Ultrasonic Pretreatment for Anaerobic Digestion: a Study on Feedstock, Methane Yield, and Energy Balance

by

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Abstract

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Dr. Bill Van Heyst

The research represents a first approach to measure the utilization potential of ultrasonic pretreatment on six different substrates: fat, oil and grease (FOG), paper sludge, ground switch grass, ground hay, ground wheat straw, and cut wheat straw. Several laboratories techniques were applied to determine the influence of ultrasonication on biogas production and yield, biogas quality, and digestibility ratio. With the data, mathematical definitions of Net Energy Balance and Net Economy Balance were computed to draw a first justification or rejection of the use of this pretreatment technology for the specific substrates. Ultrasonic pretreatment has a significant effect on biogas production and yield as well as digestibility ratio ($p$-value < 0.0001) from the early stages of digestion until as far as 50 days of digestion. Ultrasonication and macro particle size management did not influence significantly the methane ($\text{CH}_4$) content in the biogas ($p$-value = 0.1793). Also, the impact of ultrasonication on the substrate varies between all studied feedstock. Most of the ultrasonicated digestion cases studied provided a negative Net Energy and Economic Balance except for FOG where a certain window of utilization was found. In the context of an ultrasonication process retrofit upgrade, the technology looks to be more useful for substrates that are hard to digest when the retention time is, unfortunately, longer than common retention time. In the context of a new facility, a design that includes an understood ultrasonication technology has yet a small potential success depending on several variables. The ultrasonication technology for anaerobic digestion is hard to recommend due to its energy consumption that, in many cases, overshadows the energy surplus derived from its use.
Acknowledgments

It is not every day that we take a deep breath after a relative end and look behind to summarize the last times. Many important people were involved in the achievement of this study. I firstly must thank Dr. Animesh Dutta for his faith in my person, for his day to day smile, and for his loyalty to freedom and imagination that research so much need.

The wise advices and vital support of Dr. Bill Van Heyst also need to be underlined.

Je tiens aussi à remercier monsieur Jean-Claude Corbeil pour la confiance qu’il m’a démontré tout au long de ces deux dernières années. C’est par ce genre de relations que le monde de la recherche peut se rapprocher de celui du marché économique, et ainsi apporter des solutions réalistes aux problèmes complexes de la société présente.

Finally, but not with less significance, I make a node and kiss all the people who allowed me to love them since the beginning. You were part of the reason I get up in the morning and the reason I lay down at night…

“Substitute ‘damn’ every time you're inclined to write 'very'; your editor will delete it and the writing will be just as it should be.”

— Mark Twain
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<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AD</td>
<td>Anaerobic Digestion</td>
</tr>
<tr>
<td>BAP</td>
<td>Biochemical Acidogenic Potential</td>
</tr>
<tr>
<td>BMP</td>
<td>Biochemical Methane Potential</td>
</tr>
<tr>
<td>BOD$_5$</td>
<td>Biochemical Oxygen Demand (day 1 vs. day 5)</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>DD$_{COD}$</td>
<td>Degree of COD Released or Degree of Degradation</td>
</tr>
<tr>
<td>FOG</td>
<td>Fat, Oil and Grease</td>
</tr>
<tr>
<td>HHV</td>
<td>High Heating Value</td>
</tr>
<tr>
<td>ISR</td>
<td>Inoculum:Substrate ratio</td>
</tr>
<tr>
<td>LCA</td>
<td>Life Cycle Analysis</td>
</tr>
<tr>
<td>NEcB</td>
<td>Net Economy Balance</td>
</tr>
<tr>
<td>NEnB</td>
<td>Net Energy Balance</td>
</tr>
<tr>
<td>NPOC</td>
<td>Non-Purgeable Organic Carbon</td>
</tr>
<tr>
<td>OLR</td>
<td>Organic Loading Rate</td>
</tr>
<tr>
<td>OUR</td>
<td>Oxygen Uptake Rate</td>
</tr>
<tr>
<td>pH</td>
<td>potential Hydrogen</td>
</tr>
<tr>
<td>SCOD</td>
<td>Soluble Chemical Oxygen Demand</td>
</tr>
<tr>
<td>SOUR</td>
<td>Specific Oxygen Uptake Rate</td>
</tr>
<tr>
<td>STP</td>
<td>Standard Temperature and Pressure</td>
</tr>
<tr>
<td>TOC</td>
<td>Total Organic Carbon</td>
</tr>
<tr>
<td>TS</td>
<td>Total Solid</td>
</tr>
<tr>
<td>TWAS</td>
<td>Thickened Waste Activated Sludge</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile Fatty Acids</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile Solid</td>
</tr>
<tr>
<td>WAS</td>
<td>Waste Activated Sludge</td>
</tr>
<tr>
<td>$\mu$m$_{p-p}$</td>
<td>Micrometer peak to peak</td>
</tr>
</tbody>
</table>
Chapter 1 – Introduction

This chapter presents the motivators that inspired this study, explains the objectives, and explains the bases of the procedures followed.

1.1 Statement of the Problem

In order to meet today’s waste management requirements, anaerobic digestion (AD) is a well-known and beneficial solution in the suite of waste disposal options. This biological method is used for sludge stabilization, but offers several other possibilities including the following: reduction of the quantity of sludge to be disposed, destruction of many pollutants, efficient odour reduction (particularly for hydrogen sulfide), production of high quality biosolids for land application, and production of methane gas energy (Stamatelatou et al., 2011).

While popular, using AD for biomass and residue management has a number of gaps. Since the anaerobic biological reaction is so slow, its viability becomes questionable and hence institutions hesitate to invest in any form of this potentially sustainable process. To offset this reluctance, recent investments in research and subsidies for AD from the Ontario Ministry of Agriculture, Food and Rural Affair (OMAFRA) were undertaken. The difficulty lies in the cell membrane. Biomass is recalcitrant to biodegradation (Pelczar et al., 1993), meaning that digestion and cell lysis necessitates a long retention time, in the range of 30 – 60 days, for biological treatment (Metcalf and Eddy, 2003). Treating the substrate before it enters the anaerobic reactor means encouraging faster digestion as a result of the break-down of the cell walls and bacterial membranes in order to make their content substance directly available for bacterial digestion.

Potential improvements for anaerobic digestion processes have been available for more than fifteen years. It typically consists of treating the substrate with ultrasonic waves to break down the cell wall before entering the reactor (Behrend at al., 2000;
Khanal et al., 2007; Tiehm et al., 1997). Even more improvement is needed because of issues of revenue generation and gas recuperation. This study examines how different ultrasonicated substrates with low energy input influence the availability of energy cost recovery.

1.2 Objectives of Study

The aim of this study is to suggest ways to deal with the future realities of waste management by enhancing anaerobic digestion through ultrasonic pretreatment. The processes for treating sludge from wastewater treatment is also applicable for animal manure, other waste from slaughterhouses, biomass residues from the food industry and similar residues from industrial processes. The principal objectives of this study are as follows:

- Verify the methane yield and digestibility ratio for some substrate treated initially with ultrasound.
- Calculate a Net Energy Balance and Net Economy Balance when an energetic ultrasonic pretreatment is used.

Otherwise, more general and collateral consequences will be to expand the knowledge and comprehension associated with ultrasonic pretreatment and AD. Producing exergy in greater amounts and shrinking the processing times for residue reduction forms the hypothesis of the approach. It will provide a better degree of degradation of organic matter than the normal variation of between 25% and 60% (Grönroos et al., 2005). This lack of knowledge concerning application of ultrasound radiation to substrates other than wastewater sludge is a prime motivator of this research.
1.3 Scope of Study

To reach the outlined goals, the study will focus on the substrate characteristics and the specific energy of the ultrasonic wave. The substrates studied are the following:

- Fat, oil, and grease (FOG)
- Paper sludge
- Ground switch grass
- Ground hay
- Ground wheat straw
- Cut wheat straw

The choice of these substrates was made as a function of their availability as waste, their methane production potential, the difficulty in managing them in the AD process and the information available about them in the literature.

The results of a review of the literature indicate a better efficiency of the ultrasonication process had been estimated with wave amplitude of 90 μm (Wu-Hann et al., 2010), lower frequency (Lorimer and Mason, 1987), specific energy around 1500 kJ/kg TS (Bougrier et al., 2005; Rai et al., 2004; Salsabil et al., 2009), ultrasonic density of 1000 W/L (Chu et al., 2001; Závacký et al., 2010), and ultrasonic intensity of 50 W/cm² (Castro and Capote, 2007). This ultrasonication case is mainly used throughout the current study. Most of the available research on ultrasonic pretreatment requires high levels of energy input. A minimum energy input of around 1000 kJ/kg TS for breaking the cell wall has been found (Bougrier et al., 2005, Wu-Haan et al., 2010). This level provides a logical starting place to accomplish the goal of ultrasonic degradation, and suggest that positive energy recovery using these lower energy levels has greater potential of achievement. For this research on ultrasonication pretreatment, the ultrasonication treatments were performed in a batch mode for practical purposes. Figure 1.1 is the schematic procedure of the study. Figure 3.1 is a more precise complement to Figure 1.1.
Figure 1.1 - Simplistic summary of experimental procedure.
Chapter 2 - Literature Review

This chapter reviews studies related to pretreatments: more precisely the physic mechanisms involved with ultrasonic pretreatment. The ultrasonic degradability evaluation, the factors affecting the efficiency of ultrasonic degradation, and the effect of the ultrasound utilization on substrates for biogas production are also reviewed.

2.1 Pretreatment in Anaerobic Digestion

Essentially, the aim of pretreatments is to enhance the net energy gain that anaerobic digestion partially fulfills by enhancing VS destruction during digestion. Most of pretreatments work by degradation by breaking down the organic cell membranes. Indeed, pretreatments speed up the rate-limiting step of enzymatic hydrolysis of solid matters (Foladori et al., 2010). By disintegrating the solid matter, the assumption is a shorter hydrolysis period with more accessible nutrient for the methanogenic bacteria to consume. This basically means a higher biogas production in a shorter retention time. To achieve this goal, pretreatments will be managed through the techniques, the substrates and the energy applied.

Many techniques as been studied in the last quarter century, but most of them can be organized into 3 groups: physicochemical, biological and mechanical degradation. Table 2.1 summarizes elements of these techniques.

<table>
<thead>
<tr>
<th>Table 2.1 – Summary of AD pretreatments.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Technique</strong></td>
</tr>
<tr>
<td>Physicochemical: Acid or Base Addition</td>
</tr>
</tbody>
</table>
Chemical Oxidation (O$_2$, O$_3$, H$_2$O$_2$, OH, HO$_2$)  
CH$_4$ production may be retarded by more than 10 days, since the system first needs to re-adapt itself to the substrate, which, after the ozonation, contains more toxic substance (e.g. formaldehyde). (Deublein and Steinhauser, 2011).

<table>
<thead>
<tr>
<th>Process</th>
<th>Time</th>
<th>Temperature</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal:</td>
<td>160-200°C for 0.5-2 hour</td>
<td>A part of the temperature may be assured by Combine and Heat Power (CHP) unit and heat exchanger.</td>
<td></td>
</tr>
<tr>
<td>Biological:</td>
<td>Enzyme or Fungus Addition</td>
<td>Low energy requirement.</td>
<td></td>
</tr>
<tr>
<td>Mechanical:</td>
<td>Agitator Ball Mills / Rotor-Stator Degradation</td>
<td>Most of the milling methods have high energy demands (Taherzadeh and Karimi, 2008).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High Pressure Homogeniser (HPH)</td>
<td>Multiple passages of the high pressurized substrate through a valve where take place the degradation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ultrasound</td>
<td>Not hazardous for environment.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microwave</td>
<td>Microwave heats the sludge in a faster way instead of the conventional thermal methods.</td>
<td></td>
</tr>
</tbody>
</table>

In addition to the above, co-pretreatments are possible and have also been tried. The most famous blend is between thermal and chemical treatment. One is called APTMP (alkaline peroxide-thermo-mechanical pulping). Another is known as the CTMP (chemo-thermo-mechanical pulping) process. Although these methods are different, their aim remains to make available the hardly digestible matter to bacteria, which leads to solubilisation of the solid matter and subsequently to an increase in the BOD$_5$. All these techniques can increase these last goals but their efficiencies are not always the same.
Most of the researches accomplished on AD pretreatment focused on wastewater sludge substrate. That is due to the high global volume produced to be treated and the concerns related to this waste. The other few articles in relation of AD pretreatment address maize silage, corncob, ethanol by-product of maize, and other maize products. This is due to the high content of starch in the maize. This substance plays an important role in the production on methane (Deublein and Steinhauser, 2011). Regarding the exergy production and the energy used by the pretreatment, most of the research does not take into consideration the crucial positive balance. Specifically this means that, most of the time, studies do not measure the energy balance or that the pretreatment uses more energy than the extra methane energy recovered by the pretreatment by itself.

Pretreatments find their goals by reducing the digestion time with a positive net energy balance (NEnB) process. The treatment times for some methods such as alkalinity or ozone treatment normally amount to several hours, and in some cases even more than 24 h before the effect can be seen (Weemaes et al., 2000). In the case of ultrasonic pretreatment, the wait is on the order of seconds to minutes. Also, among these processes, the employment of physical treatments like ultrasound exhibits the benefit of not being hazardous to the environment (Bien and Wolny, 1997).

2.2 Ultrasonic Pretreatment

For the precise case of ultrasonic pretreatment, the ultrasonic vibrations waves are the primary cause of the degradation. Ultrasonication is an emerging and effective mechanical pretreatment method to enhance biodegradability of the sludge (Pilli et al., 2011) or other substrate. Ultrasonic degradation is a well-known method for the break-up of the microbial cells to extract intracellular material (Harrison, 1991). Ultrasonic sounds are widely accepted to be a sound which the frequency is above the human audible range; higher than 20 kHz. Despite this fact, unpleasant lower frequencies are often audible during the procedure.
2.2.1 Mechanism of ultrasonic degradation

Ultrasonic mechanisms are still not fully understood but several researches show evidences that cavitation is the crucial phenomenon that releases the intracellular matter to the aqueous phase which reduces the particles size (Behrend et al., 2000). Several other possible degradation mechanisms have been observed under the influence of the ultrasonic waves; acoustic streaming (Spengler and Christensen, 2003), capillary waves (Behrend et al., 2000), heat (Chu et al., 2001), and oxidising effect (Wang et al., 2005). According to Behrend et al. (2000), cavitation is the most important degradation mechanism and therefore, it will be the only phenomenon discussed.

Cavitation

Sound, including ultrasound, is transmitted through any physical medium by waves that compress and stretch the molecular spacing through which it passes. As the ultrasound waves cross the medium, the average distance between the molecules will vary as they oscillate about their mean position (Capelo-Martínez, 2009) and thus create voids. Those voids are the so-called cavitation bubbles (Mason and Lorimer, 1989). Thus, when a standing wave field is propagated into a liquid at high intensities, alternative high-pressure (compression) and low-pressure (rarefaction) cycles are generated. During the low pressure cycle, the diluted gases reach the vapour pressure and, thus, create small vacuum bubbles. When the bubbles attain a volume at which they can no longer keep the balance between the pressure and the viscosity forces, they implode violently during the high-pressure phase (Hielsher, 2005). Cavitation implosion produces intense local heating (≈ 5000 K), high pressure (≈ 100 atm), high heating and cooling rates (>10⁹ K/sec), and a liquid jet stream (≈ 400 km/h) (Suslick, 1998). This is why ultrasonic lysis, whereby the low-pressure and high-pressure cycles of cavitation bubbles break up the neighboring cell membrane (Wu-Haan et al, 2010), is the most interesting process for enhancement of methane generation (Wang et al., 1999).

The cavitation implosion leads to an acceleration in the hydrolysis stage. A faster hydrolysis stage leads to a reduced global retention time (Nah et al., 2000), which also provides the opportunity for more compact facility layout. Hydro-mechanical shear
forces produced by ultrasonic cavitation are predominantly responsible for sludge degradation. All these details tend to explain why cavitation is the principal degradation mechanism. To illustrate this, Behrend et al. (2000) propose the graph in Figure 2.1.

