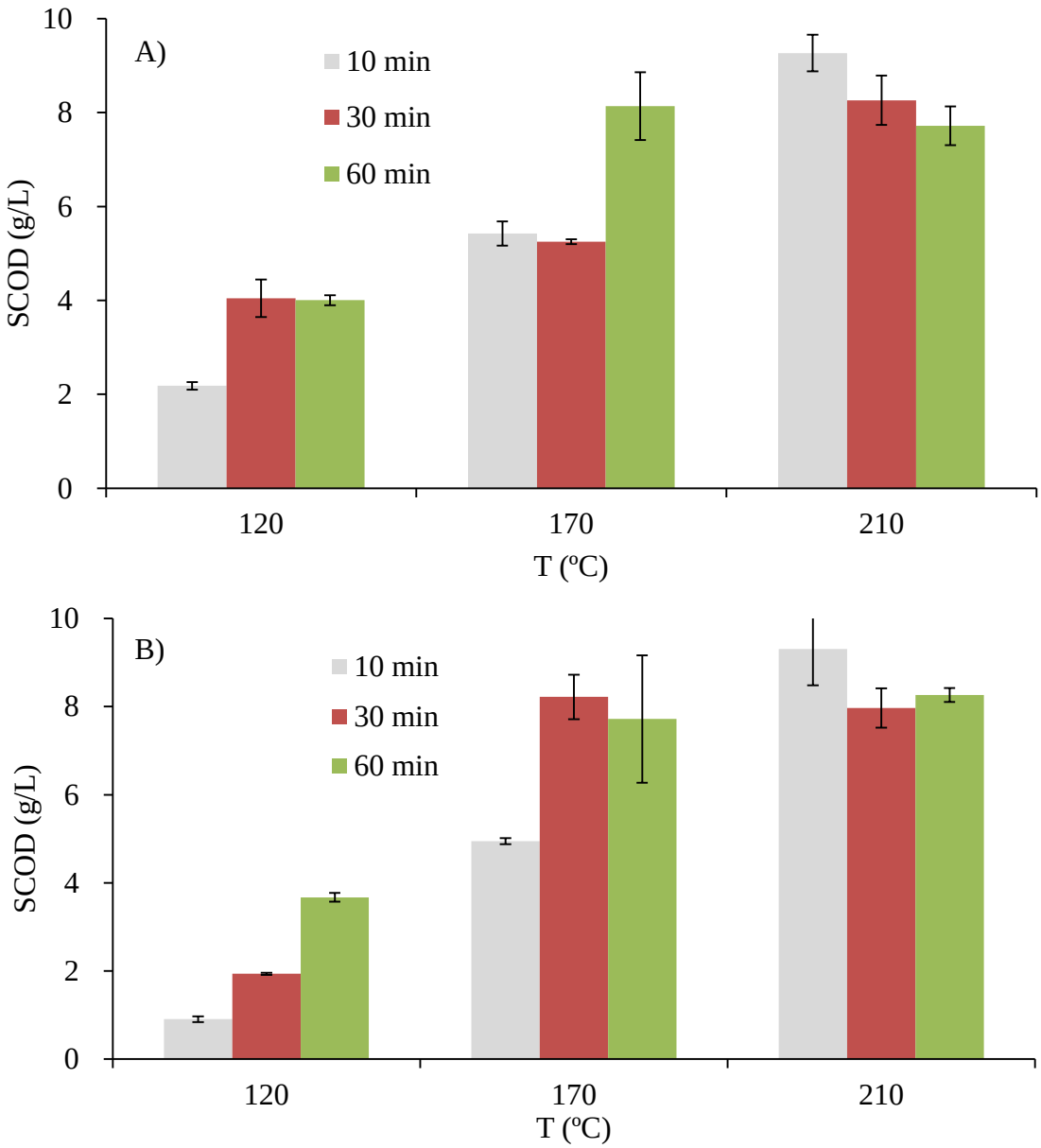


Figure 11, The effect of temperature on SCOD obtained from steam explosion (SE; A) and thermal hydrolysis (TH; B) pretreated conditions in this study

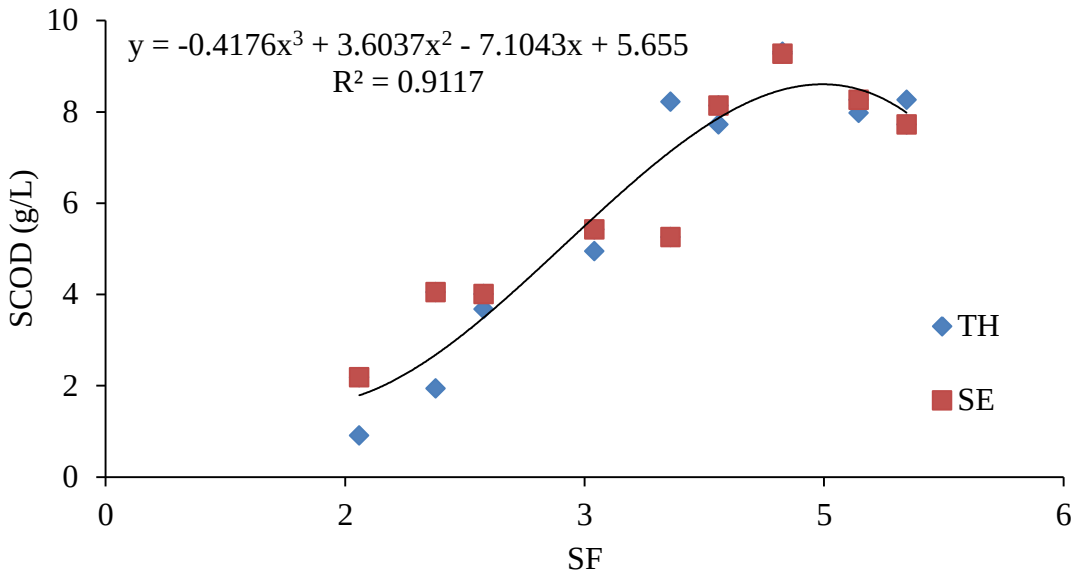


Figure 12. Correlation between SCOD from steam explosion (SE) and thermal hydrolysis (TH) pretreatments against severity factor (SF)

