

## LOW-TEMPERATURE THERMAL TREATMENT OF LIGNOCELLULOSIC WASTE PRIOR TO ANAEROBIC DIGESTION

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### ABSTRACT

This study aims to determine the effect of low-temperature thermal pretreatment on two lignocellulosic materials originating from agroindustrial activities, namely grape marc and cotton gin waste. More specifically, this paper focuses on the impact of thermal pretreatment on the solubilization and the methane potential of these two materials. Thermal pretreatment was carried out by soaking the materials in deionized water at three different temperatures, i.e. 75, 50 and 100°C. The effect of pretreatment duration was investigated in the range of 30 – 240 min. Solubilization of the materials was determined by measuring soluble chemical oxygen demand (sCOD) and total phenols concentrations in the liquid phase obtained after pretreatment. The methane potential of the solid and liquid fractions was determined through Biochemical Methane Potential (BMP) assays. The samples used in these assays were chosen on the basis of the solubilization data after pretreatment. According to the results, pretreatment temperature affects solubilization more, compared to pretreatment duration. Methane production data indicated that pretreatment favored both release of biodegradable material in the liquid phase, and digestibility of remaining solids. Thermal pretreatment at 75 °C provided the highest SMY for what concerns GM-solid substrates and CGW-liquid substrates, while the best results for GM-liquid substrates and CGW-solid substrates were obtained after pretreatment at 100 °C.

**Keywords:** anaerobic digestion, lignocellulosic, thermal, pretreatment, methane

### 1. Introduction

Anaerobic digestion is a biological process which is widely used to treat complex organic substrates in order to produce biogas (Aragaw *et al.*, 2013; Ariunbaatar *et al.*, 2014). This technology can serve as a means of both sustainable waste management and alternative energy production (Bruni *et al.*, 2010). In fact, in the last years the application of anaerobic digestion of solid substrates, particularly waste, has been intensively investigated (Ariunbaatar *et al.*, 2014).

Among the most studied solid substrates for anaerobic digestion are lignocellulosic biomass and waste. However, the effectiveness of such a process is often limited by the fact that these materials are recalcitrant to biodegradation due to their composition, consisting of cellulose, hemicellulose and lignin. For this reason, pretreatment is usually applied, aiming to enhance digestibility of lignocellulosic substrates. Pretreatment methods include physical, chemical and biological processes (Haghighi Mood *et al.*, 2013; Hendriks and Zeeman, 2009).

In the present study the effect of low-temperature (50–100 °C) thermal pretreatment on solubilization and methane potential of grape marc (GM) and cotton gin waste (CGW) was investigated, through batch hydrolysis experiments and Biochemical Methane Potential assays.

### 2. Materials and methods

#### 2.1. Substrate and inoculum

Grape marc (GM), comprising of red grape skins, seeds and stalks, was obtained from a local winery, while cotton gin waste (CGW) comprising of cotton fiber, stalks and leaves was obtained

from a cotton ginning mill. GM was initially placed in zip-lock bags and stored at -20 °C. One day before each use, appropriate amounts were transferred to 4 °C and on the day of the experiment they were comminuted without drying using a food processor. CGW was immediately dried at 60 °C and then comminuted to a particle size less than 500 µm, using a universal cutting mill. The substrates were characterized regarding total solids (TS) and volatile solids (VS) content, as well as elemental composition.

The inoculum used in this study consisted of an anaerobic suspended sludge sample originating from a mesophilic anaerobic digester of the Municipal Wastewater Treatment Facility of Chania, Crete. TS and VS contents and pH of the inoculum, as well as elemental composition of its solids, were determined. The characteristics of both the substrates and the inoculum are shown in Table 1.

## 2.2. Thermal pretreatment

Thermal pretreatment of the substrates was performed using 250 mL glass flasks. The effect of the process duration (30 – 240 min) and process temperature (50, 75 and 100 °C) was investigated. More specifically, 5 g of raw substrate were introduced in the flasks together with 100 mL of deionized water and the slurries were then agitated in an orbital shaker for 5 min for homogenization purposes. Subsequently, the flasks were covered with aluminum foil and placed in a muffle furnace previously set at the desired temperature, where they were left for each predetermined time period. The samples were then centrifuged at 3,900 rpm for 15 min and the solid and liquid fractions were collected separately. The liquid fraction was finally filtered through a 0.45 µm pore size membrane filter in order to determine sCOD (soluble Chemical Oxygen Demand) and TP (Total Phenols) concentrations. Three replicates were carried out for each trial.