![Figure 2.1](image.png)

**Figure 2.1 – Correlation of cavitation threshold, pressure amplitude, cohesive pressure and hydrostatic pressure in an ultrasonicated liquid (schematic) (Behrend et al., 2000).**

In this figure, $\hat{p}_{th}$ corresponds to the threshold pressure, $p_\infty$ is the cohesive pressure and $p_0$ is the hydrostatic pressure. Here, $p_\infty$ is linked with the saturation vapour pressure (which is linked with temperature), viscosity and surface tension of the media. Hence, to produce cavitation bubbles, $\hat{p}_{th}$ has to be higher than $p_\infty$.

A certain power needs to be reached to allow the collapse of the bubbles. However, other studies demonstrated that not every cavitation bubble causes matter degradation. Actually, it can be assumed that there are two different classes of cavitations: stable or transient. Stable bubbles are formed at low ultrasonic intensities (1-3 W/cm$^2$) and oscillate around some equilibrium size for many acoustic cycles. In the second class, transient cavitation bubbles are formed using sound intensities in excess of 10 W/cm$^2$ (Castro et al., 2007). With ultrasonication, transient cavitation bubble reactions caused the desired degradations. Figure 2.2 presents the cavitation creation model from Capelo-Martínez (2009):
Interestingly, all the processes (pressure formation, bubbles formation, etc.) lag behind the sound wave. The growth of bubbles requires a finite amount of time to occur. This time of bubble growth has been proposed by Abramov (1998) as:

$$\tau_g = 0.75T + (i - 1)T$$

Here, \( \tau_g \) is the bubble growth time, \( i \) is the number of acoustic cycles that the bubble experienced, and \( T \) is the period. Regarding the case of a stable bubble, if it is assumed that the number of acoustic cycles \( (i) \) experienced by the bubble is equal to 5000; with the common case of a 20 kHz wave, this stable bubble should exist for around 0.25 s. For the case of a transient bubble, with an \( i \) of 10 cycles before collapsing, the bubble growth time is about 0.0005 s. Still according to Abramov (1998), the duration of collapse can be expressed as:
\[ \tau_c = 0.915 R_{\text{max}} \sqrt{\frac{\rho_l}{P_0}} \]

Here, \( \tau_c \) is the duration of a collapse, \( R_{\text{max}} \) is the maximum or resonant \((Rr)\) radius, \( \rho_l \) is the density of the liquid, and \( P_0 \) is the pressure of the system. To explain the resonant cavitation bubble radius, Tiehm (2001) determined it to be a function of the ultrasound. The next equation is the relation between \( \rho \), the density of water; \( \omega_r \), the resonance angular frequency; \( R_{\text{max}} \), the resonant bubble radius; and finally \( P_o \), the hydrostatic pressure. This relation is only true for pure water and low surface tension:

\[ \rho \omega_r R_{\text{max}}^2 = 3 \gamma P_o \]

Here \( \gamma \) corresponds to the heat released upon a gas compression (Hua and Hoffman, 1997) and varies between 1.66, 1.4 and 1.33 for monoatomic, diatomic and triatomic gases, respectively. In the common case of an air bubble in water at atmospheric pressure (\( \rho = 10^3 \text{ kg/m}^3, \gamma = 1.40, P_0 = 1 \text{ atm} = 10^5 \text{ N/m}^2 \)), the resonant radius of the cavitation bubbles can be estimated as follows (Young, 1989).

\[ R_{\text{max}} \approx \frac{3.28}{f_r} \]

Here \( R_{\text{max}} \) is expressed in millimetres and \( f_r \) is the linear resonance frequency \((\omega_r / 2\pi)\) in kHz, for the common case of 20 kHz. Thus, a 20 kHz frequency would produce, in pure water, a bubble of approximately 0.16 mm in radius before it collapses (Young, 1989). The last formula also brings to light that the lower the ultrasonic frequency is, the bigger the cavitation bubbles are. Moreover, experimental results show that the bigger the cavitation bubble, the more hydromechanic shear forces are experienced and the more efficient are the degradation during collapse. Since the only model that exists applies for pure water, a reasonably valid assumption would be to keep this model for AD systems as water is always part of the ultrasonic pretreatment for AD.
Differences may occur in the number and size of the cavitation bubbles in a sludge media compared to that of a pure water system due to the high solid concentration, higher density, and the presence of dissolved gases. In addition, experimental results have shown a proportional degree of degradation (DD_{COD}) with the logarithm of the bubble radius (Tiehm et al., 2001).

Using the bubble lifetime calculation, the next progression, in common water situations, is a transient bubble time as follows:

\[
\tau_c = 0.915 R_{\text{max}} \sqrt{\frac{p_t}{P_0}} = 0.915 \left( \frac{3.28}{20kHz} \right) \sqrt{\frac{1000 \text{ kg/m}^3}{10^5 \text{ Pa}}} \approx 0.0015 \text{s}
\]

In the current case, the addition of \( \tau_g \) and \( \tau_c \) gives 0.002s (0.0015s + 0.0005s), which is smaller than the growing time of a neighbouring stable cavitation bubble (0.25s). Based on this time difference, it can be hypothesized that transient cavitation would act as a better degradation agent than stable cavitation bubbles since transient bubble implosions are extremely fast and energetic. Stable bubble cavitations are, on the other hand, fully part of the other mechanism of ultrasonic degradation.

Cavitations bubbles will occur only if the acoustic pressure \( p_0 \) is higher than the cavitation threshold pressure \( \hat{p}_{th} \) (Abramov, 1998; Show et al., 2007; Tatake and Pandit, 2002). Table 2.2 shows some values of \( p_0 \) related to the ultrasonic intensity (Mason, 1990). From this data, it is observed that the cavitation phenomenon is not linearly related with the energy required for its production. Thus, cavitation effects probably have more impact on feedstock at low energy input (with a minimum to produce transient bubbles), relative to the consumed energy, than at higher energy input. Cavitation is a complete science by itself and is not fully understood yet. The knowledge presented here is a fraction of the entire available literature. Most of this literature is related to cell extraction for COD increase which leads to larger biogas production. The
cavitation effect is also extensively used in analytical chemistry, sonochemistry, food processing and other homogenization processes.

<table>
<thead>
<tr>
<th>Intensity (W/cm²)</th>
<th>$p_0$ (atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.54</td>
</tr>
<tr>
<td>0.2</td>
<td>0.76</td>
</tr>
<tr>
<td>0.5</td>
<td>1.21</td>
</tr>
<tr>
<td>1.0</td>
<td>1.71</td>
</tr>
<tr>
<td>2.0</td>
<td>2.42</td>
</tr>
<tr>
<td>5.0</td>
<td>3.82</td>
</tr>
<tr>
<td>10.0</td>
<td>5.41</td>
</tr>
<tr>
<td>20.0</td>
<td>7.65</td>
</tr>
<tr>
<td>50.0</td>
<td>12.10</td>
</tr>
<tr>
<td>100.0</td>
<td>17.15</td>
</tr>
</tbody>
</table>
2.2.2 Component of ultrasonic equipment

Ultrasonic probe equipment (Figure 2.3) for cell homogenization has four major components; generator, converter (also called transducer), booster and probe (also called horn or sonotrode). These components have an impact on the treated matter, both individually and collectively.

**Generator and converter**

The principal task of the generator is to transform the grid AC line power to high frequency electrical energy. The generator is connected to the converter which receives the 20 kHz high voltage (if the sonicator is made to produce a frequency of 20 kHz).
Hence, the generator pulses the current through the piezoelectric ceramic/crystal component of the converter to transform this current into vibration. When a piezoelectric material (e.g. quartz, titanate zirconate, barium titanate (BaTiO₃), lead metaniobate (PbNb₂O₆)) with a specific shape is traversed by a current, its dimensions are immediately modified in proportion to the current polarity which then produces measurable amplitude at the end of the converter. This amplitude is, most of the time, manageable by the user interface generator.

The other method to produce ultrasound is by magnetostriction. Here, instead of using a piezoelectric material, the electrical energy is converted to vibration with a ferromagnetic coil attached to a vibrating piece potentially made of nickel and Terfenol-D (Clark, 1979).

**Booster and probe**

![Booster and probe](Qsonica, LLC.)

The roles of the booster and probe are to increase the amplitude of the wave (if desired) and propagate the acoustic wave into the material in contact with the probe. Since the converter motion is generally too low (15-20 μm_p-p_), the amplitude can be modified up to 5-6 times with the booster-probe stack. For this equipment, wave characteristics are only mechanically manageable. The mass, the length, the width and other dimensions of these tools are the only variables that control the amplitude and the
tip’s frequency in contact with the substrate. Both tools can magnify the amplitude through proper mass positioning by reacting as high stiffness springs. The *gain* is the name of the mass ratio between the mass above the nodal plan and the mass below the nodal plan. This nodal plan is defined to be the height in the tool where no motion and no vibration occur. According to Newton’s law, the gain of a booster or a probe can be determined as follows:

\[ F = ma \]

where \( F \) is the force, \( m \) is the mass and \( a \) is the acceleration. By energy conservation from one tip to the other,

\[ F_1 = F_2 \]

Finally,

\[ ma_1 = m_2a_2 \]

\[ \frac{m_1}{m_2} = \frac{a_1}{a_2} \]

A simpler method to calculate the gain of a probe is to divide both tip diameters as follow: \( D/d \), in the case of linear or exponential probe; \( (D/d)^2 \), in the case of stepped probe, where, \( D \) is the upper diameter of the probe, and \( d \) is the bottom diameter of the probe. Otherwise, to keep the original frequency, the length of those vibrating tools has to be in relation with the wavelength transmitted. Either the vibrating part is half the wavelength \((\lambda/2)\) and vibrates at 180° out of the phase from the vibration transmitted, or its length is a full wavelength \((\lambda)\) and it vibrates in phase with the previous vibration part. It is important to remember that wavelength \((\lambda)\) is the opposite of the frequency \((f)\) multiply by the speed of sound \((c)\) as given by:

\[ \lambda = \frac{c}{f} \]
For titanium, the propagation speed is around 5080 m/s. Regarding probes, the size of the tip in contact with the matter is also a power variable or ultrasonic intensity, which will be discussed in Section 2.2.3. The arrangement of the transducer, booster and probe is also called the stack assembly. By using this equipment, heat is generated in the media under ultrasonication. The conversion efficiency of sound energy to thermal energy can be calculated as follows (Feng et al., 2009):

\[ Q_w = m \times C_p \times (T - T_0) \]

\[ Q_u = Pt \]

\[ \eta(\%) = \frac{Q_u}{Q_w} \times 100 \]

where \( T_0 \) is the original temperature, \( T \) is the temperature after ultrasonication, \( Q_w \) is the total heat energy, \( m \) is the mass of water of the solution, \( C_p \) is the heat capacity of the water, \( Q_u \) is the actual energy consumed by the equipment, \( P \) is the ultrasound power, \( t \) is the ultrasonication time, and \( \eta \) is the thermal efficiency.

### 2.2.3 Quantification of energy consumption

Directly controlling the power consumption of the sonicator equipment is not a common way to work. Usually, the desired amplitude is selected, and then the generator provides the necessary power to keep constant amplitude regarding the media viscosity. Thus, instant power consumption increases with the amplitude, the selected booster and probe, and the stiffness of the fluid. Also, according to the first law of thermodynamics, energy conservation always occurs. From there, it can be assumed that a heavy probe will cause higher energy consumption and vice versa. This explains why most of the booster and probe are made of light metals (e.g. aluminum, titanium, vanadium) but some modern ultrasonic probes are made from glass (silica) to avoid metal traces in the solvent.
after ultrasonication. Taking into consideration the last comments, four different power definitions will be defined.

Specific input energy (SE)

The specific input energy is defined as the energy supplied per unit of mass of sludge solid matters. This power is defined as follow (Bougrier et al., 2005):

\[
SE = \frac{P \times t}{TS \times V}
\]

where: 
- \(SE\) = Specific energy in kJ/kg TS
- \(P\) = Ultrasonic power in kW
- \(t\) = Ultrasonication time in second (s)
- \(V\) = Volume of sonicated sludge in litres (l)
- \(TS\) = Total solid concentration in kg/l

Of the many available research studies that give their specific energy used, most of them range between 660 and 108 000 kJ/kg TS on a lab-scale (Bougrier et al., 2005; Salsabil and al., 2009).

Ultrasonic dose

The Ultrasonic dose is defined as the amount of energy supplied per unit of sludge volume (Thiem, 2001):

\[
Ultrasonic\ dose = \frac{P \times t}{V}
\]

where: 
- \(Ultrasonic\ dose\) is in kJ/l
- \(P\) = Ultrasonic power in kW
- \(t\) = Ultrasonication time in second (s)
- \(V\) = Volume of sonicated sludge in litter (l)

This power definition is not really used in the literature as this definition does not take into account the quantity of dry matter which is the essential compound for which
the energy is tailored. The ultrasonic dose, however, remains an essential parameter for a good industrial scale-up.

_Ultrasonic density_  
The ultrasonic density is defined as the power supplied per unit of volume of sludge:  

\[ \text{Ultrasonic density} = \frac{P}{V} \]

where:  
- Ultrasonic density is in kW/L  
- \( P \) = Ultrasonic power in kW  
- \( V \) = Volume of sonicated sludge in litter (l)

The same comment as with ultrasonic dose can be made; this definition does not take into account the TS concentration. Besides, this mathematical definition is not influenced by the duration of the pretreatment. The mean density revealed by the literature is from 50 to 8000 W/L (Grönroos et al., 2005; Nitayavardhana et al., 2008). Chu et al. (2001) has examined the effect of different ultrasonication densities at 20 kHz of frequency and maximum power input of 110 W. At 110 W/L, no degradation has been measured. The minimum ultrasonic density looks to be around 220 W/L. According to the same author, for a smaller floc particle size, the ultrasonic density is more important than time.

_Ultrasonic intensity (I)_  
Related to the probe size, the ultrasonic intensity reflects the power supplied through the tip probe area and can be calculated using the following equation (Neis et al., 2000):

\[ \text{Ultrasonic Intensity} = \frac{P \times t}{A} \]

where  
- Ultrasonic intensity is in W/cm²  
- \( P \) = Ultrasonic power in kW
\[ t = \text{Ultrasonication time in second (s)} \]

\[ A = \text{Surface area in cm}^2 \]

This variable will increase with the amplitude provided by the converter in proportion to the power consume by the generator. To create cavitation, Lorimer (1990) found a threshold intensity value of 0.4 W/cm\(^2\). More recently, Tiehm et al. (2001) found cavitation bubbles at intensity as low as 0.1 W/cm\(^2\). Most of this cavitation produces stable cavitation bubbles which do not really lead to cell degradation. Nevertheless, 10 W/cm\(^2\) are required for transient cavitations bubbles and therefore cause noticeable degradation (see section 2.2.1 - Cavitation).

### 2.3 Ultrasonic Degradability Evaluation

The expected outcome of ultrasonic pretreatment is the cell wall destruction and hence, the release of the intracellular materials into the aqueous phase. This changes the physical, chemical and biological properties of the solution during the ultrasonication process. According to Guwy (2004), anaerobic biodegradability can be determined by the volume of gas produced, by the amount of substrate depletion or by the formation of intermediates and end products by the different micro-organisms groups. Several methods have been implemented in order to assess anaerobic biodegradability and can be classified in two major groups: (1) gasometric methods and (2) substrate analysis consumption and temporary by-product formation methods. These approaches can thus be used more specifically for ultrasonic degradation with the exception that, there will be a need to link the changes in gas formation and temporary by-product formation (or depletion) to the ultrasonic pretreatment.

#### 2.3.1 Gasometric methods

Gasometric methods are the most commonly used approaches to determine the anaerobic biodegradation in the literature (Guwy, 2004). In such methods, two main ideas predominate to measure the gas production. One is to keep a constant volume and
measure the resulting increase in pressure. The second is to keep a constant pressure and measure the increasing volume. For these established volumetric and manometric methods, the principals and relatively affordable equipment required are some syringes, volume displacement devices, manometer and valves.