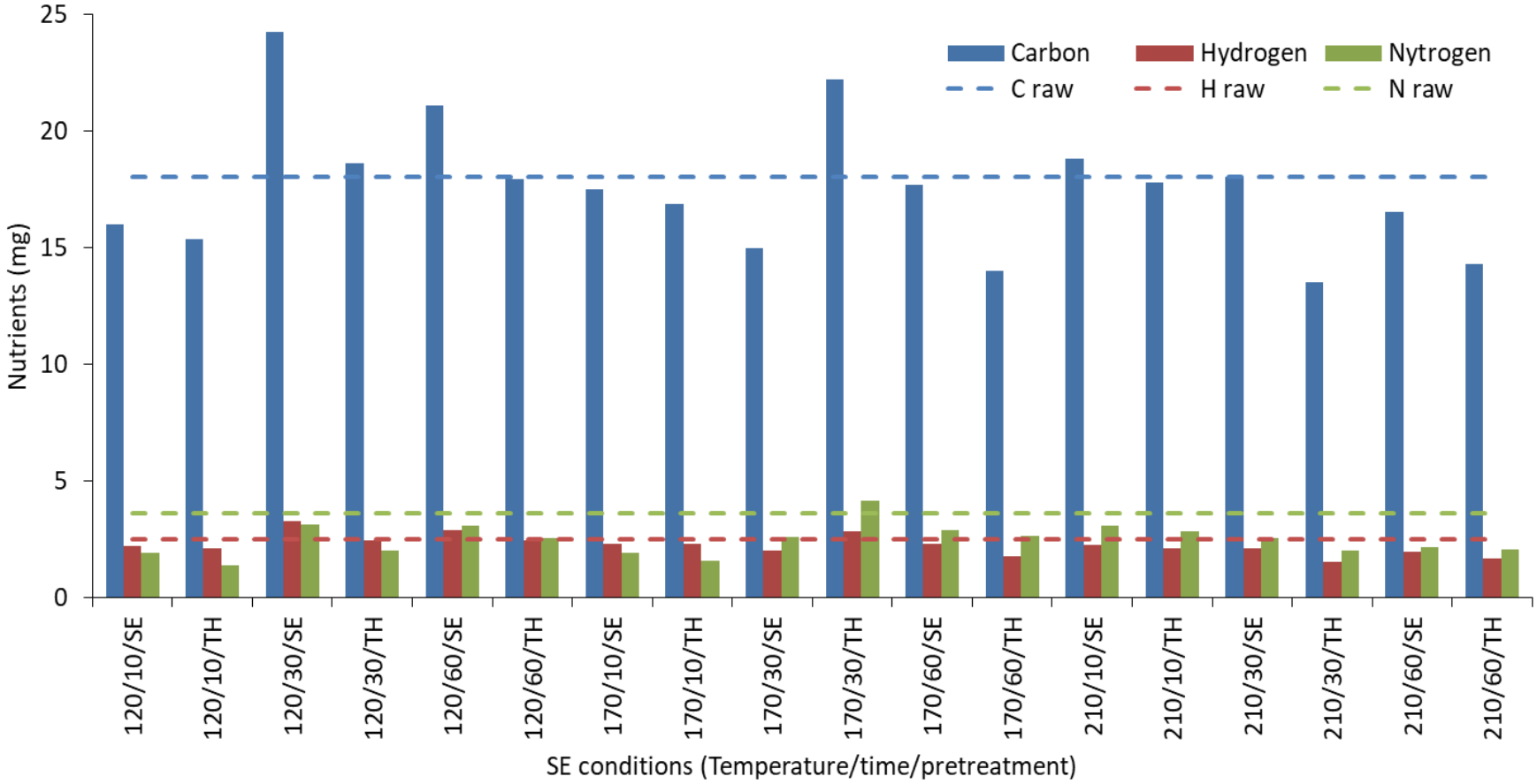


Figure 13. Carbon (C), hydrogen (H) and nitrogen (N) mass measured from pretreated conditions' total solid fraction as compared with raw *Eichhornia crassipes*

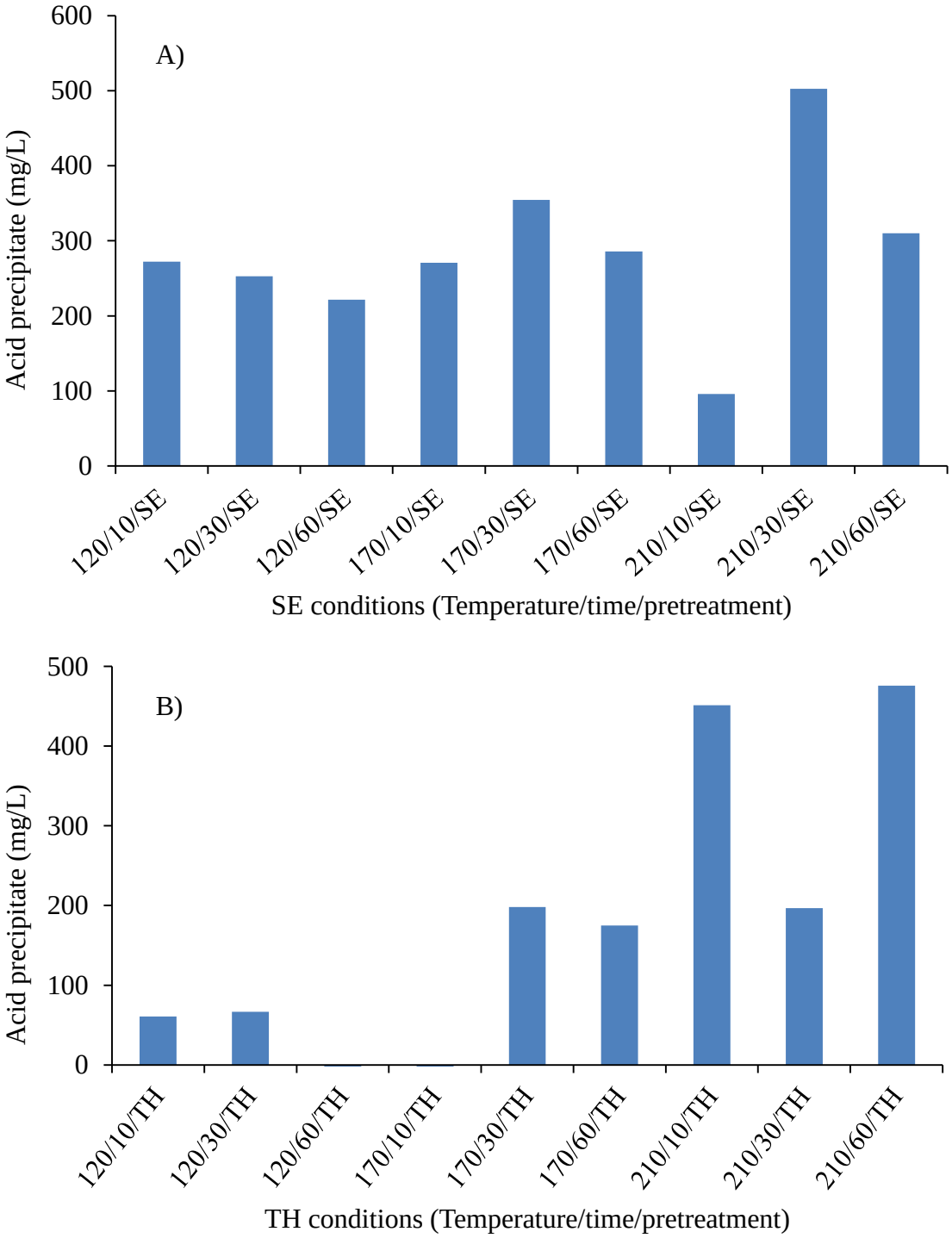


Figure 14, Acid precipitate obtained from the different steam explosion (SE; A) and thermal hydrolysis (TH; B) pretreatment conditions during this study