## 2.3. Biochemical methane potential (BMP) assays

Both solid and liquid fractions obtained after pretreatment were used for the BMP assays. The experimental apparatus for BMP assays consisted of 250 mL conical flasks covered with rubber stoppers. Three PVC (Polyvinyl chloride) tubes were inserted in the stoppers, which allowed N<sub>2</sub> flushing in the flasks, methane measurement and sampling for pH measurement once a week.

The working volume for the BMP assays was set to 50 mL and the inoculum quantity was maintained constant at 15 gVS/L and 7 gVS/L for solid and liquid samples, respectively. The substrate to inoculum ratio (SIR) on a VS basis ( $\text{gVS}_{\text{substrate}}/\text{gVS}_{\text{inoculum}}$ ) for solid samples and on a COD/VS basis ( $\text{gCOD}_{\text{substrate}}/\text{gVS}_{\text{inoculum}}$ ) for liquid samples was set to 0.5 for GM and to 0.25 for CGW. These values were chosen on the basis of the results of a previous study (Pellera and Gidarakos, 2014). Blank assays (SIR=0), containing only the inoculum were also performed.

BMP assays were carried out by firstly introducing the inoculum and substrates in the flasks and then bringing the total volume to 50 mL with deionized water. After adjusting the pH of the mixture at  $7.8 \pm 0.05$ , the flasks were covered with the rubber stoppers and finally flushed with N<sub>2</sub> for 2 min. The reactors were finally placed in an incubator set at 35 °C. Methane production was measured daily for the first seven days of incubation and subsequently every 2 days. All the assays were performed in duplicate.

## 2.4. Analytical methods

Total Solids (TS) and Volatile Solids (VS) contents were determined according to APHA (American Public Health Association) method 2540G. Elemental analysis (C, H, N, S) of the substrates was performed using an EA300 Euro Vector elemental analyzer, via flash combustion at 1020 °C. Oxygen content was determined by difference, considering the VS content of each sample. sCOD of the liquid fractions obtained from thermal pretreatment was determined through APHA method 5220C, while TP were determined according to Folin-Ciocalteu's method. Methane production was determined by means of volume displacement using a 11.2% KOH solution.

### 3. Data analysis

Specific methane yields (SMY) are obtained by subtracting the ultimate cumulative methane production of the inoculum (mL CH<sub>4</sub>) from the ultimate cumulative methane production of each assay, and by subsequently dividing it by the added amounts of VS of substrate. These values were then converted to STP values by applying Equation 1 (Xie *et al.*, 2011).

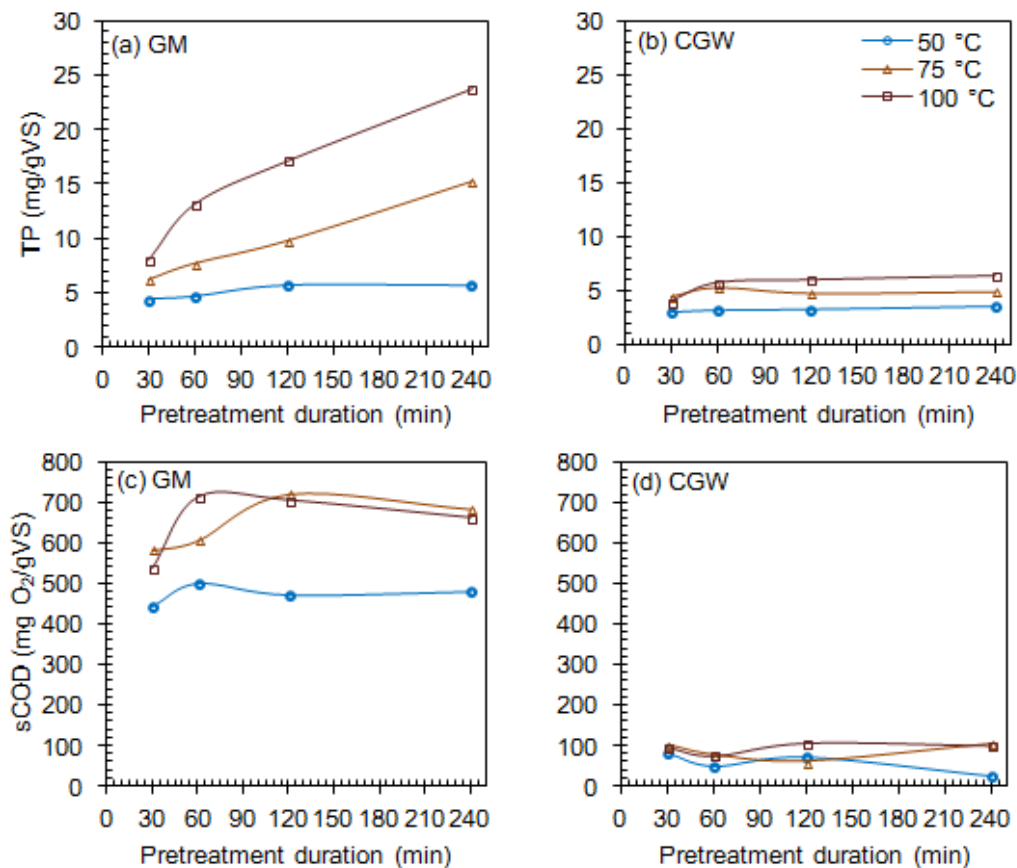
$$V_{STP} = (P \cdot V \cdot T_{STP}) / (P_{STP} \cdot T) \quad (1)$$