For liquid displacement methods, carbon dioxide is easily diluted in water which introduces an important volume/pressure error if the biogas is in contact with pure water. Thus, the use of saline barrier (NaCl - 200 g/l and citric acid – 5 g/l, (ISO/DIS 14853, 1997)), of highly acid solution (H2SO4 - 5% (Sponza, 2003)) or alkaline solution (NaOH - 2.5% (Gonzalez-Gil et al., 2002)) can be used instead of pure water to limit the gas expansion. To avoid the CO₂ dilution issue related with the water displacement method, one recognized technique is the one proposed by Owen et al. (1979) which avoids the use of water by using glass syringes. Many gasometric protocols had been proposed in the literature but no one has reached the status of a recognized standard. Today, both volumetric and manometric methodologies have been implemented as automated devices, using optical or electric sensors and pressure transducers to reduce the labour necessity (Rozzi and Remigni, 2004).

Biochemical methane potential (BMP) test

BMP test is the most utilized test related to AD. This simple handlings as well as economical and modest equipment requirement have made the method attractive. On the other hand, the big disadvantage is the long-time of execution ranging between 30-60 days. This assessment essentially provides the biogas yield. Daily gas production can also be measured as the collection of the gas is typically done on a daily basis. This would help to measure the best retention time of the specific substrate. The general accepted gazometric method has been developed by Owen et al. (1979) which is based on the earlier work of Miller and Wolin (1974). The authors described an incubated serum bottle method in which the biogas produced is measured by a lubricated syringe placed horizontally. The method uses a 5–50 cm³ glass syringe, flushed with a 30:70 mixture of CO₂ and N₂ gas, connected to the serum bottle via a three way valve for gas collection.
The biogas produced is measured by allowing the syringe plunger to move and equilibrate between the pressure in the serum bottle and atmospheric pressure at the incubation temperature.

The variable with the largest uncertainty involved with this technique is the inoculum:substrate ratio (ISR) used for the preparation of the BMP bottles. This variable is not well documented by the users of this technique which may explain some of the conflicting data reported in the literature to the effectiveness of various pretreatment by underestimating or overestimating the biodegradability of the biomass. The inoculums amount and fermentation time can significantly affect the methane yield. It is important that the effects of ISR and fermentation time are addressed whenever studies are undertaken to determine the methane yield from complex organic substrate such as biomass to ensure that accurate results are obtained (Hashimoto, 1989).

2.3.2 Substrate analysis consumption and temporary by-product formation

Others methods of substrate analysis try to link the biogas production to non-specific parameters such as chemical oxygen demand (COD), biochemical oxygen demand for day 1 to day 5 (BOD₅), total organic carbon (TOC), dissolved organic carbon (DOC), oxygen uptake rate (OUR), protein measurements (Lowry et al., 1951), carbohydrate (Dubois et al., 1956), NH₃ measurement, or any other biomass indication found in the substrate. In order to develop quicker anaerobic biodegradability assessments, these parameters have been linked to biogas production. Since the temporary by-product formation (or biomass depletion) occurs before the methanogenesis, it is assumed that the biodegradability can be estimated faster than by the use of gasometric method. On the other hand, the equipment required for substrate analysis and temporary by-product formation are a lot more expensive and elaborate (e.g. gas chromatography, mass spectrometry, etc.).
Biochemical acidogenic potential (BAP) test

One of the principal methods for the measurement of a temporary by-product formation is the BAP test. Instead of waiting the 30, 40 and sometimes 50 days required for the almost total biogas formation during the biochemical methane potential (BMP) test, BAP test last between 6 and 10 days and measure the volatile fatty acid (VFA) production during acidogenesis. Since a correlation between acid formation and methane formation is known, this gasometric method can now be used as anaerobic biodegradability estimation (Kianmehr, 2010).

BOD, COD, TOC, OUR and SOUR assessment

Originally, this collective of tests were developed to measure the “life” concentration in so-called clear and clean water. In the specific case of biochemical (or biological) oxygen demand (BOD), the measurement represents the amount of dissolved oxygen (DO) consumed by microorganism for the biochemical oxidation of organic matter and inorganic matter. BOD tests measure the oxygen consumption in actual biological break down of a biomass sample. The accepted standard is BOD₅ which is the change in dissolved oxygen between the first day and the fifth day at an incubation temperature of 20°C. The chemical oxygen demand (COD) is also an index of the amount of organic material but this test uses a chemical oxidizing agent to measure all the oxygen consumption. Thus COD value gives a measure of the total organic content, whether or not it is biodegradable. Therefore, the BOD/COD ratio is a guide to the proportion of the organic materials present which are biodegradable (Bitton, 1994). The presence of organic carbon that does not respond to either BOD or COD test makes them suitable for the measurement of TOC. TOC is more convenient and direct expression of total organic content than either BOD or COD, but does not provide the same kind of information (TOC Manual, Shimadzu, Tokyo).

In addition, oxygen uptake rate (OUR) and specific oxygen uptake rate (SOUR) measurements have been used in wastewater treatment plants to monitor biological activity of conventional BOD removal systems. The most common use of these
measurements by plant operators has been to determine if the organisms are alive/viable. These test represents the oxygen consumption rate (mg of oxygen / g VSS (or TS) / hour) and are based on the same theories as that for BOD/COD. The advantage of these tests is that they are less time consuming than BOD₅.

2.4 Factors Affecting Efficiency of Ultrasonic Degradation

Since cavitation is the main mechanism of ultrasonic degradation, this section explains the factors influencing the cavitation phenomenon and degradation factors.

Sludge characteristics

Similar to the case of anaerobic digestion, the nature of the residues used is a variable that influence the reaction faced with ultrasonic waves. Some substrates will be easier than others to release their internal matter in aqueous phase. As for common AD, organic matter is more and more difficult to be degraded according to the row: sugar – protein – fat – hemi-cellulose – cellulose – lignin (Deublein and Steinhäuser, 2011).

Also, cavitation bubbles can be more intense by using a solvent with low viscosity, low surface tension and high vapour pressure. These last liquid characteristics mean low natural cohesive force acting within the fluid (Thompson and Doraiswamy, 1999). But since normal AD process has water as imposed solvent, this way of enhancing the process is not to undertake.

Frequency of the field waves

The higher is the frequency, the more power is required to keep the same cavitation energy and the better is the net energy balance (NEnB) at low frequency (Lorimer and Mason, 1987). The following table indicate the intensity at an equivalent depth for different frequency (Mason, 1990):
Table 2.3 – Frequency and intensity relationship (Mason, 1990).

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Intensity (W/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>20.0</td>
</tr>
<tr>
<td>1 000</td>
<td>30.7</td>
</tr>
<tr>
<td>20 000</td>
<td>112.0</td>
</tr>
</tbody>
</table>

This behaviour might be explained by the higher frequencies being attenuated more rapidly than lower frequencies (section 2.4 - *Operation of ultrasonic equipment components and attenuation phenomenon*). The other theory behind the idea of prioritizing lower frequencies on higher frequencies comes from the period of the wave. Mathematically, a lower frequency generates longer period since:

\[ f = \frac{1}{\tau} \]

where \( f \) is the frequency and \( \tau \) is the period. Therefore, regarding the common case of 20 kHz ultrasound waves, the period is 50 μs, this lead to a rarefaction gas time of 25 μs and finally a potential growing period of 12.5 μs. The increase in frequency lowers the odds of a cavitation bubble formation. A longer growing period helps reach the estimated growing time (\( \tau_g \)) for effective cavitation (section 2.2.1 - *cavitation*).

However, higher frequencies may actually increase the number of free radicals in the system because, although cavitation is less violent, there are more cavitational events and thus more opportunities for free radicals to be produced (Crum, 1995). In addition, the shortened bubble lifetime may increase the amount of free radicals which are able to escape from the cavitation site to the bulk mixture, where they facilitate the bulk reaction. It is contended that the optimum frequency is system specific and depends on whether intense temperatures and pressures are required (thus enhanced by lower frequencies) or if the rate of single electron transfer is more important (enhanced by higher frequencies) (Thompson and Doraiswamy, 1999). For AD, oxidising effects is surely not a
determining effect. For most of the biogas applications, lower frequencies (20-30 kHz) are usually chosen because of the reasons previously explained.

Energy input and ultrasonication time

As discussed in section 2.2.3, ultrasonication energy can be classified in 4 groups; specific energy (kJ/kg TS), ultrasonic dose (kJ/L), ultrasonic density (W/L) and ultrasonic intensity (W/cm²). These mathematical definitions refer to the amplitude of the wave, ultrasonication time, stack assembly, and matter content managed through the electrical consumption. More precisely, intensity is the variable most linked to the amplitude of the transmitted wave. Furthermore, amplitude is known as the variable who manages the sonochemical effect. High amplitudes lead to high ultrasonication intensities. To achieve the cavitation threshold, a minimum intensity is required (section 2.2.1 - cavitation). In many cases, the cavitation threshold is related to transient bubbles creation in a specific substrate. The existence of the cavitation threshold also suggests that higher amplitudes are not always necessary to obtain better results. A general rule is to increase the amplitude when working with a sample of higher viscosity, such as blood. The higher the viscosity, the more the resistance of the sample to the ultrasonic movement occurs (Capelo-Martínez, 2009). On the other hand, too high intensity will first lead to large bubble, where many of which coalesce into even larger, longer-lived bubbles that hinder the transport of acoustic energy through the liquid (Capelo and Capote, 2007). The step after the occurrence of this phenomenon is called decoupling and happens when the probe is no longer able to maintain the contact with the liquid throughout the cycle. This phenomenon highly reduces the ultrasonic efficiency.

Regarding the time variable, the normal assumption has been empirically proven many times; the longer is the ultrasonication the better is the degradation. On the other hand, an optimized balance can be studied since particle size and degradation rate are lower with higher ultrasonication time. Besides, some results suggest that ultrasonication could be set for less than a minute for energy cost optimisation (Show et al., 2007).
Regarding ultrasonication intensity and ultrasonic density, the same authors also found that with a specific energy of 40 kWh/kg TS and an increase of the SCOD by 1.2 times at a ultrasonication density of 180 W/L, higher SCOD was released by 1.4 and 1.9 times, at 330 and 520 W/L, respectively. This indicated that higher ultrasonication density could exert stronger cavitational forces, obtaining a better disruption when using the same amount of energy. The general agreement in the literature expresses that ultrasonic density is more important than ultrasonication time for efficient sludge degradation (Khanal et al, 2007). This suggests that ultrasonication density is a vital operating parameter to be considered in order to achieve a cost-effective ultrasonication (Show et al., 2007).

*Temperature of sonicated substrate*

The general conclusion in the scientific literature tends to a decrease of the cavitation effect with a temperature increasing. Since sonicator equipment does not have a perfect energy transmission, and heat is also generated by other mechanical cavitations frictions, bubbles energy collapsing diminish with ultrasonication time. This behavior can be partially explained by the natural characteristics of the gas has saturated vapour pressure increase with temperature. This leads to an easier bubbles formation due to the decreasing cavitation threshold. Cavitations bubbles will be more present but their own collapsing should be less energetic. Besides, the cavitations bubbles formed will contain more solvent vapor with higher temperature. This diluted vapour act as cushion for the implosion and thus use the enthalpy generation from this same implosion to condensate into the liquid phase (Thompson and Doraiswamy, 1999) (section 2.4 - *Gas concentration and particulate matter traces*). If the solvent reaches its boiling point, a larger number of cavitation bubbles is produced, which acts as barrier to sound transmission and nullify the efficiency of ultrasound energy (Moholkar et al., 1996). According to Mason (1990), the temperature at which cavitation in pure water reaches maximum intensity is 35°C. Otherwise, the temperature increase rate is almost proportional to the increase in ultrasonic density; 110, 220, 330 and 440 W/L generates
temperature increase rates by 0.15, 0.28, 0.43 and 0.51°C/s, respectively (Chu et al., 2001).

*Operation of ultrasonic equipment components and attenuation phenomenon*

The ultrasonic equipment also plays a role for a good cavitation/degradation. The stack assembly is important in relation to the amplitude of the waves produced. The probe choice will also influence the ultrasonic intensity transmitted to the media. Also, the depth of the probe in the sonicated substrate and the physical containment of this substrate would also have a non-negligible influence. The choice of the substrate container (height:depth ratio, general shape and material) partially controls for a good stirring caused by the acoustic streaming and heat. Also, wear of the probe needs to be taken into account. Even with normal usage, the tip of the probe tends to erode, to lose some metal particles which lead in irregular surfaces and hence a bad acoustic transmission. This wear also causes a variation in the system frequency which can lead to generator failure.

Otherwise, the acoustic field has limitation regarding is field transmission. Ultrasound intensity varies with distance, from its source due to the attenuation promoted by viscous forces, which results in the heating of the liquid. Grönroos et al. (2005) measured the ultrasonic propagation (attenuation) by hydrophone. They agreed that ultrasound efficiency decrease non-linearly with power input in sludge at small distances from the transducer and can be altered with sufficient mixing or flow. As example, Figure 2.12 shows that at 2.3% TS sludge, 400 W power input, the attenuation begins to be felt significantly around 4 cm from the transducer.
External hydrostatic pressure

Theoretically, an increase in the ambient reaction pressure generally results in an overall increase in the sonochemical effect because of the decrease in the vapor pressure of the mixture. Decreasing the vapour pressure increases the intensity of the implosion, thus increasing the ultrasonic energy produced upon cavitation (Thompson and Doraiswamy, 1999).

However, the experimentation demonstrates that this last theory is true until a certain limit of pressure. This is caused by the partial suppression of cavitation which is the main mechanism of power dissipation. A slight increase of the mean droplet size can be observe around $1.5 \times 10^5$ Pa, but seems to also be in relation with flow rate (Behrend and Schubert, 2001). No real clear positive or negative effect correlating this with hydrostatic pressure has been demonstrated yet.

Gas concentration and particle matter traces

Gas concentration and particle matter play an important role since they act as bubble nuclear sites. As gases are removed from the reaction mixture because of the implosion of the cavitation bubbles, initiation of new cavitational events becomes
increasingly difficult. Bubbling gases through the mixture facilitates the production of cavitation bubbles, but the type of gas used is important. As a general rule, a gas with a high specific heat ratio and monoatomic formula gives a greater cavitational effect than a low specific heat ratio and polyatomic gas (Thompson and Doraiswamy, 1999; Castro and Capote, 2007). Since air is the common gas blend linked to substrate degradation for ultrasonic pretreatment with anaerobic digestion, an optimization on this would probably be a mistake. This would increase the complication level of the process.

Generally, presence of gas in the liquid will lower the cavitation threshold and reduce the intensity of the shock waves produced. In simpler words, the gas is acting as a cushion that absorbs a part of the collapsing energy. Otherwise, Behrend and Schubert (2001), concluded their experimentation by saying that gas saturation or partial degassing prior to emulsification lead to a shift in a maximum energy density and less intense cavitation action. When looking at their scattered results, there is no clear effect of the gas content on the droplet disruption process for a constant energy density. On the other hand, Castro and Capote (2007) found that degassing a liquid raises the cavitation threshold which is positive for degradation.

**TS content and particle size**

Logically, a higher amount of solid to treat will decrease the ultrasonic dose and density. Also, different authors have observed that a too high solid concentration diminishes the extraction efficiency. This might be caused by a too low solvent volume to extract and dissolve the compound efficiently under cavitation (Gatidou et al., 2007). Too high TS concentration seems to decrease the cavitation whereas the best TS ultrasonication seems to generally be around 3% (Show et al., 2007). On the other hand, Neis et al. (2000) reported that the efficiency of sludge disruption by ultrasonication increased with solids content. This means that still some misunderstandings are related to solid concentration of sonicated samples. Otherwise, in general, small particle sizes are recommended to increase the solid–liquid interface and thus to increase the extraction.
efficiency. If possible, samples may be ground to a fine powder before extraction (Capelo-Martínez, 2009).

2.5 Effect of Ultrasonicated Substrates on Biogas Production

As previously explained in section 2.1, the first goal of ultrasound pretreatment is to increase the sludge biodegradability in order to enhance the methane production and reduce the retention time. Ultrasonication density, ultrasonication intensity and ultrasonication time are the three most utilized and researched parameters that influence the degradation. When searching the current scientific literature on ultrasonication, using waste activated sludge (WAS) or any other biomass or residues as a substrate, all experiment published have registered an increase of biogas production in AD context. The results of those experiments are fairly hard to compare due the differences in substrate used, power definitions used (power consumption, specific energy, dose, intensity, density, ultrasonication time), evaluations of ultrasound degradation used (gasometric test (BMP, BAP), or other physical, chemical and biological evaluation changes done (particle size analysis distribution, microscopic image evaluation, BOD$_5$, COD, SCOD, DD$_{XXX}$, $\eta$, etc.). Furthermore, many scientific articles do not reveal details on some of those important factors.