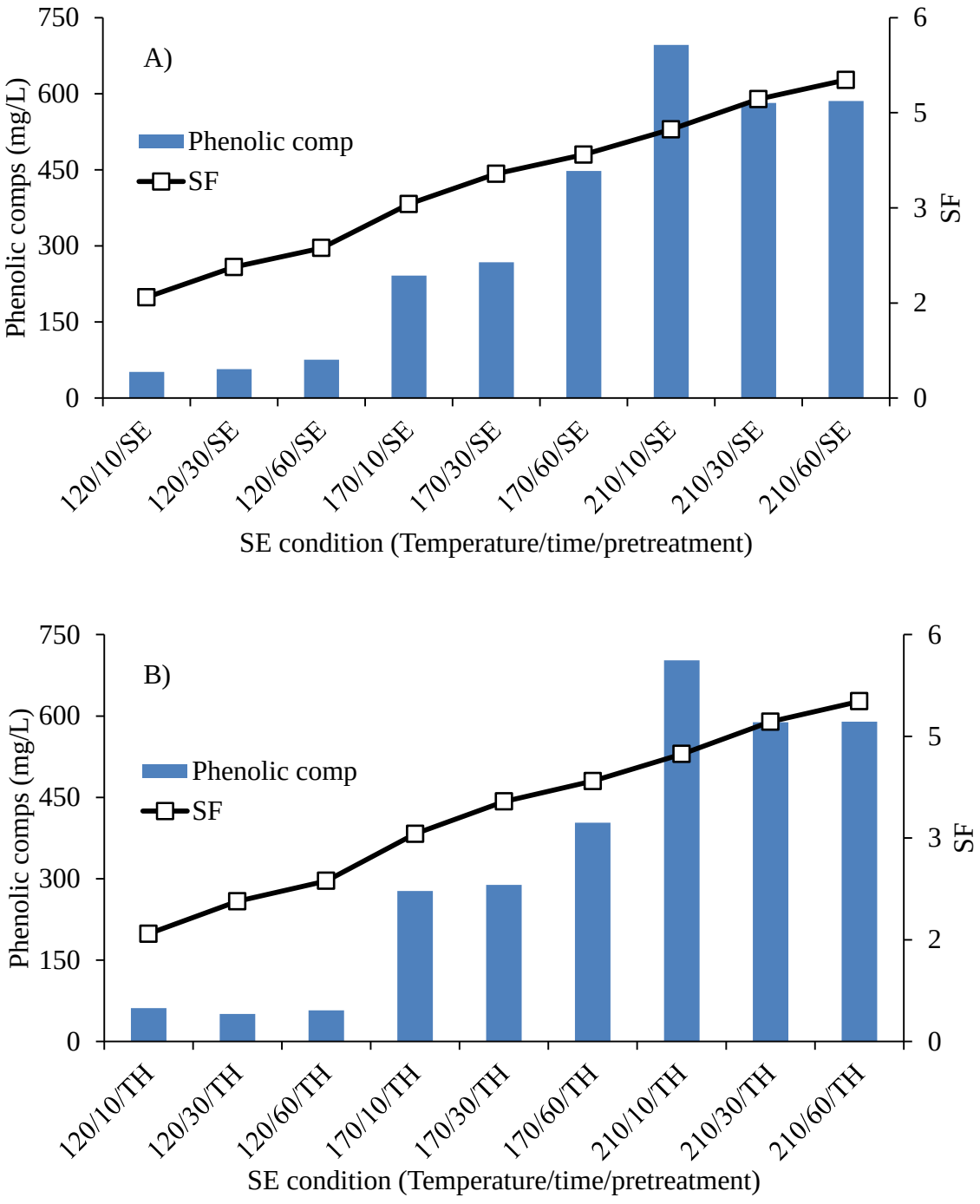


Figure 15, Phenolic compounds produced for steam explosion (SE; A) and thermal hydrolysis (TH; B)