where  $V_{STP}$ ,  $P_{STP}$  and  $T_{STP}$  are methane volume, atmospheric pressure and temperature at STP conditions, respectively, and  $V$ ,  $P$  and  $T$  are methane volume, atmospheric pressure and temperature at the time of measurement.

### 4. Results and discussion

#### 4.1. Thermal pretreatment

As it can be seen in Figure 1, organic material release appears to be positively affected by pretreatment temperature, since increased values of both TP and sCOD are observed with increasing temperatures. More specifically, TP release shows a relatively even increase with temperature, while sCOD release increases between 50 and 75 °C and begins to stabilize around the same levels between 75 and 100 °C. On the other hand, the effect of pretreatment duration on solubilization differs depending on the examined parameter as well as on the substrate. In fact, as pretreatment time increases, TP release in the case of GM has a generally increasing trend, while for CGW the values remain constant after the first 60 min. For what concern sCOD it is noticed that in all cases, after 2 h of pretreatment, equilibrium has already been attained. Therefore, this pretreatment duration was chosen as optimum and adopted to produce the materials used in the BMP assays.



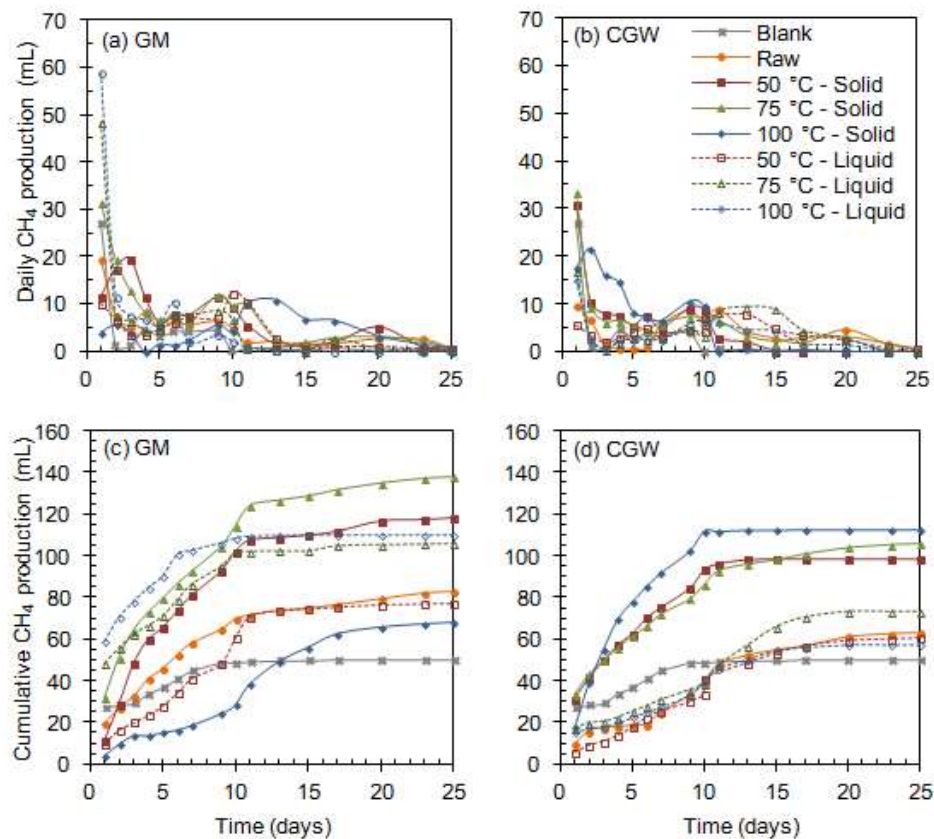
**Figure 1:** TP (a and b) and sCOD (c and d) release after pretreatment as a function of pretreatment duration and pretreatment temperature