In the published literature, the mean biogas production increment is between 15% and 60% (Závacký et al., 2010; Wang et al., 1999). Biogas production is closely correlated with COD and SCOD increases, which is proportional with particle size reduction itself depending on ultrasonication time, intensity (amplitude) and density. Bougrier et al. (2005) found that a minimum of 1000 kJ/kg TS (20 kJ/l) is necessary to break the cell membrane. Under these conditions, the energy is used to reduce flocs size, which also has a positive, but less significant, impact on biogas production. According to their results, it does not seem interesting to have a supplied energy higher of 7000 kJ/kg TS since biogas generation remains constant pass this point. Rai et al. (2004) found a different optimal level of energy supplied, 3000 kJ/kg TS, where the biggest particle size
reduction was observed. Other research concludes that high ultrasound power together with short treatment time is more efficient than low ultrasound power with long treatment (Grönroos et al., 2005). Hearn et al. (2008) found that wave amplitude is more influential in the particle size reduction process than ultrasonication time. Moreover, Neis et al. (2008) proved that ultrasonication of substrate prior to AD did not only increase the biogas production but also made the entire process faster. Another study showed a same VS degradation efficiency in one third the retention time (20 days to 8 days) when using ultrasound (Nickel, 2002). This means that this technology could reduce drastically the reactor size (which is the most expensive part of the AD facility) or increase the power of a biogas plant, and this, with the same substrate to digest. Based on the same idea, instead of reducing the retention time, the organic loading rate (OLR) could be increased in proportional fraction. Tiehm et al. (2001) added that biogas produced by a digester using ultrasonic pretreatment is not only formed in a higher amount but the biogas itself is also more concentrated in methane gas, therefore of higher quality. Hiraoka et al. (1984) observed this same behaviour with thermal sludge degradation. No complete study has been done at this point on this matter.

Otherwise, according to Wu-Hann et al. (2010), important attention should be given to amplitude since their results confirmed that cumulative methane production is proportional with the amplitude used. From their results, the most efficient amplitude seems to be somewhere between 50 and 160 μm_p-p_.

Regarding ultrasonic density, Závacký et al (2010), use in their experiment an energetic efficiency density of 250 W/L. This number is in accordance with the results from Chu et al. (2000) who found that a minimum density of 220 W/l was needed to decrease the particle size, which lead to better biogas production.
2.6 Summary of the Literature Review

This literature review allowed us to notice the fundamental potential of the ultrasonic pretreatment. Also, it does not require any chemicals addition so that this degradation technique is not hazardous for environment. Ultrasonication constitutes an effective mechanical pretreatment method that enhances biodegradability of sludge from various sources. The anaerobic process of co-producing sustainable energy in the form of bio-methane has some gaps. It is as hard to make it a profitable process, which might be come from a too low biogas production rate. The potential benefits that come with pretreating the substrate are a shorter retention time and a higher biogas production which is caused by a higher degradation of the biomass. Therefore, with this upgraded hydrolysis, more nutrients are released more quickly into the aqueous phase for the next steps of AD. This processing opens the door to a better biogas production through nutrients balance and ratio. Since then, a multitude of pretreatment had been developed and all of them have benefits and disadvantages.

Additionally, because many other residues requests to be managed in a proper way and because energy efficiency is more and more crucial in our contemporary world, this research focused on unstudied residues and biomass for a positive ultrasonic net energy balance (NEnB) and net economic balance (NEcB). Regarding the history of the ultrasonic pretreatment, the major efforts have been tailored to waste activated sludge (WAS) coming from waste water treatment plant (WWTP). Also, in most of the cases, the studies did not take into account the NEnB and NEcB which are trivial for industrial scale-up. This study aims to partially fulfill the lack in the scientific literature related to other sonicated residues for a positive NEnB and NEcB.
Chapter 3 – Methodology

This section presents the material and the methods used to assess the original characteristics of the studied samples and their subsequent degradation in absence of oxygen. The mathematical definitions for energy balance and statistical analysis are also explained.

3.1 Samples and Inoculum Collection

The inoculum used was a digester effluent (digested thickened waste activated sludge (TWAS)) obtained from the Guelph Wastewater Treatment Plant (Guelph, Ontario, Canada). The wheat straw and hay were obtained from the Ontario Veterinary College (OVC) large animal clinic at the University of Guelph campus. The hay used, a common forage, represents a medium quality hay as animal food and its composition was equally fractioned between alfalfa, clover, and timothy grasses. Traces of other unknown grasses were also observed. The switch grass was provided by the Crop Science department of the University of Guelph and harvested from the Elora Research Centre fields. The harvested switch grass represents a common and normal switch grass sample. The switch grass specie was Cave in Rock. The paper sludge used was donated by Domptar Papermill in Windsor, Québec. The used paper sludge was a mixture of 50% primary sludge and 50% secondary sludge. Finally, the FOG utilized was spent vegetable oil from the University Centre restaurants from University of Guelph campus.

3.2 Samples Characterization

3.2.1 Total solid (TS) and volatile solid (VS) concentration

In order to determine the TS and VS concentrations, substrate samples were first dried at 103°C for a minimum of 6 hours to obtain their dry solids concentration. The ratio obtained from the difference between the dried and the wet solid masses provided the TS concentration for each feedstock. Next, the dried solids were incinerated at 550°C for a minimum of 3 hours. The residues after incineration represented the inorganic dry solids. The difference between the dry solid and the inorganic dry solids represented the
volatile solids fraction. This method follows the Standard Methods 2540B and 2540E (APHA, 1995) presented in Appendix A.

3.2.2 Lignin, cellulose & hemicellulose determination
The method used to determine lignin, cellulose and hemicellulose concentration is the one developed by Harper and Lynch (1981). In the 3-step gravimetric analysis, lignin was firstly dissolved in a solution of acetic acid and sodium chlorite. The remaining solids were washed with water, acetone and ether and then weighed to determine the cellulose content. Secondly, the solids were soaked in a KOH solution, washed with acetic acid, water, acetone and ether and then weighed to calculate, by mass difference, the fraction of hemicellulose. Finally, the solids remaining were ashed at 550°C and weighed to measure the cellulose fraction. The complete procedure is presented in Appendix B.

3.2.3 Grinding
This research also aimed to determine the impact of particle size both on ultrasonication and digestion. Hence, wheat straw was studied in both states: ground and cut. Hay and switch grass were only studied in a ground state. A screen was used during the grinding process to provide a particle size of 1 mm or less while the cutting process was executed with scissors to have a particle size that was roughly between 1 and 4 cm. The grinder used for all grinding processes was made by IKA (model: MF 10 basic). Importantly, even though this procedure is a mechanical pretreatment for AD, the energy consumed by the grinder was not taken into account for the energy balance analysis.

3.2.4 Calorific value
The calorific value defined as the maximum available energy in a biomass sample was determined using an IKA C200 bomb calorimeter. After calibration with benzoic acid (26.460 MJ/kg), known masses of dry wheat straw, hay, switch grass, paper sludge, and FOG were burned in the bomb. Since the amount of water in which the bomb was immersed was known and since the heat capacity of water is known, the water
temperature increase was used to estimate the calorific value of the tested samples. The complete procedure for assessment of calorific value is presented in Appendix C.

3.3 Ultrasonic Pretreatment

In order to improve uniformity of the samples, to facilitate ultrasonic processing, and to improve potential degradation, wheat straw, hay and switch grass samples were diluted to approximately 2% TS. Paper sludge and FOG were also diluted in deionised water but to a concentration of 2.3% and 3% TS, respectively. The calculations for the preparation of those solutions are given in Appendix J.

The ultrasonic processor utilized was from Qsonica, model Q500 (Qsonica, Newton, Connecticut). This equipment can deliver a maximum power of 500 Watts at a frequency of 20 kHz. The stack equipment of ultrasonic processor used had a 3-16 $\mu$m$_{p-p}$ converter, a 3:1 gain booster and a 2:1 gain probe of 2.54 cm (1 in) diameter. With this equipment, the amplitude could thus be modulated from 6 to 90 $\mu$m$_{p-p}$.

The ultrasonication chamber used for batch ultrasonication was a common borosilicate 500 ml glass beaker. The lowest 3 cm of the probe were immersed in the solution. This depth was enough to avoid air introduction and scum formation in the media (according to sounds produced and visual observations) which would reduce the acoustic transmission and therefore the ultrasonication efficiency. Also, this depth was shallow enough to allow the entire sample to be mixed by acoustic streaming and cavitation. The diameter of the beaker (approximately 13 cm) allowed the half wave length (around 12.7 cm at 20 kHz) to be fully created in this container. The diameter of the beaker was selected to avoid the introduction of unwanted wall effects.

Table 3.1 shows the characteristics of the ultrasonication treatment that was performed for each substrate studied. In every case, a maximum power corresponding to 90 $\mu$m was applied. Only the ultrasonication time varied as a function of the composition.
of the substrate. A ultrasonication time of 40 seconds was used for cellulosic matter (e.g. hay, wheat straw, and switch grass). This time was chosen to achieve a specific energy supplied around 2000 kJ/kg TS, which is higher than the energy required to breakdown cells (1000 kJ/kg TS, Bougrier et al., 2005), and low enough to increase the chance of a positive net exergy creation.

For paper sludge and FOG, the ultrasonication treatment causes an emulsification of the feedstock into water and hence the selected ultrasonication time was determined by the time required to destroy any viewable floc and to suspend all the solids in the water phase. To accomplish this, intervals of 20 and 5 seconds were chosen for paper sludge and FOG, respectively. The ultrasonic procedure is detailed in Appendix D.

<table>
<thead>
<tr>
<th>Table 3.1 – Ultrasonication treatment performed on each feedstock.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground Wheat Straw (1 mm)</td>
</tr>
<tr>
<td>Amplitude [μm]</td>
</tr>
<tr>
<td>Ultrasonication Time [s]</td>
</tr>
<tr>
<td>Power [W]</td>
</tr>
<tr>
<td>Total Energy [J]</td>
</tr>
<tr>
<td>Specific Energy [kJ/kg TS]</td>
</tr>
<tr>
<td>Ultrasonic dose [kJ/L]</td>
</tr>
<tr>
<td>Ultrasonic density [W/L]</td>
</tr>
<tr>
<td>Ultrasonic intensity [W/cm²]</td>
</tr>
</tbody>
</table>
3.4 Degradation Assessment

3.4.1 Total organic carbon (TOC)

TOC was measured using a total organic carbon analyzer (TOC-V CSN/CSH, Shimadzu Corporation, Kyoto, Japan). The oven temperature was set to 720 °C and the internal gas flow to 150 ml/min at a pressure of 200 kPa. All NPOC values were derived from 4 measurements out of the 6 taken with the two most extreme measurements rejected based on their variance by the TOC analyzer. The mean and the confidence intervals (95%) were thus based on four measurements. Regarding the quality control of the measurements, blank, laboratory fortified blank, procedural blank, and repeatability measurements were completed and the associated graphs are available in Appendix L.

In order to determine the quantity of organically bound carbon, the organic molecules must be broken down to single carbon units and converted into a single molecular form that can be measured quantitatively. TOC methods utilize heat and oxygen to convert organic carbon to CO₂. The CO₂ is measured by a non-dispersive infrared (NDIR) analyzer. The complete method for TOC measurements is presented in Appendix E and refers to Standard Methods 5310B (APHA, 1995).

Solutions preparation

Prior to ultrasonication, feedstock solutions were prepared on a total solid (TS) basis. The solution concentrations and details are given in Table 3.2.

<table>
<thead>
<tr>
<th>Solution preparation details.</th>
<th>Straw</th>
<th>Hay</th>
<th>Paper Sludge</th>
<th>Switch Grass</th>
<th>FOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of water [ml]</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Mass of wet solid [g]</td>
<td>6.63</td>
<td>6.70</td>
<td>33.55</td>
<td>7.06</td>
<td>27.05</td>
</tr>
<tr>
<td>Solution [m/m %]</td>
<td>2% TS</td>
<td>2% TS</td>
<td>3% TS</td>
<td>2% TS</td>
<td>10% TS</td>
</tr>
</tbody>
</table>
Manipulations

A- Particle size effect

A1 - Cut
One half of each solution was sonicated and the other half remained unsonicated. For the sonicated solutions, ultrasonication was performed immediately after the solution preparation. NPOC measurements were also done a few seconds following the ultrasonication. Particle sizes remained unchanged for the FOG and paper sludge feedstock and were cut to 1 to 4 cm for plants origin feedstock size.

A2 - Ground to 1 mm
Similarly to manipulation A1, one half of each solution was sonicated and the other half was unsonicated for manipulation A2. Ultrasonication was performed immediately after the solutions preparation and NPOC measurement is also done a few seconds following the ultrasonication. For wheat straw, hay, and switch grass, the mean particle size was reduced to 1mm.

B- Effect of soaking time (no grinding)

B1 - Action order: 6h of soaking - no ultrasonication - NPOC measurements
Solutions settled during 6 hours at ambient temperature, with constant air contact, before NPOC measurements. NPOC measurements were taken after soaking time.

B1 - Action order: 6h of soaking - ultrasonication - NPOC measurements
Prior to ultrasonication, feedstock solutions settled during 6 hours at ambient temperature, with constant air contact. NPOC measurements were performed immediately after the ultrasonication.

B2 - Action order: 6h of soaking - no ultrasonication - 6h of soaking - NPOC measurements
Solutions settled during 12 hours at ambient temperature, with constant air contact, before NPOC measurements. NPOC measurements were taken after soaking time.

B2 - Action order: 6h of soaking - ultrasonication - 6h of soaking - NPOC measurements

Solutions settled during 6 hours at ambient temperature, with constant air contact, then sonicated, followed by a second 6 hours settling period, and finally analyzed for NPOC measurements.

N.B: In all the cases where ultrasonication was not involved in the manipulation, the unsonicated period was replaced by a strong manual stirring period of 40 seconds.

**Statistics**

Standard deviations calculated by the TOC analyzer based on the four measurements were relatively small due to the precision of the carbon analyzer used. The total experimental standard deviation was estimated and varied between 2.0% and 4.5%. These variations included the standard deviation of the TOC analyzer, the measured machine repeatability, and the bias error of the machine. Differences of less than 5% were assumed to be non-significant.

**3.4.2 BMP assays**

BMP assays were performed as per Owen et al. (1979), with slight modifications that included using Wheaton 500 mL bottles with rubber seals and moulded aluminum caps.

Each bottle was filled with 50 mL of inoculums (digested TWAS), 50 mL of nutrient solution, and between 0.5 and 2 grams of raw feedstock sample with the goal of having a maximum of 0.5 g VS to digest. This mass constraint was used to avoid acidification of the media and thus the death of bacteria in the bottles. Since the ultrasonication process required a feedstock/water solution, the sonicated feedstock
characteristics needed to be analyzed for TS after ultrasonication to put a known mass of VS in sonicated BMP bottles. To achieve this, the solid phase was separated from the sonicated water after the ultrasonication of the cellulosic feedstock, then filtered with a Whatman filter (#541) using a vacuum pump (-10 kPa) for 1 minute and finally weighed before being added to the BMP bottles. Obviously, the TS solid content of the sonicated feedstocks was different than the unsonicated one due to the recent intimate contact with water. Hence, TS was also determined for this watery sonicated feedstock. To better represent a real life situation, in addition to the sonicated solid fraction put in the bottles, a sonicated water fraction was added in the same proportion as in the original sonicated solution concentration.

For the paper sludge and FOG, a perfect emulsification was assumed in order to put a similar amount of feedstock in the sonicated bottles as the unsonicated ones. Each calculation regarding the content of every BMP assays is explained in Appendix G.