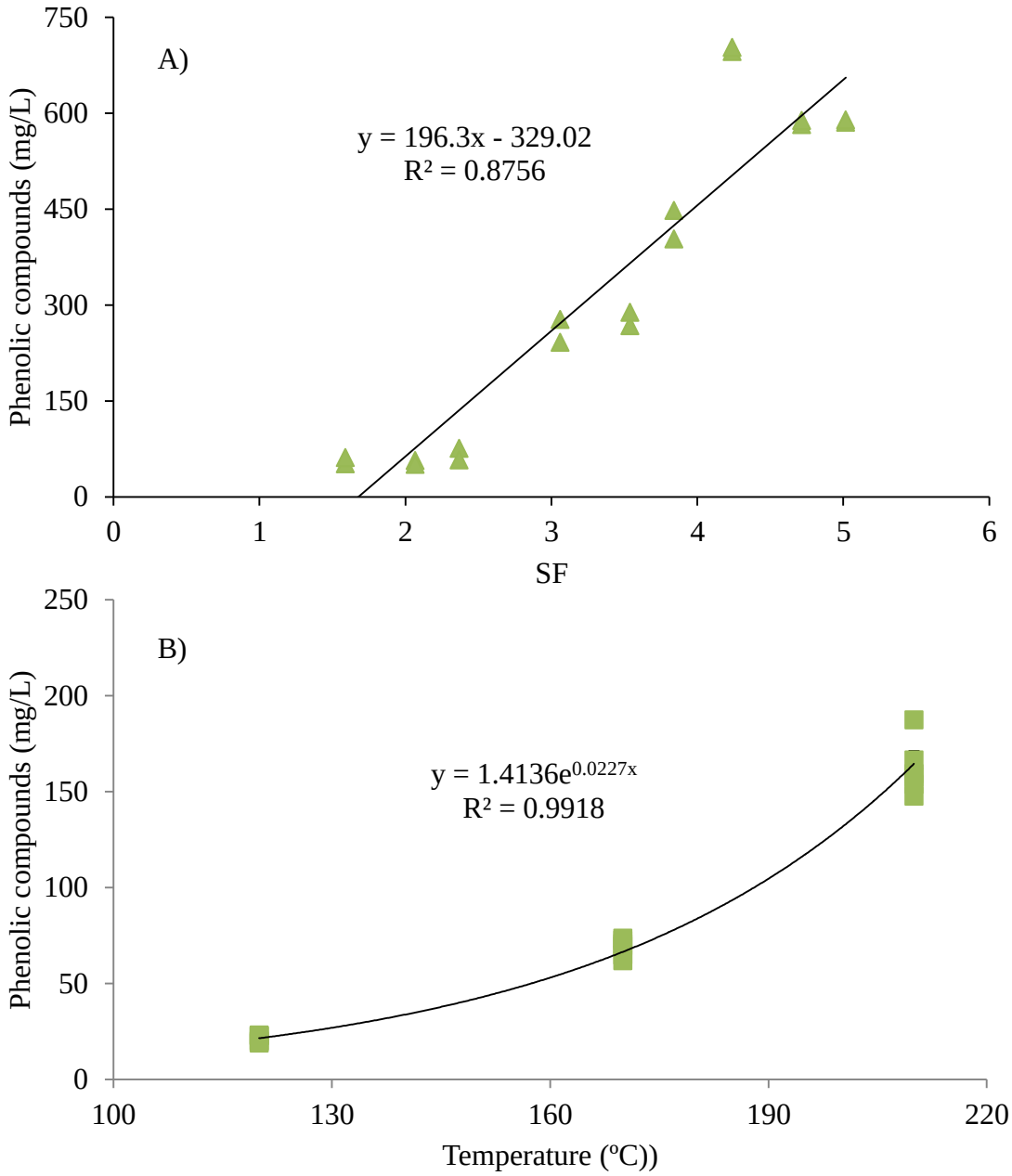


Figure 16, Correlation between phenolic compounds generated and pretreatment conditions for both steam explosion and thermal hydrolysis:
A) Correlation with severity factor (SF)
B) Correlation with temperature