**Table 1:** Characteristics of inoculum and substrates

Parameter	Inoculum	GM	CGW
TS (%)	2.25	28.1	70.6
VS (%)	1.59	25.8	52.9
VS/TS	70.5	92.0	75.0
pH	7.8	3.6	5.6
Empirical formula	-	$C_{27.2}H_{42.2}O_{14.6}N$	$C_{23.7}H_{38.9}O_{19.7}N$
ThCOD ( $mgO_2/gTS$ )	-	1546	1601
Elemental composition			
C (%)	33.7	48.8	32.7
H (%)	0.2	6.3	4.5
N (%)	4.0	2.1	1.6
S (%)	0.0	0.0	0.0
O (%)	24.1	34.8	36.2
C/N	8.4	23.2	20.4

#### 4.2. Biochemical methane potential assays

Figure 2 depicts the results obtained from the BMP assays. A first observation is that methane production for pretreated solid samples was much higher than that of raw samples, indicating a positive effect of thermal pretreatment on materials solubilization. For the majority of samples the peak in methane production was observed at the beginning of incubation. This behaviour may be attributed to the availability of readily biodegradable organic matter in the substrates (Aragaw *et al.*, 2013), which was enhanced by pretreatment. A slight lag phase of a few days was observed only for the assays containing solid GM pretreated at 50 and 100 °C and solid CGW pretreated at 100 °C.



**Figure 2:** Daily (a and b) and cumulative (c and d) methane production of raw and pretreated substrates

Table 2 presents the SMY of the BMP assays. These values reveal more information on the effect of pretreatment on methane production, which in fact was different for the two materials used. More specifically, in the case of GM, and in particular for solid substrates, the increase in pretreatment temperature caused an increase in SMY up until 75 °C, while the value for 100 °C was found much lower, even compared to the raw substrate. SMY of liquid substrates on the other hand, showed an increasing trend in the whole temperature range tested. The exact opposite behaviour was observed in the case of CGW, with a continuous increase being noticed for solid substrates, while regarding liquid substrates, the peak value was the one corresponding to 75 °C.

The effect of pretreatment temperature on methane production was most likely a result of a combination of several phenomena. Initially, pretreatment caused substrate solubilisation, i.e. the disruption of the materials matrices, leading not only to the release and transport of organic matter from the solid to the liquid fraction (Fernández-Cegrí *et al.*, 2012), but also to an increase in the bioavailability of the organic matter still remaining on the solid fraction. The former might have contributed to the lower SMY of the solid GM substrate pretreated at 100 °C, as well as the higher SMY of the respective liquid substrate. The latter on the other hand, may be a suitable explanation for the results of CGW samples and more specifically, for the increasing SMY of solid substrates as the temperature increases.

Lower methane yields at higher temperatures, for both solid and liquid substrates, may eventually be a result of the release or/and formation of certain inhibiting and recalcitrant compounds caused by the breakage of chemical bonds (Mood *et al.*, 2013), although most of these substances are usually released at higher pretreatment temperatures (>100 °C) (Appels *et al.*, 2010). Moreover, in the case of GM in particular, treating this sample at 100 °C, may have also caused further damage to the material by destroying the available organic matter still present on the solid matrix.

**Table 2:** SMY of solid (NmLCH<sub>4</sub>/gVS<sub>substrate</sub>) and liquid (NmLCH<sub>4</sub>/gCOD<sub>substrate</sub>) substrates

Substrate	Raw samples	Pretreated samples					
		50 °C - Solid	75 °C - Solid	100 °C - Solid	50 °C - Liquid	75 °C - Liquid	100 °C - Liquid
GM	78.40	164.04	210.12	35.90	129.34	268.25	282.62
CGW	62.07	231.00	265.56	296.87	79.04	220.35	45.51

## 5. Conclusions

This study focused on evaluating the effect of low temperature (50–100 °C) thermal pretreatment on solubilization and methane potential of grape marc (GM) and cotton gin waste (CGW). It was demonstrated that while pretreatment duration did not significantly affect materials solubilization in terms of sCOD and total phenols released from the solid matrix, contrarily the variation in pretreatment temperature had a much greater effect. Moreover, according to the results the effect of the latter parameter on the methane potential of the two lignocellulosic materials was different. In the case of GM, pretreatment at 75 °C provided the best results for what concerns solid substrates, while 100 °C worked better for liquid substrates. The opposite is true in the case of CGW, with the 100°C–solid substrate and the 75°C–liquid substrate having the highest SMY.

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