After the inoculums, Owen’s nutrient solution (Owen et al., 1979), and feedstock to digest were added to the bottles, they were filled with deionised water up to 250 mL. The Owen’s nutrient solution includes resazurin compound that change the color of the solution when oxidized by oxygen. This particularity of the method is part of the quality control of the test. They were then purged of oxygen using 30% CO$_2$ and 70% N$_2$ gas for 3 minutes prior to being sealed with a rubber seal and an aluminum cap and then placed into an incubator at 36±1 °C. Biogas production was measured using a 60 mL glass syringe every time the biogas production was estimated to be close to the maximum capacity of the syringe. The biogas was collected as long as a significant amount of biogas was produced from the substrate under digestion. Total collection period varied between 30 and 50 days, depending on the feedstock. The bottles were inverted and gently shaken before every collection of biogas. All assays were replicated in triplicate. Each set of trials was run in parallel with three blank assays. The blank assays contained Owen’s nutrient solution and inoculum, but no feedstock sample, and were used to
determine the amount of biogas supplied by the inoculum alone. The complete BMP procedure is presented in Appendix F.

3.5 Biogas Composition Analysis

The biogas produced by wheat straw (sonicated and cut; unsonicated and cut; sonicated and ground; unsonicated and ground) was analyzed for CH$_4$ and CO$_2$ proportions using gas chromatography (Agilent technologies; GC – TCD model 6890N). After calibration and standards establishment, biogas from each biogas collection was injected to follow the evolution of CH$_4$/CO$_2$ production trend across the duration of the AD experiment. The biogas analysis procedure, calibration curves and standards are presented in Appendix H.

3.6 Digestibility Ratio

The digestibility ratio is a measure of the energy of the digestion process compared to the total energy available in the feedstock as defined by:

$$\text{Digestibility ratio [\%]} = \left( \frac{P \ [\text{mL}] \times Q \times C \ [\text{J/mL}]}{m \ [\text{g}] \times HHV \ [\text{J/g}]} \right) \times 100$$

where $P$ is the net biogas production, $Q$ is the quality (fraction of CH$_4$) of the biogas, $C$ is the energy content of pure methane (38.2 J/ml), $m$ is the TS mass of feedstock used for digestion, and $HHV$ is the high heating value of the feedstock.

3.7 Net Energy and Economy Balance

With most of the feedstock studied, ultrasonication causes a higher biogas production at a certain time, and thus a higher biogas yield. It is uncertain, however, if the surplus energy production compensates for the energy cost of the ultrasonication?
The answer to this question would justify the use or the avoidance of the ultrasonic pretreatment in biogas production. It is a question of positive or negative net energy production.

For the Net Energy Balance (NEnB) and Net Economy Balance (NEcB) calculations, the first information needed is the biogas production surplus. Since CO₂ does not contribute to the flame temperature, the biogas surplus needs to be multiplied by the biogas quality. The average of the quality of the biogas was assumed at 60% CH₄ for all studied feedstock (based on experiments to be discussed in Chapter 4). Since the energy content of pure methane is approximately 38.2 MJ/m³ (STP), the energy surplus produced can be calculated. From the energy surplus value, the energy used for the ultrasonic treatment was subtracted. The efficiency of the ultrasonic equipment was also studied as explained in Section 2.2.2. Roughly, by using the sonicator for 40 seconds at maximum amplitude in a 300 mL water-feedstock solution, a temperature increase of 7.8 °C was measured. This represents an energy output of 9700 J which is 80% of the electricity used by the sonicator (if only water is taken into account in the calculation). The 12500 J used by the sonicator was transferred in kinetic and potential energy in the stack assembly, thereby causing cavitation and intense mixing with the water, leading to a temperature increase of the solution. For anaerobic digestion purposes, the heat should not be considered as “lost” since the digester requires heat to operate efficiently. Therefore, if approximately 80% of the electrical energy was converted into heat in the water solution, the remaining 20% of the energy was actually lost as sound energy and through other dissipation mechanisms. In this study, to be conservative, it was assumed that 25% of the energy used by the sonicator was lost. Hence, this energy fraction was subtracted from the one produced by the surplus methane generation from the sonicated samples. Finally, with these considerations, if the result of the subtraction was positive, the ultrasonication technology was more likely to be worthwhile. On the other hand, if the result of the subtraction was negative, it would not be worthwhile to use the ultrasonication technology.
NEnB [J] = (P [ml] × Q × C [J/ml]) - (E × (100 - ηultrasonic))

where \( E \) is the energy consumed by the sonicator, and \( η_{sonicator} \) is the energy efficiency of the sonicator (75% was assumed). For the specific case of the Net Economy Balance, the energy quality difference between methane gas and electricity was taken into account. In other words, to produce electricity with bio-methane, an efficiency factor of 35% is required for the energy conversion which comes from the use of the Otto cycle as the typical conversion system. If the aim is electricity production, the process may be supported by the Ontario Feed in Tariff (FIT) program. At the time of writing, the Ontario government pays 0.185 $/kWh of electricity produced by an anaerobic digester, for an average sized facility (>100 kW ≤ 250 kW). The cost of electricity, for the sonicator use, was assumed to be 0.065 $/kWh (current average consumer cost for electricity).

\[
\text{NEcB} [\$] = \left( 0.185 \ [\$/kWh] \times \left( \frac{P \ [mL] \times Q \times C \ [J/mL] \times η_{engine}}{100 \times 3600} \right) \right) - \left( 0.065 \ [$/kWh] \times \frac{E \ [J] \times (100-η_{sonicator})}{100 \times 3600} \right)
\]

where \( η_{engine} \) is the energy efficiency of the engine used to transform chemical energy to electricity (35% was assumed). These two terms can be used to define the net energy balance with the first definition, Net Energy Balance, taking into account the energy produced while the second definition, Net Economic Balance, is an indicator of the economic feasibility of the digester.

3.8 Experimental Procedure

To be able to achieve the goals of this study (Section 1.2), twelve different feedstock samples were used and analyzed using seven techniques: TS and VS determination, calorific value, TOC measurements, ultrasonication, BMP assays, lignin, cellulose and hemicellulose determination, and finally biogas analysis. Figure 3.1 shows the sequence of those techniques.
3.9 Statistical and Graphing Analysis

Means and p-values in this study were calculated using the statistical software SAS (Statistical Analysis System, version 9.3). Since SAS is applicable only for population distribution statistical theory, standard deviation and confidence interval were computed using an Excel spreadsheet so that the sample distribution statistical theory could be applied. Thus, the t-distribution table and the following equation were used to calculate every confidence interval (CI):

\[
CI = t \times (s / \sqrt{n})
\]

\[
\bar{x} \pm t \times (s / \sqrt{n})
\]

where CI is the confidence interval, \(\bar{x}\) is the sample mean, \(t\) is the \(t\)-ratio coming from the student’s \(t\)-distribution, \(n\) is the sample size, and \(s\) is the standard deviation computed with the following equation:

\[
s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}
\]

where \(x\) is the observed value for each respondent. A 95% confidence interval was maintained throughout the research. In measuring the biogas volume generation on a periodic basis, the error in the volume estimation from a previous measurement will propagate into the current measurement as there is only a finite quantity of feedstock available for conversion. Thus, in many of the graphs presented in the Chapter 4, the term “augmented” confidence interval is used. This adjusted term was used to account for the cumulative error effects in the biogas volume measurements (i.e. the experimental parameter with the largest lab error). To show this cumulative error, \(\pm 1\) milliliter of biogas volume was added to each single measurement in addition to the definition of the confidence interval, given above. Also, due to the experiment type, repeated measure
analysis was done in all cases to measure if a difference in the means between the pretreated and not pretreated group was significant. The SAS programming term “proc mixed”, which includes ANOVA, was used with Tukey’s definition. This definition was selected because the data set was pairwise between unsonicated and sonicated groups. A coding example used for the data can be found in Appendix K. Graphs were created using gnuplot 4.6, an open source software.
Figure 3.1 – Summary of experimental procedure.
Chapter 4 - Results and Discussions

The data provided in Table 4.1 summarizes the results of values for TS, VS, and high heating value (HHV) for each raw feedstock. Also, the lignin, cellulose and hemicellulose content were measured for the case of wheat straw. These results show a similar TS, VS, and HHV for all forages: 90% TS, 93% VS and 19 MJ/kg TS. As paper sludge was wet and low in VS, the results indicate that it would not likely be seen as a good feedstock for AD. The FOG utilized had the highest calorific value per mass of TS which was 40 MJ/kg TS and did not contain water and ashes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wheat Straw</th>
<th>Hay</th>
<th>Switch Grass</th>
<th>Paper Sludge</th>
<th>FOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solid [%]</td>
<td>90.8 ± 0.4</td>
<td>89.8 ± 0.8</td>
<td>85.5 ± 0.9</td>
<td>21.7 ± 0.3</td>
<td>99.7 ± 0.6</td>
</tr>
<tr>
<td>Volatile Solid [%]</td>
<td>94.9 ± 0.7</td>
<td>91.5 ± 1.7</td>
<td>91.9 ± 0.6</td>
<td>62.5 ± 0.4</td>
<td>100 ± 0.0</td>
</tr>
<tr>
<td>HHV [MJ/kg TS]</td>
<td>18.59 ± 0.25</td>
<td>19.26 ± 0.10</td>
<td>19.13 ± 0.13</td>
<td>12.35 ± 0.15</td>
<td>39.97 ± 0.32</td>
</tr>
<tr>
<td>Lignin content [%]</td>
<td>8.4 ± 0.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cellulose content [%]</td>
<td>37.7 ± 12.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hemicellulose content [%]</td>
<td>44.9 ± 0.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

### 4.1 Effects of Ultrasonication on TOC

Figures 4.1 to 4.4 present the amount of NPOC released from the paper sludge, switch grass, hay and wheat straw based on whether the sample was sonicated or unsonicated as well as by manipulation type (see Section 3.4.1). As FOG is a special case, Figure 4.5 illustrates the NPOC generated for both the sonicated and unsonicated samples in comparison to the maximum NPOC values from the four other feedstock types. Details on the NPOC generated for each feedstock are discussed below.
**Paper Sludge**

Figure 4.1 shows the graph for paper sludge NPOC results for the various treatments. Organic carbon released from the low organic matter concentration of paper sludge seemed to react well with ultrasound degradation. With manipulation A1 (not ground, no soaking time), unsonicated paper sludge dissolved 8.96±0.11 mg/L NPOC while sonicated paper sludge dissolved 45.1±1.02 mg/L NPOC.

Paper sludge was not ground in the experiments due to high level of moisture content (28.5% TS), therefore manipulation A2 was not conducted. With 6 hours of pre-sonication soaking (manipulation B1), the NPOC level was 23.17±0.42 mg/L for unsonicated solution and 52.41±0.58 mg/L NPOC for sonicated solution. Similarly, for manipulation B2 (pre and post-sonication soaking), NPOC level was 34.26±0.73 mg/L for unsonicated solution and 82.78±0.95 mg/L NPOC for sonicated solution. For manipulation A1, B1 and B2, organic carbon of paper sludge solution had increased by 403%, 126%, and 142%, respectively, with the use of sonication. Paper sludge reaction thus presents a significant degradation under ultrasound pretreatment. The degradation level provided by sonication was still maintained 12 hours after the solution preparation. These finding suggest that sonication effects tend not to dissipate with time on paper sludge. In other words, the organic carbon present naturally dissolves in water with soaking time.
Figure 4.1 – NPOC ultrasonic release for paper sludge.

Figure 4.2 – NPOC ultrasonic release for switch grass.
Figure 4.3 – NPOC ultrasonic release for hay.

Figure 4.4 – NPOC ultrasonic release for wheat straw.
Switch Grass

Figure 4.2 contains the graph with the switch grass NPOC results. For manipulation A1, the unsonicated solution dissolved 12.35±0.12 mg/L NPOC while the sonicated solution yielded 34.75±0.28 mg/L NPOC. When ground switch grass was used (manipulation A2), 288.6±12.35 mg/L NPOC and 334.6±3.23 mg/L NPOC were observed for the unsonicated and sonicated solution, respectively. With pre-sonication soaking time of 6 hours, (manipulation B1), 148.5±1.22 mg/L NPOC and 189.2±4.41 mg/L NPOC were found when unsonicated and sonicated, respectively. With pre- and post-sonication soaking times were used (manipulation B2), organic carbon concentrations increased marginally over the B1 manipulation results with the unsonicated solution producing 176.3±3.23 mg/L NPOC and the sonicated solution producing 202.8±2.71 mg/L NPOC.

For manipulation A1, A2, B1 and B2, organic carbon of the sonicated switch grass solutions showed increases of 181%, 16%, 27%, and 15%, respectively, over the unsonicated solutions using the same manipulations (see Figure 4.2). With manipulations A2, B1, and B2, the amount of increase in the NPOC generated is minimal and thus may indicate the potential difficulty of switch grass to be degraded by anaerobic digestion. Also, with sonication, switch grass dissolved approximately the same amount of organic carbon into water over the unsonicated samples as did the paper sludge samples in spite of the fact that the paper sludge contained two thirds the organic matters (VS) of switch grass.

Hay

The hay substrate released its organic carbon more easily than switch grass. With manipulation A1 (not ground, no soaking time), unsonicated hay dissolved 252.1±0.46 mg/L NPOC while sonicated hay had dissolved 507.9±0.94 mg/L NPOC. The highest organic carbon concentration was found by grinding the hay (manipulation A2). Indeed, 1687±3.89 mg/L NPOC and 1837±4.17 mg/L NPOC was found for unsonicated and sonicated ground hay, respectively. These results are shown graphically in Figure 4.3. When allowing the hay to soak for 6 hours before sonication, the NPOC dissolution was improved (manipulation B1), but it did not improve the ultrasonic degradation. Pre-sonication soaking time manipulation released
1039±1.63 mg/L NPOC and 1085±6.18 mg/L NPOC, for unsonicated and sonicated treatments, respectively. With manipulation B2, (pre- and post-sonication soaking times before NPOC measurement), organic carbon concentration increases slowly. The NPOC level was 1101±7.97 mg/L for the unsonicated solution and 1105±1.71 mg/L NPOC for the sonicated solution. For manipulation A1, A2, B1 and B2, the organic carbon of hay solution resulted in increases of 101%, 9%, 4% (not significant) and 0% (not significant), respectively for the sonicated versus the unsonicated samples (see Figure 4.3). In addition, unsonicated results reached a similar carbon level after 12 hours in water than sonicated results (manipulation B2).

Wheat Straw

Figure 4.4 graphs the NPOC results for wheat straw. Following manipulation A1 (not ground, no soaking time), unsonicated wheat straw dissolved 51.15±0.55 mg/L NPOC while sonicated straw dissolved 155.4±2.17 mg/L NPOC. These values increased with the grinding treatment to 334.7±1.19 mg/L and 393.2±0.51 mg/L NPOC for unsonicated and sonicated treatment, respectively (manipulation A2). When the straw was soaked for 6 hours previous to sonication, the organic carbon concentrations measured were 364.8±1.19 mg/L and 375.2±1.48 mg/L NPOC for unsonicated and sonicated, respectively (manipulation B1). The highest organic carbon concentration was found with 6 hours of pre- and post-sonication soaking times (manipulation B2). Organic concentration of 421.0±2.78 mg/L and 453.8±1.41 mg/L NPOC were found for unsonicated and sonicated treatments, respectively. For manipulation A1, A2, B1, and B2, organic carbon of wheat straw solution, sonication resulted in increases of 204%, 17%, 3% (not significant) and 7%, respectively.

FOG

The case of FOG was special as a feedstock due to the effects of ultrasonication on it. This feedstock does not have presented an organic and complex barrier of lignin, cellulose and hemicellulose as straws, hays and most other plant origin feedstock. The reaction to ultrasound pretreatment observed with the vegetable oil-water solution was thus akin to homogenization of the solution and thus the triglycerides dissolution was different than organic cellular degradation seen with the forage feedstock. The variables measured for this experiment was the NPOC
dissolved in water. The unsonicated oil-water solution had only 4.832±0.79 mg/L NPOC since oil is mostly insoluble with water. After sonication, the resulting solution was creamy in texture and had 2031±151 mg/L NPOC. This was simply due to the dissolution of long-chain trans-fatty carbon acids in water. This homogenization reaction might have had an influence on AD. While unsonicated FOG would create a layer on top of most reactors, sonicated FOG would be, most likely, part of mixture in the tank. Figure 4.5 gives the NPOC results for FOG as well as the maximum NPOC generated by “A” manipulations for the other feedstock as a comparison.