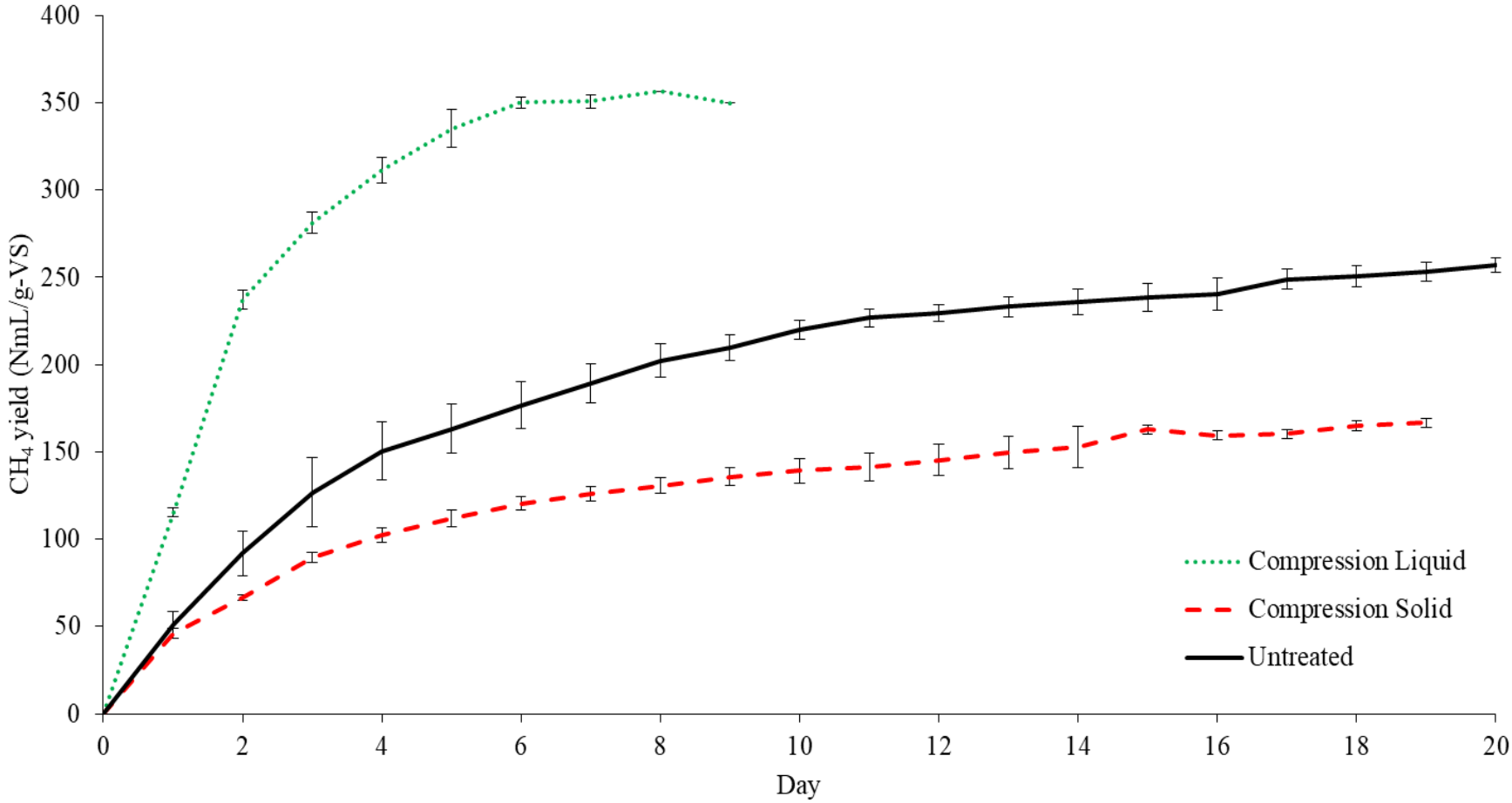
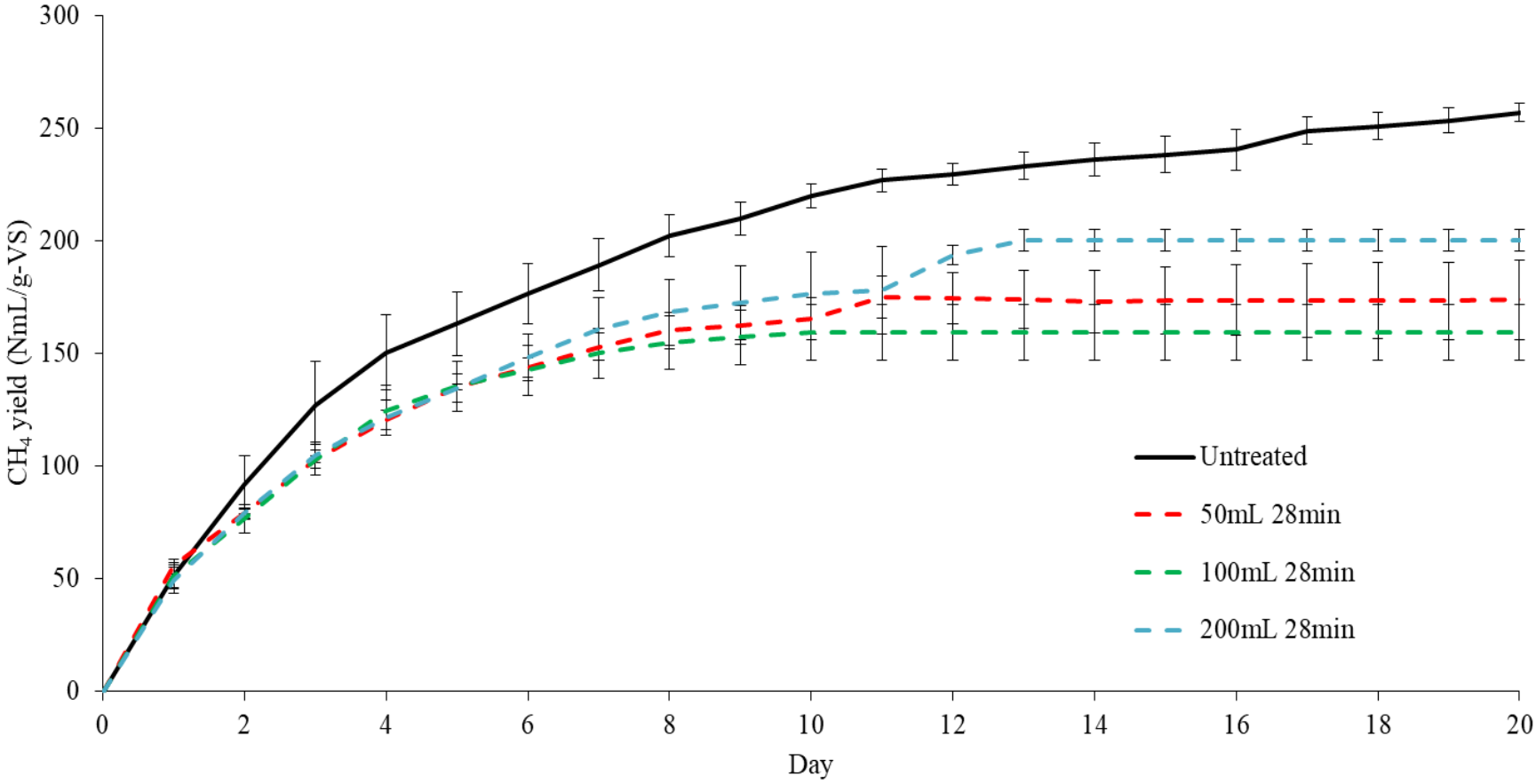
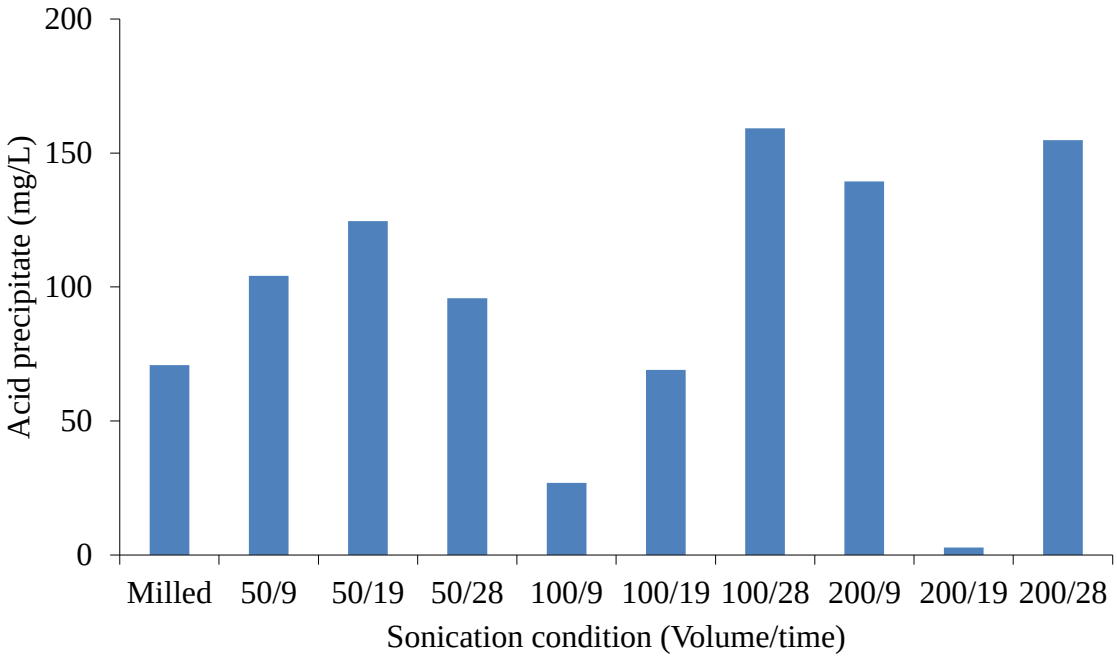


Figure 17. CH_4 yield of the compressed and untreated conditions tested on AD batch experiments



Fiure 18. CH_4 yield of the sonicated and untreated conditions tested on AD batch experiments