![Figure 4.5 – NPOC release for FOG, paper sludge, switch grass, hay, and wheat straw.](image)

**Summary of the effects of ultrasonication on TOC dissolution**

Ultrasonic pretreatment increased the organic carbon in the solvent for FOG, paper sludge, switch grass, hay, and wheat straw. For all the studied feedstock, the organic carbon concentrations for unsonicated matter tended to reach the same organic carbon concentration with the soaking time factor. For the specific case of AD, these results suggest that there is a speed up of the hydrolysis step since dissolved organic carbon plays an important role in methane production.
Figure 4.5 indicates that, for manipulation A1, FOG dissolves more organic carbon in water than all the other feedstock tested. For the paper sludge and the forages, the ultrasonic pretreatment improved the organic carbon released in the solvent. Between manipulations A1 and A2, the NPOC increase is less than 100%. Hence, the first pretreatment (grinding) does not readily improve the efficiency of the second pretreatment (sonication).

4.2 Effects of Ultrasonication on Lignin, Cellulose and Hemicellulose Degradation

Since wheat straw contains leaves, internodes, and cores in a similar fraction then lignin, hemicellulose, and cellulose, these plant sections were averaged on their natural mass fraction in the plant (internodes: 60.9%; leaves: 35.5%; nodes: 3.6%). They are presented has a whole plant in Figure 4.6. The same data with confidence intervals added can be found in Table 4.2.
Figure 4.6 indicates that the mass fraction measured for lignin, cellulose and hemicellulose, in both cases of unsonicated and sonicated treatment, are similar. The mass lost due to sonication appears to be mostly due to the water soluble fraction. This last compound is recognized to mainly be soluble elements coming from ashes. The failure in the differentiation between unsonicated and sonicated samples probably comes from the inability to adapt the experimental materials and methods to be suitable for the present purpose (choices of filters and type of filtration, manipulation of samples, etc.). Also, since lignin and holocellulose (cellulose and hemicellulose) have a low solubility, the filter choice should have done in relation with the acceptable degraded particle size of the wheat straw. Even though sonication would attack the matter and reduce the particle size, holocellulose and lignin would still be part of the sample and thus would have a measurable weight. Hence, the question of interest would be: “At what particle size should a sonicated sample be considered to be degraded enough?” The answer to this question would be useful to select the filter size.

| Table 4.2 – Sonication effect on water soluble matter, lignin, hemicellulose and cellulose on unsonicated and sonicated wheat straw sample with confidence intervals (95%). |
|---|---|---|
| **Sonicated Step [%]** | — | 10.7±2.9 |
| **Water Soluble Matter [%]** | 10.2±3.5 | 5.5±0.1 |
| **Lignin [%]** | 8.3±0.7 | 6.1±1.8 |
| **Hemicellulose [%]** | 37.6±12.3 | 33.3±0.3 |
| **Cellulose [%]** | 44.9±0.4 | 44.2±3.0 |
| **Total [%]** | 101 | 99.8 |

Measuring lignin is complicated by the extensive cross linkages with cellulose and hemicellulose and the insolubility of those polymers (Hatfield et al. 1994). Also, some of the conditions and steps in the detergent fiber methods are critical in obtaining accurate results. Among these are: subsampling, drying, grinding, sample amount, standardization of reagents, removal of starch and nitrogen contamination, timing and temperature of refluxing, transferring residues, washing fibrous residues, type of filtration vessel and weighing method. The abundance of fibers method is further complicated by the modifications of each method that are
commonly used. Since fiber is defined by the method used to isolate it, it should be clear that modifications have the potential for defining a new fiber value that is not comparable to the parent method. The sensitivity of fiber values to each method suggests that fiber methods must be followed exactly to be reproducible (Mertens, 1992).

With these last comments, regarding the development of a suitable technique coupled with the shadow of important variability in the results, efforts for the study of ultrasonic pretreatment have been invested on other techniques. There was no significant conclusion to be drawn from this experiment.

### 4.3 Effects of Ultrasonication on Biogas Production and Yield

Figures 4.7 to 4.12 present the biogas production, in units of ml, as a cumulative function of time for FOG, paper sludge, ground switch grass, ground hay, ground wheat straw, and cut wheat straw. In each figure, the experimental data points are shown with a 95% augmented confidence interval to assess the impact of error propagation in the cumulative trend lines. The shaded areas between the unsonicated and sonicated trend lines represent the enhanced biogas produced of the sonicated sample over that of the unsonicated sample for a given time. Figure 4.13 presents the biogas production (in ml) for all feedstock studied to facilitate comparisons between the different feedstock.

Figures 4.14 to 4.19 present the biogas production, in units of m³/kg VS, as a cumulative function of time the substrates studied with Figure 4.20 presenting a comparative graph of all the feedstock studied. Table 4.3 also summarizes the yield of biogas (m³/kg VS) and the retention time required to achieve a yield of 50%, 80% and 100% of the maximum biogas yield. Detailed discussions on the results for each feedstock are given in the subsections following the graphs. In the discussion section, when the term “maximum yield” is used, it always refers to the yield found on the last retention time day.

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Figure 4.7 – Biogas production from FOG.
Figure 4.8 – Biogas production from paper sludge.
Figure 4.9 – Biogas production from ground switch grass.
Figure 4.10 – Biogas production from ground hay.
Cumulative biogas production from GROUND WHEAT STRAW and control with respective augmented confidence intervals (95%)

Figure 4.11 – Biogas production from ground wheat straw.
Figure 4.12 – Biogas production from cut wheat straw.
Figure 4.13 – Biogas production from FOG, paper sludge and studied forages.
Figure 4.14 – Biogas yield from FOG.
Figure 4.15 – Biogas yield from paper sludge.
Figure 4.16 – Biogas yield from ground switch grass.
Figure 4.17 – Biogas yield from ground hay.
Figure 4.18 – Biogas yield from ground wheat straw.
Figure 4.19 – Biogas yield from cut wheat straw.
Figure 4.20 – Biogas yield from FOG, paper sludge, and all studied forages.
Table 4.3 – Net biogas yield for 50%, 80% and 100% of the maximum biogas yield for each feedstock with their respective confidence interval. In parenthesis, the corresponding retention time in hours.

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>50%</th>
<th>80%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsonicated FOG [m³/kg VS]</td>
<td>0.78±0.03 (150)</td>
<td>1.24±0.01 (275)</td>
<td>1.56±0.01 (825)</td>
</tr>
<tr>
<td>Sonicated FOG [m³/kg VS]</td>
<td>0.83±0.02 (75)</td>
<td>1.33±0.03 (125)</td>
<td>1.66±0.01 (825)</td>
</tr>
<tr>
<td>Unsonicated Paper Sludge [m³/kg VS]</td>
<td>0.29±0.01 (75)</td>
<td>0.46±0.01 (150)</td>
<td>0.58±0.01 (825)</td>
</tr>
<tr>
<td>Sonicated Paper Sludge [m³/kg VS]</td>
<td>0.30±0.12 (36)</td>
<td>0.48±0.04 (125)</td>
<td>0.60±0.01 (825)</td>
</tr>
<tr>
<td>Unsonicated Ground Switch Grass [m³/kg VS]</td>
<td>0.25±0.01 (375)</td>
<td>0.40±0.01 (650)</td>
<td>0.50±0.01 (1200)</td>
</tr>
<tr>
<td>Sonicated Ground Switch Grass [m³/kg VS]</td>
<td>0.24±0.02 (375)</td>
<td>0.38±0.01 (625)</td>
<td>0.48±0.01 (1200)</td>
</tr>
<tr>
<td>Unsonicated Ground Hay [m³/kg VS]</td>
<td>0.24±0.01 (100)</td>
<td>0.38±0.01 (400)</td>
<td>0.48±0.01 (1200)</td>
</tr>
<tr>
<td>Sonicated Ground Hay [m³/kg VS]</td>
<td>0.30±0.01 (100)</td>
<td>0.47±0.02 (400)</td>
<td>0.58±0.01 (1200)</td>
</tr>
<tr>
<td>Unsonicated Ground Wheat Straw [m³/kg VS]</td>
<td>0.26±0.01 (200)</td>
<td>0.41±0.01 (450)</td>
<td>0.51±0.01 (1200)</td>
</tr>
<tr>
<td>Sonicated Ground Wheat Straw [m³/kg VS]</td>
<td>0.34±0.02 (300)</td>
<td>0.54±0.02 (500)</td>
<td>0.67±0.01 (1200)</td>
</tr>
<tr>
<td>Unsonicated Cut Wheat Straw [m³/kg VS]</td>
<td>0.21±0.01 (525)</td>
<td>0.33±0.01 (750)</td>
<td>0.41±0.01 (1200)</td>
</tr>
<tr>
<td>Sonicated Cut Wheat Straw [m³/kg VS]</td>
<td>0.25±0.01 (475)</td>
<td>0.39±0.02 (675)</td>
<td>0.49±0.01 (1200)</td>
</tr>
</tbody>
</table>

Control (Unfed) versus Fed

Prior to looking at the behaviour of biogas production for all the feedstock considered, it is important to note the significant difference in biogas production between fed and unfed (control) BMP assays bottles as depicted in Figures 4.7 to 4.12. In all cases, the fed BMP assays bottles significantly produced more biogas \((p\text{-value} \leq 0.0001)\) than the unfed bottles (control) within the first 24 h of incubation. This was an important part of the quality control assessment.

FOG

FOGs are quick and easy to digest. Figure 4.7 shows that most of the FOG available in the samples was transformed after 300 h (12 days) of retention. The rest of the biogas production after 300 h is mostly due to that produced by the inoculum. FOGs are also a dense source of energy as evidence by the highest yield of approximately 1.6 m³ biogas/kg VS (see Figure 4.14), which was more than three times the production of any other feedstock studied.
Regarding the sonication process, the emulsification caused by cavitation appears to be positive for the methanogens. Instead of having a distinct water-oil layer where the bacteria can feed, with sonication, their food is literally available everywhere due to the emulsification reaction. This model may explain the rapid acceleration of the FOG digestion after sonication. It is important to note that the biogas production did not increase globally throughout the entire digestion time with the ultrasonication only serving to speed up the digestion time. This behaviour is evident in Figure 4.7, which shows explicitly that there is no reason to use sonication pretreatment with FOG if the retention time of the digester is more than 300 h. More precisely, the main advantage of ultrasonication on FOG can be seen between 72 h and 192 h (3 to 8 days). Also, a very interesting peak of the sonicated curve of biogas production can be seen on Figure 4.14 at 156 h to 168 h (6.5-7 days) of retention time. At this time, 86% of the maximum yield had been produced (~1.41 m$^3$ biogas/kg VS). If a digester design was required for a FOG feedstock, this retention time would probably have higher chances to justify the use of ultrasonic pretreatment on a life cycle analysis (LCA) point of view. Table 4.3 illustrates that, when aiming for a production of 80% of the maximum biogas yield, sonication allows the retention time to be reduced by a factor of 2. This situation would even allow a higher biogas yield (unsonicated 1.24 m$^3$ biogas/kg VS; sonicated 1.33 m$^3$ biogas/kg VS). The $p$-value between sonicated and unsonicated yield means was smaller than 0.0001 from day 2 to day 9, inclusively. Hence, the pretreatment had a significant impact on the digestion for this period.

**Paper Sludge**

For the paper sludge feedstock, the same kind of behavior was observed as with FOG but at a smaller order of magnitude. This could be explained by the fact that the paper sludge was less dense in energy than FOG (paper sludge: 12 MJ/kg dry versus FOG: 40 MJ/kg). Paper sludge was also quick to digest. Most of the digestion was achieved after 144 h (6 days) (see Figure 4.8). Following this retention time, the sonicated and unsonicated curves followed the same pattern as the control curve. Figure 4.15 shows that the sonicated yield curve plateaued between 144 h and 288 h (6 to 12 days) at 0.52 m$^3$ biogas/kg VS. These results show that there would be no reason to design a digester for paper sludge with retention time greater than 144 h (6 days). The large variability in the first hours of the sonicated curve (Figure 4.15) was caused by
the death of the micro organisms in one of the three bottles. This bottle was probably over-stimulated by a too high food availability causing acidification of the media. The \( p\)-value between sonicated and unsonicated yields and biogas production means was smaller than 0.0001 from 48 to 96 h (2 to 4 days), inclusively. Hence, the pretreatment had a significant impact on the digestion for this period.

**Ground Switch Grass**

Switch grass, a cellulosic matter, similarly to the other forages studied in this experiment, displayed a different behavior regarding the digestion and sonication than the two, non-cellulosic matters, namely FOG and paper sludge. The digestion times were long and the sonication effect varied depending on the lignin, cellulose and hemicellulose content, which is closely related to the plants age and species. Switch grass has a high crop yield, but since the plant is only harvested once a year, at the end of the season, the lignin content is relatively high. This high lignin content seemed to inhibit the sonication effect on the digestion (Figure 4.9). The lignin limits cell wall (fiber) digestion by imposing a physical barrier to the bacteria and the concentration of both fiber and lignin increases the maturation state of the plant (Van Soest, 1978; Chaves et al., 2002a). The lignin inhibits the digestion rate and extends the digestion time, especially when the proportion of lignin in fibers begins to increase (Chaves et al., 2002b).

The \( p\)-values, for the entire retention time on ground switch grass biogas yield, were higher than 0.05. Thus, there was no significant difference found in the mean biogas production between unsonicated and sonicated yield. When looking at the yield in Figure 4.16, there seems to have a numerical reduction of biogas yield when feedstock was sonicated but this was not significant. The conclusion in this case was that sonication had little to no effect on the anaerobic digestion of ground switch grass. Moreover, no apparent physical degradation was achieved by sonication on ground switch grass.

**Ground Hay**

Since the maturation of the hay used in this experiment was significantly lower than that for switch grass, the lignin content was probably lower in this feedstock. A significant effect of
sonication was notable on biogas yield from entire study period, from with the first 24 h up to 1200 h (day 1 to day 50) (p-values ≤ 0.0001). This experiment did not achieve complete digestion of the feedstock. Nevertheless, it would be logical to believe that no more biogas would be produce by the sonicated sample then the unsonicated one at the end of a complete digestion period. Since the digestion process was slow for ground hay, the enhanced production due to the sonication effect was not completed over the 1200 h (50 days) digestion period used in this experiment. This explains why both, sonicated and unsonicated yield, were at their maximum at 1200 h of 0.49 m$^3$ biogas/kg VS and 0.59 m$^3$ biogas/kg VS, respectively (see Figure 4.17).

**Ground Wheat Straw**

The ground wheat straw feedstock had the highest sonicated forage biogas yield of 0.67 m$^3$ biogas/kg VS after 50 days of retention time (RT). In comparison, the unsonicated ground wheat straw yielded 0.51 m$^3$ biogas/kg (50 days of RT) VS which represent an increase of 32% due to the sonication process. The difference in the mean production between sonicated and unsonicated yield curves was significant even in the first 24 h (day 1) (p-values ≤ 0.0001; see Figure 4.18). The sonicated and unsonicated biogas production behavior observed for this feedstock was similar to the one when hay was used as feedstock.

**Cut Wheat Straw**

Cut wheat straw had an unsonicated biogas yield of 0.41 m$^3$ biogas/kg VS and a sonicated one of 0.49 m$^3$ biogas/kg VS, at 1200 h (50 days) of digestion. The difference in the means between sonicated yield curve and unsonicated yield curve was significant as early as 48 h (day 2) (p-values ≤ 0.0001; see Figure 4.19). The unsonicated biogas yield reached 80% of production (0.33 m$^3$ biogas/kg VS) in 744 h (31 days) of retention (see Table 4.3). When this feedstock was sonicated, a higher yield was reached (0.39 m$^3$ biogas/kg VS) in 672 h (28 days).

In comparison to the ground forages used, the cut wheat straw forage was the only one where a negative yield was noticeable right from the beginning (see Figure 4.19). This phenomenon can be explained by the difficulty that the bacteria experience in accessing their food when in the cut form. The sonication influence on cut wheat straw did not achieve a level
of physical degradation to increase the surface area for the bacteria to attach in the early stage of biogas production.

Summary of the effects of ultrasonication on biogas production and yield

In all cases, the fed BMP assays bottles significantly produced more biogas ($p$-value $\leq 0.0001$) than the unfed bottles (control). For FOG, paper sludge, ground hay, ground wheat straw and cut wheat straw, there was a specific window of time where the sonicated feedstock had a significant higher yield than the unsonicated feedstock. The biogas yield for ground switch grass, probably due of its too high level of lignin, was not significantly influenced by the sonication process. This increase in yield over a select period of time using sonication thus allows a net energy balance (NEEnB) and net economy balance (NEcB) analyses to be conducted for all feedstock with the exception of switch grass. Unfortunately, no comparative study was found for discussion purposes.

4.4 Influence of Ultrasonication on Biogas Content

Biogas produced by wheat straw (unsonicated and cut; sonicated and cut; unsonicated and ground; sonicated and ground) were analyzed for CH$_4$ and CO$_2$ content. Figure 4.21 shows the CH$_4$ fraction along the 1200 h (50 days) of experiment. The first 10 days were slightly chaotic probably due to the generally irregular start of the bio-process in all feedstock. The BMP method itself also contributes to this phenomenon since several biogas samples were needed before the majority of the N$_2$ and CO$_2$ gases, previously introduced in the bottles, were removed.

After statistical analysis with SAS, the $p$-value associated with the difference between these four groups equaled 0.1793 (proc mixed). This $p$-value reveals that neither sonication pretreatment nor particle size management influenced the CH$_4$ fraction in the biogas. This fact remains true for the entire experimental period. Confidence intervals were relatively small due to the precision of the gas chromatography analyzer and thus are not visible when plotted on the graph. The average confidence interval was in the order of 0.5% of the measured values. With these results, it was decided that further calculation where a biogas quality percentage was
required, a somewhat conservative value of 60% CH$_4$ would be used independently of the treatment applied to the feedstock.

Figure 4.21 – Biogas quality for all studied cases of wheat straw.

4.5 Effects of Sonication on Digestibility Ratios

As stated in Section 3.6, the digestibility ratio is a measure of the energy of the methane produced by the digestion process compared to the total energy available in the feedstock. By looking at the cumulative digestibility ratio as a function of time, an indication of how much energy is extracted from the feedstock can be obtained by the end of the digestion experiment. Figures 4.22 to 4.27 depict the behaviour of the digestibility ratio with time for the feedstock considered in the experiments. A table summarizing the results (Table 4.4) and a more detailed discussion of the behaviour of the unsonicated and sonicated samples for each feed stock follows the graphs.
Figure 4.22 – Digestibility ratio for FOG.
Figure 4.23 – Digestibility ratio for paper sludge.
Figure 4.24 – Digestibility ratio for ground switch grass.
Figure 4.25 – Digestibility ratio for ground hay.
Figure 4.26 – Digestibility ratio for ground wheat straw.
Figure 4.27 – Digestibility ratio for cut wheat straw.
Table 4.4 – Digestibility ratio for 50%, 80% and 100% of the maximum digestibility ratio for each feedstock with their respective confidence interval. In parenthesis, the corresponding retention time in hours. The last column represents the digestibility ratio increase due to the sonication process for 80% of the maximum digestibility ratio.

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>50%</th>
<th>80%</th>
<th>100%</th>
<th>Increase [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsonicated FOG [%]</td>
<td>45±1 (150)</td>
<td>71±1 (275)</td>
<td>89±2 (800)</td>
<td>7</td>
</tr>
<tr>
<td>Sonicated FOG [%]</td>
<td>48±1 (75)</td>
<td>76±1 (125)</td>
<td>95±2 (800)</td>
<td></td>
</tr>
<tr>
<td>Unsonicated Paper Sludge [%]</td>
<td>34±1 (75)</td>
<td>54±1 (150)</td>
<td>67±2 (800)</td>
<td>7</td>
</tr>
<tr>
<td>Sonicated Paper Sludge [%]</td>
<td>36± (75)</td>
<td>58±1 (1506)</td>
<td>72±2 (800)</td>
<td></td>
</tr>
<tr>
<td>Unsonicated Ground Switch Grass [%]</td>
<td>29±1 (400)</td>
<td>46±1 (700)</td>
<td>57±2 (1200)</td>
<td>-4</td>
</tr>
<tr>
<td>Sonicated Ground Switch Grass [%]</td>
<td>28±1 (400)</td>
<td>44±1 (700)</td>
<td>55±2 (1200)</td>
<td></td>
</tr>
<tr>
<td>Unsonicated Ground Hay [%]</td>
<td>27±1 (100)</td>
<td>42±1 (450)</td>
<td>53±2 (1200)</td>
<td>19</td>
</tr>
<tr>
<td>Sonicated Ground Hay [%]</td>
<td>32±1 (100)</td>
<td>50±1 (400)</td>
<td>63±2 (1200)</td>
<td></td>
</tr>
<tr>
<td>Unsonicated Ground Wheat Straw [%]</td>
<td>30±1 (300)</td>
<td>48±1 (475)</td>
<td>60±2 (1200)</td>
<td>29</td>
</tr>
<tr>
<td>SonicatedGround Wheat Straw [%]</td>
<td>39±1 (300)</td>
<td>62±1 (500)</td>
<td>78±2 (1200)</td>
<td></td>
</tr>
<tr>
<td>Unsonicated Cut Wheat Straw [%]</td>
<td>24±1 (550)</td>
<td>38±1 (775)</td>
<td>48±2 (1200)</td>
<td>21</td>
</tr>
<tr>
<td>Sonicated Cut Wheat Straw [%]</td>
<td>29±1 (500)</td>
<td>46±1 (700)</td>
<td>58±2 (1200)</td>
<td></td>
</tr>
</tbody>
</table>

FOG

The highest improvement in the digestibility ratio caused by sonication on FOG was observed between 150 h and 175 h (day 7). During this day, the sonicated feedstock reached a digestibility ratio of 80% while the unsonicated feedstock had digested only 50% of the substrate. This represents an increase of 60% in the digestibility ratio between the unsonicated and sonicated samples (see Figure 4.22). This retention time also corresponded to the best yield of the sonicated feedstock (see Figure 4.14). After 200 h (day 8), the sonicated feedstock slowly reached the unsonicated behavior (for the digestibility ratio as well as the biogas yield). The final digestibility ratio after 300 h followed the same behaviour for the both the sonicated and unsonicated samples thus indicating that the ultrasonication process no longer produced a difference in the yield as previously discussed.
**Paper Sludge**

Similarly to the process discussed in the previous section, sonication had a brief but smaller effect on paper sludge. Since paper sludge was easy to digest, the digestibility ratio improved continuously, but had a small impact throughout the entire digestion experiment (see Figure 4.23). Digestibility ratio of 54±1 % and 58±1 % were reached for unsonicated and sonicated cases, respectively, after 150 h (6 days) of retention. This represented 80% of the maximum digestibility ratio achieved over the course of the experiments.

**Ground Switch Grass**

The digestion ratio, as with the biogas production and the biogas yield for ground switch grass, was not significantly improved by sonication at any time during the digestion ($p$-value $\geq 0.05$) (see Figure 4.24). A digestibility ratio of 45% was reached, for the unsonicated and the sonicated feedstock, after 700 h (28 days) of retention. This represented 80% of the maximum digestibility ratio achieved over the course of the experiments.

**Ground Hay**

After 1200 h (50 days) of digestion, the sonicated ground hay was digested at 63±2% while the unsonicated ground hay was digested at the lower level of 53±2%. The significant digestibility ratio difference ($p$-value $\leq 0.0001$) stayed true from the early period of digestion until the end of the experiment (Figure 4.25). The 80% of the maximum digestibility ratio was reached at 450 h (day 18) and 400 h (day 16) for unsonicated and sonicated cases, respectively.

**Ground Wheat Straw**

At the end of the 1200 h (50 day) experiment, the digestibility ratio measured for unsonicated and sonicated were 60±2% and 78±2%, respectively (see Figure 4.26). This feedstock reacted the most to ultrasonication process. A constant increase of 29% is maintained between unsonicated digestibility ratio and sonicated digestibility ratio at 50%, 80%, and 100% of the maximum digestibility ratio.
Cut Wheat Straw

The cut wheat straw digestibility ratio was also influenced by sonication (see Figure 4.27). At 80% of the maximum digestibility ratio, an increase in the digestibility ratio of 21% was recorded from the unsonicated to the sonicated feedstock (38±1% to 46±1%, respectively). The maximum digestibility ratio measured (at 1200 h or 50 days) for unsonicated and sonicated was 48±2% and 58±2%, respectively (see Table 4.4).

Summary of the effects of ultrasonication on digestibility ratio

Sonication positively and significantly influenced the digestibility ratio of FOG, paper sludge, ground hay, ground wheat straw and cut wheat straw at some point during the retention time. These results are in agreement with the biogas production and biogas yield results presented in Section 4.3. Since switch grass was too hard to be physically degraded by cavitation, this last feedstock did not show significant improvement on the digestibility ratio when sonicated. Unfortunately, no comparative study was found for discussion purposes.

4.6 Energy and Economic Balance Analyses

A cumulative net energy and economic balance analyses (NEnB and NEcB, respectively) was performed for each of the ultrasonicated substrates to determine if there was a pronounced gain in energy production and derived cash flow by using the sonication process over the unsonicated samples. Figures 4.28 to 4.33 depict the behaviour of these indices with the NEnB given on the left hand axis in units of kJ/kg of feedstock and the NEcB on the right hand axis in units of $/kg of feedstock. Figures 4.34 and 4.35 give the comparison of all the NEnB and all the NEcB, respectively, on a single plot to facilitate a comparison between the different feedstock. A more detailed discussion for each feedstock follows the graphs.
Figure 4.28 – NEnB and NECB for FOG.
Figure 4.29 – NEnB and NEcB for paper sludge.
Figure 4.30 – NEnB and NECB for ground switch grass.
Figure 4.31 – NEnB and NEcB for ground hay.
Figure 4.32 – NEnB and NEcB for ground wheat straw.
Figure 4.33 – NEnB and NECB for cut wheat straw.
Figure 4.34 – NEnB for FOG, paper sludge, and all studied forages.
Figure 4.35 – NEcB for FOG, paper sludge, and all studied forages.
**FOG**

For the NEnB and NECB indices, ultrasonication of FOG showed positive results in the early stages of digestion. The optimal retention time should be 100 h if the design of a digester was based on a FOG feedstock with ultrasonication technology. At this retention time, sonication caused a net energy balance of 12,000 kJ/kg of FOG and a net economic balance of 0.22 $/kg of FOG (see Figure 4.28). Since most of the FOG was digested within 300 h (12 days), sonicated or not, the positive NEnB and NECB seen after 300 hours is due to the cumulative mathematical definition used. Thus, the flat line seen on Figure 4.28, after 300 hours of retention time, can be explained by the absence of biogas produced in surplus (or in deficit) due to the sonication process.

**Paper Sludge**

Even though sonication of paper sludge improved the first hours of digestion, this improvement was not sufficient to justify the use of the sonication pretreatment as the NEnB and NECB peaks remained negative during the entire retention time.

**Ground Switch Grass**

Since ground switch grass did not show positive results when treated with ultrasound, the NEnB and NECB follow the same trend. Figure 4.30 displays no positive energy production or economic values and thus sonication only added a cost to the process without creating any tangible benefits. The important conclusion with this feedstock is that there is no possibility where the use of ultrasonication should be recommended.

**Ground Hay**

Even though the NEnB and NECB of ground hay always increased along the 1200 h (50 days) experiment, the final calculations remained negative (NEnB = -1020 kJ/kg and NECB = -0.018 $/kg (see Figure 4.31)). Thus the use of ultrasonication with ground hay cannot be justified.
**Ground Wheat Straw**

As with ground hay, the use of ultrasonication pretreatment for ground wheat straw cannot be justified. This forage was the most influenced by the sonication, but because of the resulting NEnB = -360 kJ/kg and NEcB = -0.006 $/kg at 1200 h (day 50), sonication pretreatment should not be conducted (see Figure 4.32).

**Cut Wheat Straw**

Cut wheat straw had similar biogas production results as with the ground wheat straw but at a lower order of magnitude. This resulted in more negative NEnB and NEcB calculations for cut wheat straw in comparison to ground wheat straw (see Figure 4.33). Once again, these results contraindicated potential utilization of sonication with this feedstock.

**Summary of energy balance analysis**

For FOG, a certain window of utilization of ultrasonication was found when sonication produced more energy and increased the economic benefit. For a digester with a FOG retention time around 100 hours, ultrasonication would be interesting since both NEnB and NEcB are positive. Sonication of paper sludge also had a peak, but its height was not sufficient bump the NEnB and the NEcN to positive values. While sonication of FOG and paper sludge generated NEnB and NEcB peaks at the beginning of the digestion time, the best situation for sonicated forages was at the end of the digestion. Within all forages studied, none reached a positive NEnB or NEcB. The comparison of all feedstock for NEnB and NEcB are presented on Figure 4.34 and Figure 4.35, respectively. Finally, no comparative study was found for discussion purposes.

### 4.7 Effects of Particle Size on Ultrasonication TOC, AD, Biogas Content, Digestibility Ratios, and Energy Balance

**TOC**

Grinding is a pretreatment by itself. In Figures 4.2, 4.3, and 4.4, switch grass, hay, and wheat straw NPOC releases were impacted by grinding in manipulation A2. Indeed, for these
three forages, grinding treatment increase the NPOC release more than the sonication pretreatment itself. Also, for the cases of switch grass and hay only, even with 6 h of pre- and post-sonication soaking time (manipulation B1 and B2) grinding was still the pretreatment that released the most organic carbon into the water. For wheat straw, the soaking time effect appeared to have a greater impact on the NPOC release than with the other two forages. For all forages, grinding did not improve the sonication effect on NPOC release.

**BMP assays**

According to the results shown in Figures 4.11, 4.12, 4.18 and 4.19, ground wheat straw was a better feedstock to digest than cut wheat straw. For both substrate sizes, sonication had a significant effect on biogas production and yield. When looking at 80% of the maximum yield, the produced yield by unsonicated ground wheat straw (0.41±0.01 m³/kg VS in 456 h or 19 days) was similar to the sonicated cut wheat straw (0.39±0.02 m³/kg VS in 672 h or 28 days), but the unsonicated ground wheat straw reached this yield 216 h (9 days) earlier (see Table 4.3). Once again, this finding illustrates that particle size reduction from the order of a centimeter to millimeter had a greater impact on digestion than sonication which produces particle size reduction at smaller order of magnitude.

**Biogas content**

As seen in Section 4.4, particle size did not influence significantly ($p$-value = 0.1793) the CH$_4$ fraction contained in the biogas produced.

**Digestibility ratio**

Digestibility ratios were also influenced by the particle size management. From 50%, 80% and 100% of the maximum digestibility ratio (from hour 500 to hour 1200), sonication increased the digestibility ratio by 29% for ground wheat straw and by 21% for cut wheat straw (see Table 4.4). The 8% of digestibility ratio surplus caused by grinding of the feedstock was significant ($p$-value ≤ 0.0001). Also, to reach 80% of the maximum digestibility ratio, ground wheat straw required 475-500 h (19-20 days) (sonicated and unsonicated) while cut wheat straw required approximately 750 h (30 days) (sonicated and unsonicated). This acceleration of the
digestibility ratio represents a potential increase in the global power of a digester. Furthermore, by comparing, always at 80% of the maximum digestibility ratio, for both the unsonicated ground and cut wheat straw, a digestibility ratio increase of 26% could be computed (48±1% at 475 h (19 days) and 38±1% at 775 h (31 days), respectively) due the particle size reduction. Using the same process, but with sonicated ground and cut wheat straw, a digestibility ratio increase of 35% was computed (62±1 at 500 h (20 days) and 46±1 at 700 h (28 days), respectively). By using the repeated measures of statistic theory, these two last paired groups were significantly different ($p$-value ≤ 0.0001).

Energy balance

Since the grinding pretreatment itself increased the biogas production of wheat straw (for the same retention time), the ground sonicated wheat straw had a higher yield than the cut sonicated wheat straw. Despite these improved results, the ground sonicated wheat straw did not achieve positive NEnB and NEcB. In other words, the advantage given by the grinding pretreatment is not sufficient to overcome the energy cost caused by the ultrasonic pretreatment (see Figure 4.32 and Figure 4.33). As explained in Section 3.2.3 – Grinding, the energy used to cut or grind the samples was not taken into account in the NEnB and NEcB calculations. This was to focus on the ultrasonication process.