Fiure 19. Acid precipitate from milled and sonication conditions

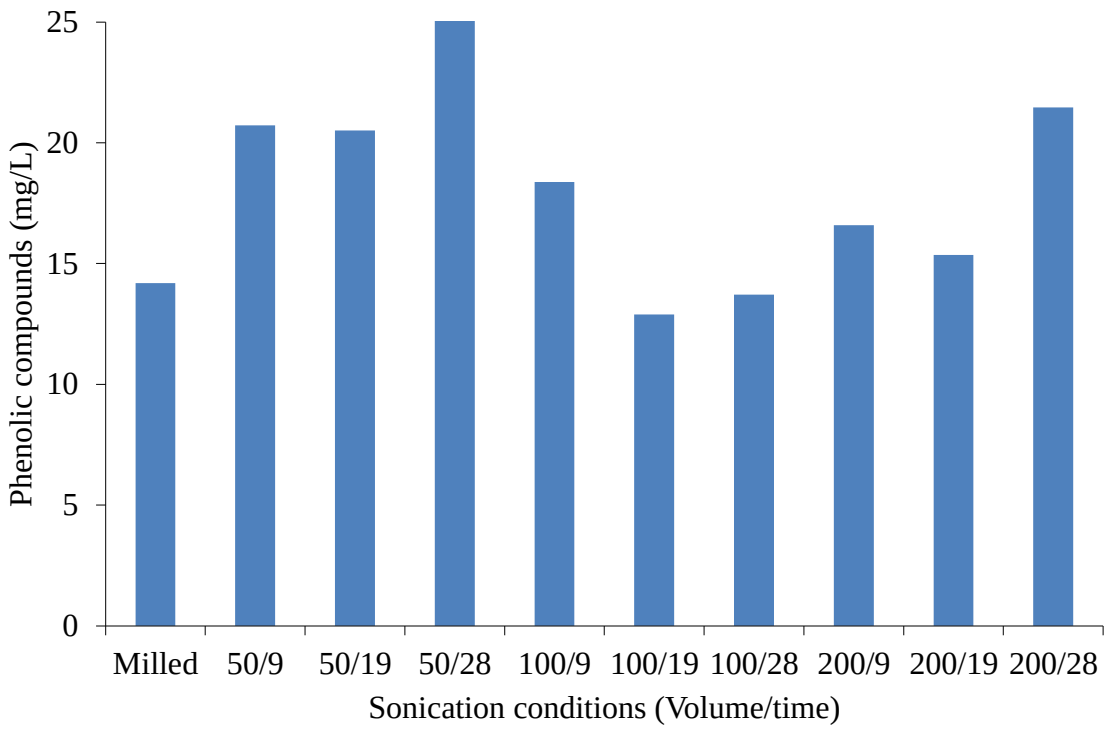


Figure 20. Phenolic compounds concentrations from sonication conditions

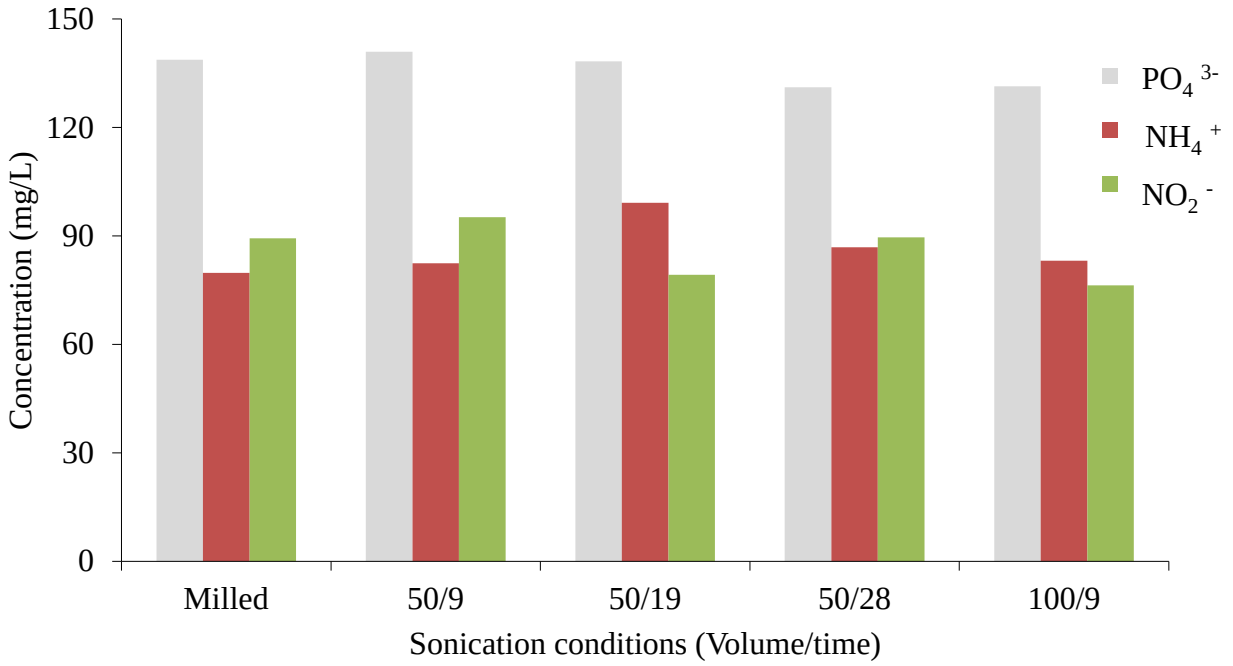


Figure 21. Soluble nutrients concentration from untreated and sonication conditions

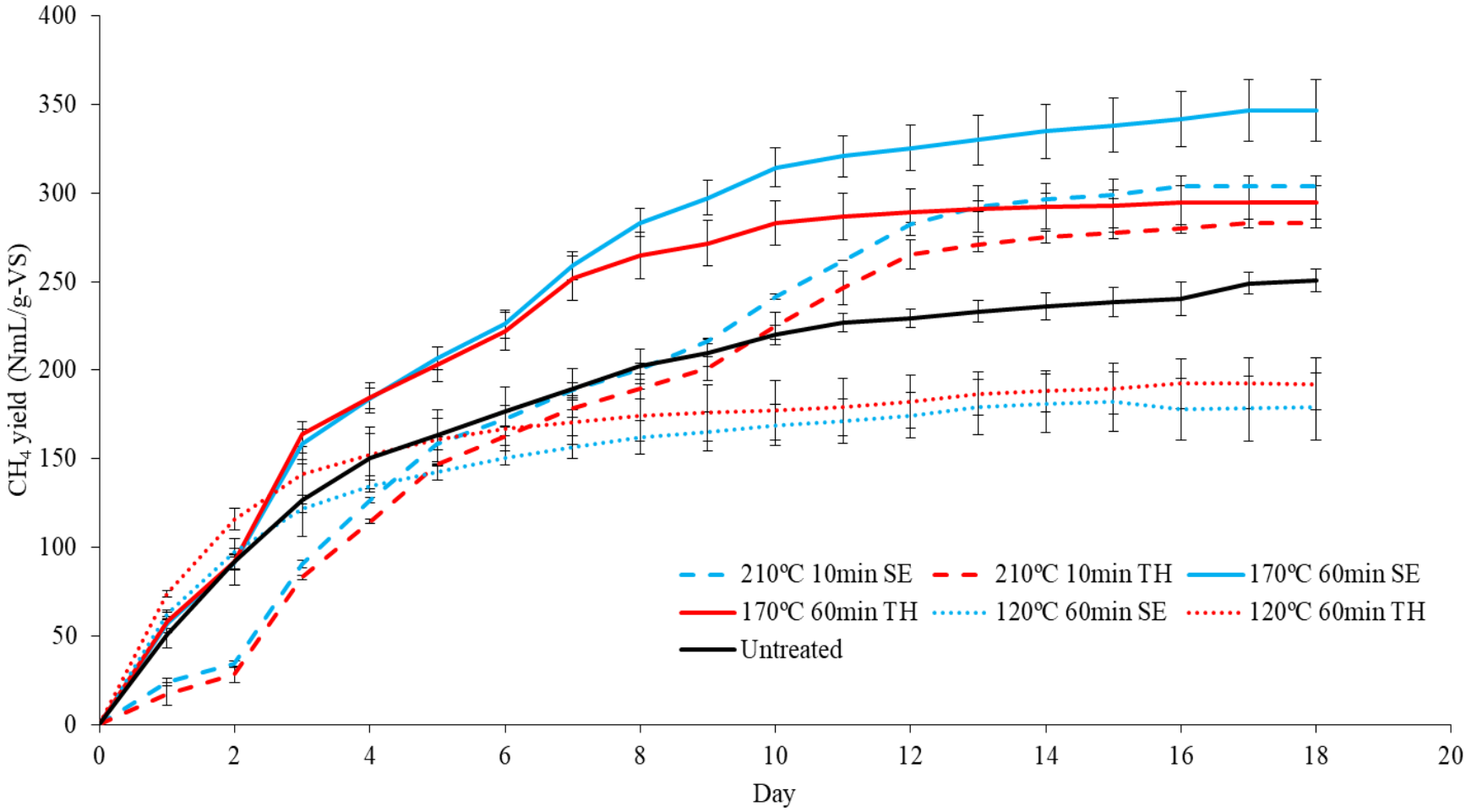


Figure 22. CH_4 yield of the steam exploded (SE) and untreated conditions tested on AD batch experiments.

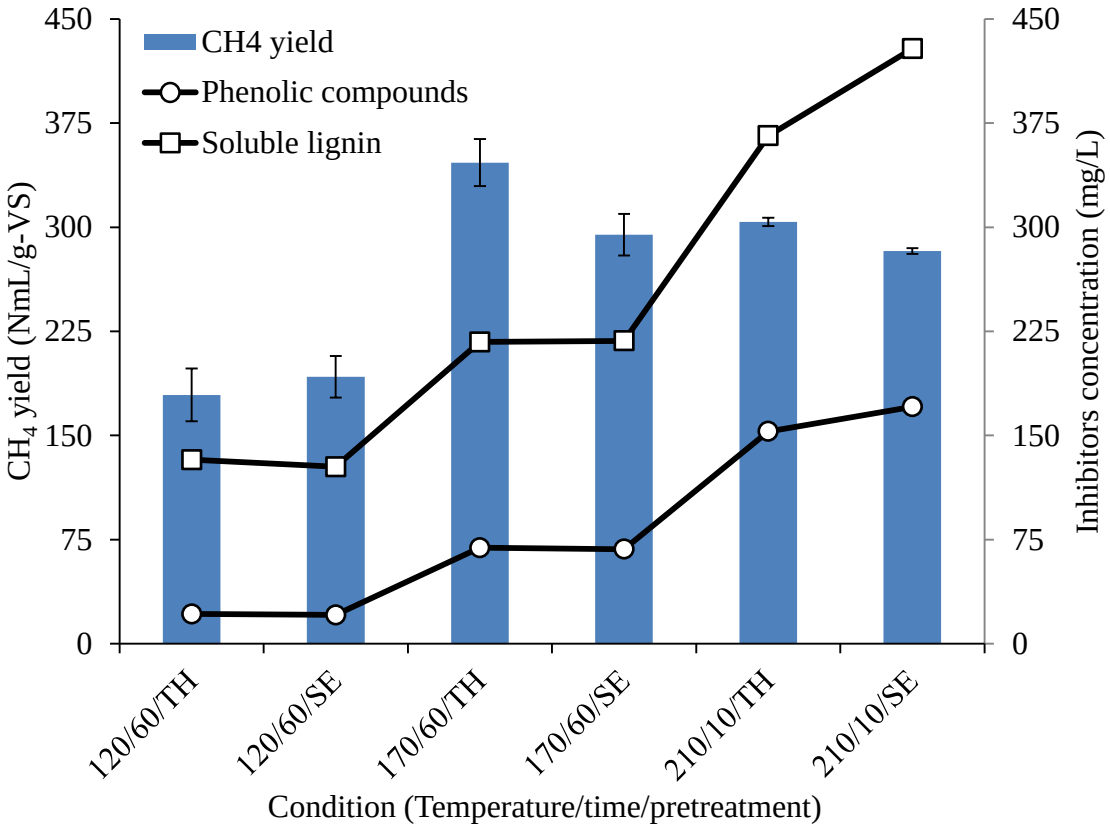


Figure 23. CH_4 yield, phenolic compounds and soluble lignin concentrations from steam exploded AD samples

Table 1. *Eichhornia crassipes* spreading all over the world

Location	Reference
Europe (Belgium)	Verbandt (1990)
North America (USA)	Center et al. (2001)
South America (Brazil, Amazon basin)	Cilliers et al. (1996); Center et al. (2001); Carlini et al. (2018)
Africa (Egypt, Nile river)	Elserafy et al. (1980); Cilliers et al. (1996); Carlini et al. (2018)
Asia (India, Papua New Guinea, Australia, Philippines)	Beshir and Bennett (1984); Jayanth (1988); Cilliers et al. (1996); Center et al. (2001); Nigam (2002); Carlini et al. (2018)

Table 2. Comparison of pretreatment methods.

Pretreatment	Advantage	Disadvantage	Reference
Physical			
Milling	Increase the surface area of biomass	High energy demand	Taherzadeth and Karimi (2008) Izumi et al. (2010)
Microwave	No risk of inhibitors formation Short treatment time	High cost	Passos et al. (2013) Travaini et al. (2016)
Steam explosion	Cost effective Hydrolysis of hemicellulose Short treatment time	Risk of inhibitors formation	Taherzadeh and Karimi (2008)
Thermal hydrolysis	Cost effective Hydrolysis of hemicellulose Non required special equipment	Risk of inhibitors formation	Kist et al. (2018b)
Compression	Simple operation	Remaining nutrient-rich solid phase	Akely et al. (2010) Kolawole et al. (2011)
Sonication	Delignification Hemicellulose and cellulose hydrolysis	Novel pretreatment. Mechanisms not fully understanded	Bussemaker and Zhang (2013) Luo et al. (2013)
Chemical			
Alkali	High delignification ability Short treatment time	High cost Risk of inhibitors formation	Carrere et al. (2016) Taherzadeh and Karimi (2008)
Acid	Hydrolysis hemicellulose Short residence time	High cost Risk of inhibitors formation	Carrere et al. (2016) Taherzadeh and Karimi (2008)
Ozone	Low generation of inhibitory compounds Operation at ambient temperature Short treatment time	Large amount of ozone required; high cost	Sun and Cheng (2002) Travaini et al. (2016) Taherzadeh and Karimi (2008)
Biological			
Fungi	Delignification Low energy demand No chemical demand	Low treatment rate Long treatment time	Taherzadeh and Karimi (2008) Sun and Cheng (2002)

Table 3. Sonication conditions in this study

Time (min)	Volume (mL)	Specific Energy (MJ/kg-TS) *
9.5	50	285.0
19.0	50	570.0
28.7	50	855.0
9.5	100	142.5
19.0	100	285.0
28.7	100	430.5
9.5	200	71.3
19.0	200	142.5
28.7	200	215.3

* See equation on Materials and Methods

Table 4. Steam explosion and thermal hydrolysis conditions in this study

Temperature (ºC)	Time (min)	Severity Factor *
120	10	1.6
120	30	2.1
120	60	2.4
170	10	3.1
170	30	3.5
170	60	3.8
210	10	4.2
210	30	4.7
210	60	5.0

* See equation on Materials and Methods

Table 5. Characteristics of *Eichhornia crassipes* used on this this study

Parameter	Chemical composition
TS (g/g)	0.05
VS (g/g)	0.04
VS/TS	0.81
TOC (g/kg-wwt)	54.00
Carbon (% TS)	37.71
Hydrogen (% TS)	5.23
Nitrogen (% TS)	7.60
Lignin (% TS)	9.04
Hemicellulose (% TS)	31.13
Cellulose (% TS)	28.02

Table 6. Total solids (TS), volatile solids (VS) and moisture content (%) of raw substrate and compressed fractions (liquid and solid fractions)

	TS (%)	VS (%)	Moisture (%)
Raw substrate	4.79	3.86	95.21
Liquid fraction	0.69	0.21	99.31
Solid fraction	10.72	9.47	89.28

Table 7. Compression TCOD and SCOD

Sample	TCOD (g/L)	TCOD (g/L)
Liquid fraction (g/L)	3.24	2.39
Solid fraction (g/kg*)	70.53	-

*kg of raw substrate

Table 8. VS mass balance for compression pretreatment

	VS Balance		
	Mass (g)	%	g-VS
Raw substrate	373.84	100	14.43
Liquid	247.78	66	0.52
Solid	112.69	30	10.67
Total sample	360.47	96	11.19
Loss	13.37	4	3.24

Table 9. TCOD mass balance for compression pretreatment

	TCOD balance		
	TCOD(g/kg-wwt)	Raw mass (g)	TCOD (g)
Raw substrate	54.00	100	5.40
Solid	70.53	30	2.13
Liquid	3.24*	66	0.21
Loss	-	4	3.06

*g-TCOD/L

Table 10. Simulation parameter using the modified Gompertz's equation for CH_4 yield results of AD batch experiments

Pretreatment		a*	b*	c*
Untreated		232	35	-0.40
SE	210ºC 10min SE	313	30	0.36
	210ºC 10min TH	288	29	0.53
	170ºC 60min SE	339	42	-0.19
	170ºC 60min TH	291	45	-0.13
	120ºC 60min SE	172	36	-0.47
	120ºC 60min TH	178	52	-0.20
Sonication	50mL 28min	168	30	-0.44
	100mL 28min	159	34	-0.21
	200mL 28min	182	30	-0.40
Compression	Liquid	336	132	0.16
	Solid	146	24	-0.61

*a, b and c indicate the stabilization point, the kinetics factor and the inhibition factor, respectively. These values are calculated according to the modified Gompertz's equation as shown on the matherials and methods section

Table 11. Compression CHN results compared with raw substrate

Condition	Carbon (% TS)	Hydrogen (% TS)	Nitrogen (% TS)
Raw water hyacinth	37.71	5.23	7.60
Compression Solid	42.51	5.81	5.02

Table 12. Different pretreatment's AD comparison based on CH_4 yield

Condition		CH_4 yield (NmL/g-VS)	Improvement (NmL/g-VS)	Improvement (%)	Time (d)
Untreated		252	-	-	19
SE	210ºC 10min SE	285	34	13.4	16
	210ºC 10min TH	283	31	12.4	17
	170ºC 60min SE	347	95	37.9	17
	170ºC 60min TH	293	41	16.3	14
	120ºC 60min SE	179	-72	-28.8	18
	120ºC 60min TH	192	-59	-23.6	16
Sonication	50mL/28:45min	174	-78	-30.8	11
	100mL/28:45min	159	-92	-36.7	10
	200mL/28:45min	199	-53	-21.0	13
Compression	Liquid	350	98	39.1	8
	Solid	167	-85	-33.7	19

Table 13. AD's raw substrate consumption and ratios with CH4 production

Condition	VS added	Sample added	Raw substrate (g)	CH_4 prod (mL)	CH_4/raw substrate (mL CH_4/g)	Operation time (d)	CH_4/d (mL/d)	CH_4/d·g-raw
Untreated	1.03	26.80	26.80	273	10.2	23	11.9	0.44
210°C 10min SE	1.50	90.32	48.30	427	8.8	16	26.7	0.55
210°C 10min TH	1.50	92.67	49.55	423	8.5	17	24.9	0.50
170°C 60min SE	1.50	90.88	48.60	519	10.7	17	30.5	0.63
170°C 60min TH	1.50	109.11	58.35	438	7.5	14	31.3	0.54
120°C 60min SE	1.16	56.54	30.23	207	6.9	18	11.5	0.38
120°C 60min TH	1.16	68.79	36.79	223	6.1	17	13.1	0.36
Compression liquid	0.46	220.19	333.62	165	0.5	8	20.6	0.06
Compression solid	1.16	12.22	40.72	193	4.7	19	10.2	0.25
50mL 28min	1.03	140.16	28.03	180	6.4	20	9.0	0.32
100mL 28min	1.03	147.17	29.43	165	5.6	10	16.5	0.56
200mL 28min	1.03	143.77	28.75	207	7.2	13	15.9	0.55

Table 14. AD's CH_4-production/COD ratios

Condition	SCOD (g/L)	TCOD (g/L)	Cumm*-CH_4 /SCOD	Cumm*-CH_4 /TCOD
Untreated	-	54.0	-	5.06
210ºC 10min SE	9.3	-	46.06	-
210ºC 10min TH	9.3	-	45.45	-
170ºC 60min SE	8.1	-	63.80	-
170ºC 60min TH	7.7	-	56.72	-
120ºC 60min SE	4.0	-	51.75	-
120ºC 60min TH	3.7	-	60.67	-
Compr liquid	2.4	3.2	69.01	50.92
Compr solid	-	70.5	-	2.73
50mL 28min	1.2	-	151.06	-
100mL 28min	1.2	-	135.03	-
200mL 28min	1.1	-	191.65	-

* Total amount in NmL-CH_4 produced during the batch AD experiment