Summary of the effect of particle size management

Grinding pretreatment had a significant effect on biogas production and yield ($p$-value ≤ 0.0001). On the other hand, when combined, both pretreatments had a diminished impact if used as second pretreatment, without regard to which one is first or second. Therefore, if this second pretreatment was used alone, its impact was greater.
Chapter 5 - Conclusions

This research produced the following significant findings:

(i) Pre-sonicated substrates release more organic carbon (NPOC) into the solvent than unsonicated substrates for FOG, paper sludge, switch grass, hay and wheat straw.

(ii) For the same retention time and the entire digestion period, sonicated substrates had a significant higher biogas yield than unsonicated substrates for FOG, paper sludge, ground hay, ground wheat straw and cut wheat straw (p-value ≤ 0.0001). This finding was not found for ground switch grass.

(iii) The biogas quality was not influenced by particle size management and sonication pretreatment (p-value ≥ 0.05).

(iv) Sonication positively and significantly influenced, at some point during the digestion time, the digestibility ratio of FOG, paper sludge, ground hay, ground wheat straw, and cut wheat straw (p-value ≤ 0.0001). Sonication did not influence the digestibility ratio of ground switch grass.

(v) Except for sonicated FOG, none of the sonicated feedstock studied reached a positive Net Energy Balance or Net Economy Balance. FOG had a small window of utilization when sonication could be used with energy and economic benefits.

The sonication of the studied FOG, paper sludge, switch grass, hay, and wheat straw had a positive and significant impact on biogas production. Nevertheless, in most cases, the use of this technology is hard to recommend due to its energy consumption that overshadows the energy surplus generated from its use.
Chapter 6 - Recommendations

Based on the current study, the following recommendations are given to help improve the results of future experimental endeavours:

(i) Further testing with cellulosic matter should include a way to confirm, observe and measure the cell’s explosion and/or degradation after the pretreatment.

(ii) Future work should look at the biogas production of different blend fractions of the studied feedstock. For instance, a blend of sonicated FOG and sonicated forages should be tried to know if the retention time of the cellulosic matter could be reduced by the use of this new recipe.

(iii) Results of BMP assays should be taken as an estimate of biogas production and should not be used for final design considerations. The test sometimes underestimates and sometimes overestimates the yield compared to the reality in larger scale anaerobic digestion.

(iv) If further higher industrial scale researches are undertaken, a certain form of energy balance must be calculated to justify or reject the ultrasonic pretreatment technology under controlled and known conditions.
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APPENDIX A – Solid Measurement Procedure

Solids determinations are performed based on procedures in Standard Methods (Methods 2540 B, E and G for Solid and Semisolid Samples). Total solids are measured according to Standard Method 2540B. A clean evaporating dish is heated to 550 ± 50°C for one hour in a muffle furnace then cooled to room temperature in a dessicator. The cool dish is weighed before transferring a 50 mL sample (if liquid) of sludge to it and the dish and sample are then reweighed. The sample is heated to dryness overnight in a drying oven at 103 – 105°C then cooled to room temperature in a dessicator. The dish containing the dried residue is then weighed again and total solids calculated according to the following equations:

\[
Total \, Solids \left(\frac{g}{L}\right) = (B - A) \times \frac{1,000}{\text{sample volume, mL}} \tag{1}
\]

where \( A = \) mass of dish, g
\( B = \) mass of dried residue + dish, g

\[
Total \, Solids \, (\%) = \frac{D}{C} \times 100\% \tag{2}
\]

where \( C = \) mass of wet solids (g)
\( D = \) mass of dry solids = \( B - A \) (g)

In order to determine the volatile solids content according to Standard Method 2540E, the dried residue of the sample from the total solids determination is ignited in a muffle furnace at 550°C for one hour. The dish is then cooled and stored in a dessicator until it reaches room temperature. The ashed sample is then reweighed and the volatile solids determined:

\[
Volatile \, Solids \left(\frac{g}{L}\right) = (B - E) \times \frac{1,000}{\text{sample volume, mL}} \tag{3}
\]

where \( B = \) mass of residue + dish before ignition (g)
\( E = \) mass of residue + dish after ignition (g)

\[
Volatile \, Solids \, (\%) = \frac{F}{D} \times 100\% = \frac{VS\left(\frac{g}{L}\right)}{TS\left(\frac{g}{L}\right)} \times 100\% \tag{4}
\]

where \( D = \) mass of dry solids = \( B - A \) (g)
\( F = \) mass of ignited volatiles = \( B - E \) (g)
APPENDIX B – Lignin, Cellulose & Hemicelluloses Determination Procedure

From Harper SHT and Lynch JM (1981)

1. Sample preparation
Samples of wheat straw were dried at 60°C (16 h). Straw was cut at either side of the nodes and the leaves and internodes separated. The leaf base was removed from each node by hand to leave the node 'core'. Samples contained (by weight) 60.9% internodes, 31.9 % leaves, 3.6 % leaf bases and 3.6 % node 'cores'.

2. Gravimetric analysis
Chemical components were determined by sequential extraction followed by weighing, using methods modified from those described by Allen et al. (1975). Subsamples (about 1 g) were cut into 1 cm lengths with the nodes and internodes split longitudinally, then dried at 60°C (16 h) and weighed into 125-ml conical flasks.

2.1. Hot-water-soluble materials
Water (75 ml) was added and boiled gently for 1 h. The water was changed and boiled again for 1 h. The straw was washed once with cold water, dried at 60°C overnight (minimum 15 h) and weighed.

2.2. Lignin
Water (30 ml), 10 % aqueous acetic acid (2 ml) and sodium chlorite (0.6 g) were added. Samples were heated at 75°C for 1 h and more acid (2 ml) and sodium chlorite (0.6 g) then added. After 2h samples were washed with water (five times), acetone (twice) and ether (once), then dried at 105°C (90 min) and weighed.

2.3. Hemicelluloses
24% KOH (20 ml) was added and left in air for 2 h at 20°C. Samples were then washed with water (five times), 5% aqueous acetic acid (once), water (once), acetone (once) and ether (once), then dried at 105°C (90 min) and weighed.

2.4. Cellulose
The weight of the residue, corrected for ash content, was taken as cellulose.
APPENDIX C – Calorific Value Procedure

Company: IKA
Model: C200

Starting
- Turn on the bomb calorimeter by flicking the on/off switch at the back of the machine.
- Have a sufficient amount of tap water at room temperature available (each test uses approximately 2L of water.)

Calorific measurement/calibration
- Weigh the fuel mass to burn at a precision of 4 digits after the comma (0,0000 g).
- Record the mass of the fuel in the calorimeter.
- Set up the bomb: put the fuel in the crucible, make touching the cotton to the fuel, close and seal the bomb and fill the bomb with pure oxygen at a minimum pressure of 3000 kPa.
- Put the electrical connector on the bomb.
- Put the bomb in the calorimeter.
- Fill up the calorimeter exterior tank with room temperature tap water.
- Close the calorimeter and start the process.

Depending on the chosen mode (automatic or manual), the calorific value will be expressed by the machine or will need to be calculated with the known increase of temperature of water.

When calibration mode is chosen instead, the machine will expressed the heat capacity (C [J/K]) of the whole system. The fuel to use for this step is benzoic acid and his calorific value is 26, 460 MJ/kg).
- When the process is done by the calorimeter and all the required data is known, the calorimeter can be open, the water will be automatically pumped out, the bomb can be removed and his pressure can be released with the right tool.
- Open the bomb and make all the fuel had been burned. In a case of unburned fuel, the test has to be canceled.
- Wash carefully all the parts of the bomb before next use.

Shutting down:
- Flick the on/off switch, at the back of the calorimeter, in off position.
- Make sure the oxygen gas cylinder valve is closed.
APPENDIX D – Ultrasonication Procedure

No ultrasonic procedure is standardized in the scientific community yet. Thus, the handling presented here has been developed for our specific needs and had been inspired by the other available research on the subject.

Apparatus
- Graduated cylinder
- Ultrasonication chambers (beakers)
- Sonicator (generator, converter, booster, probe, stack assembly stand)
- Alcohol thermometer
- Scale (+/- 0.01g)

Before ultrasonic treatment and reagents
1. Prepare the sample to be sonicated (volume, TS concentration, desired particle size).
2. Screw the probe, booster and converter with sufficient torque force.
3. Maintain the stack assembly in a vertical position by the converter case.
4. Record the original sample temperature.

Ultrasonic treatment procedure
1. Introduce the probe at a minimum depth of 1.5 X the probe diameter in the sample to be sonicated.
2. Select the desire amplitude (%) and ultrasonication time of the treatment.
3. Perform the ultrasonication.

N.B.1: The depth probe rule is to avoid the scum formation and air introduction in the media which reduces the acoustic transmission.
N.B.2: Aerosols can be created during ultrasonication,
N.B.3: Due to the unpleasant noise, ear protection should be worn.

After ultrasonic treatment
1. Record the sample temperature.
2. Record the energy consumption (J).
APPENDIX E – Total Organic Carbon Procedure
Calibration curve and QC standard TOC determination

1. You may wish to dilute your (stock) standard solution depending on the range you wish
to measure. Do so as necessary.
2. There is no need to make more than one calibration standard, as the TOC machine can
dilute the standard internally. There are restrictions to the automatic dilution that the TOC
machine can perform. Dilutions up to 50 fold are possible. Dilutions of less than 20 are
recommended by experienced users.
3. Pour the standard solution into a 125 mL Erlenmeyer flask and cover with parafilm.
Puncture a small hole to fit the sampling tube from the TOC machine into the standard
solution.
4. Perform 4-5 different dilutions of this same standard;
5. Using the original undiluted standard solution, perform quantitative dilution(s) of
appropriate magnitude as to be useful for QC tests. In other words, this (or these)
standards should be in the expected measurement range for the samples you will be
analyzing. Consult the dilution table below to decide which dilution(s) will be most
helpful for your QA program.

<table>
<thead>
<tr>
<th>V pipet (mL)</th>
<th>Volume of Florence Flasks (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td>25.0</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td>3</td>
<td>8.3</td>
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<tr>
<td>4</td>
<td>6.3</td>
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<td>4.2</td>
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<td>3.6</td>
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<td>8</td>
<td>3.1</td>
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<td>10</td>
<td>2.5</td>
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<td>15</td>
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<td>20</td>
<td>1.3</td>
</tr>
<tr>
<td>25</td>
<td>2.0</td>
</tr>
<tr>
<td>30</td>
<td>1.7</td>
</tr>
<tr>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Preparation of Standard Curve:
The plot and r2 value are computed for you in the TOC machine. Transcribe them into your
calibration records.
References:
TOC Manual, Shimadzu, Tokyo.

Measurement Procedure
   - Check Dilution Water
   - Check Acid;
   - Check Drain Vessel Water Level (located on floor - if full, pour down drain with lots of water);
   - Check Humidifier Water Level.
3. Open gas cylinder. Check to make sure that the delivery pressure is not below 300 psi, preferably 400 psi is a minimum for good results. Notify the Lab Technician (Joanne) when pressure is below 500 psi.
4. Turn on the TOC by pressing the ON button on bottom right corner of instrument.
5. Once initialization is finished, press Measure Sample button on keypad. You are now in the ‘measurement parameters’ page.
6. Press F5 (NPOC) to measure TOC [unless high VFA in sample then use TC - IC = TOC].
7. Enter the Sample Name using the keypad then press Enter.
8. To set up calibration curve options, press down arrow (V) (you should now be in the TC menu). With your cursor in the first field “Calib. 1st”, you have the option of which calibration curve you wish to use. Press F1 (Calibration curve) to see the details of each calibration curve. Each curve has a number associated with it. When you are done choosing the curve, return to the ‘measurement parameters’ page by pressing F1 (cancel) again.
   Press the number on the key pad corresponding to the calibration curve you wish to use. You may want to use the same one you always use, or the most recent calibration or the most accurate one. Press ‘enter’ after keying in the digit.
   Press down until you reach the “Inj #” field. This is the minimum number of injections that the machine will make its determination for the parameter in question. Press ‘2’ then ‘enter’.
   Similarly, press down once more and you will be in the “Max. Inj #” field. This allows the machine to make another determination if the criteria (standard deviation and coefficient of variation) are not satisfied by the first two injections. Press ‘3’ and ‘enter’.
   Then press the right arrow (>) and you will be in the next menu (IC). Once again chose the calibration code as you have done with the TC, then the number of injections (2) and max. number of injections (3). Press enter after each entry or it will not register.
9. To start analysis, press Next button (bottom right corner of keypad) and place tubing in sample. Press Start button (bottom left corner of keypad). Wait for analysis to finish.

10. For the next sample, type in the sample name followed by Enter, Next, place tubing in sample and then press Start.

11. When you have finished the last sample, press Measure Sample button. This will return you to main screen. Press F1 (Standby Option) key. Press F5 (Power OFF) key. Press F6 (Execute) key. Press F6 (Yes) key.

12. When the gas flow indicator shows no flow, close the gas cylinder.

13. Do not turn off the TOC machine by pressing the power button. It will continue to run its fan to cool down the furnace. When the furnace reaches a suitable temperature, the TOC will shut itself off.

**IF YOU ARE USING THE TOC MACHINE, YOU MUST FOLLOW THESE STEPS WHEN ANALYZING YOUR SAMPLES**

1. **FRESH MILLI-Q WATER** (i.e. the Milli-Q sample must be replenished after each use, and not be allowed to sit out on the counter for multiple days! MUST BE RUN THROUGH THE MACHINE AS FOLLOWS:
   - 2 INJECTIONS OF ‘TC’ AND ‘IC’ PRIOR TO STARTING ANALYSES
   - 1 INJECTION OF ‘TC’ AND ‘IC’ AFTER EVERY 5 SAMPLES
   - 2 INJECTIONS OF ‘TC’ AND ‘IC’ AFTER FINISHING ANALYSES

2. **SAMPLES MUST BE PARTICULATE FREE:** MEANING, IF REQUIRED, ALL SAMPLES SHOULD BE FILTERED THROUGH A 0.45 μm FILTER PRIOR TO ANALYSIS
APPENDIX F – BMP Procedure
The BMP test as defined by Owen et. al (1979) was developed to determine the methane potential of potential feedstocks for an anaerobic digester in a cheap and effective way. The BMP test is a batch scale test which estimates the total biogas production of a feedstock in an optimum environment.

Apparatus
- Drying Oven
- Ashing Oven
- Scale (+/- 0.01g)
- 500 mL Wheaton serum bottle, with rubber seals and metal lids
- Glass syringe with 20 mm gauge needles
- pH meter
- 35°C incubator
- N₂/CO₂ (70:30 v/v) gas supply
- Gas standards
- Various glassware
- 60 ml glass syringe

Reagents
- Ammonium Phosphate (NH₄)₂HPO₄
- Calcium Chloride Dihydrate CaCl₂-2H₂O
- Ammonium chloride NH₄Cl
- Magnesium Chloride Hexahydrate MgCl₂-6H₂O
- Potassium chloride KCl
- Manganese Chloride MnCl₂-4H₂O
- Cobalt Chloride CoCl₂-6H₂O
- Boric Oxide H₃BO₃
- Copper (II) Chloride Dihydrate CuCl₂-2H₂O
- Sodium Molybdate Na₂MoO₄-2H₂O
- Zinc Chloride ZnCl₂
- Ferrous Chloride FeCl₂-4H₂O
- Resazurin
- Biotin
- Folic Acid
- Pyridoxine hydrochloride
- Riboflavin
- Thiamin
- Nicotinic Acid
- Pantothenic acid
- Vitamin B12 (cyanocobalamin)
- cyanocobalaminB₁₂
- p-aminobenzoic acid
- Thiocetic acid

As summarized in Table 1, the following solution must be created to prepare the stock solution. Stock 1 (S1) is used to detect oxygen contamination, as resazurin appears pink when oxidized. Stock 4 (S4) is used to provide a reducing environment.
<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Compound</th>
<th>Concentration (g/L)</th>
<th>Volume Needed per 3L (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Resazurin</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>S2</td>
<td>(NH₄)₂HPO₄</td>
<td>26.7</td>
<td>5.4</td>
</tr>
<tr>
<td>S3</td>
<td>CaCl₂-2H₂O</td>
<td>16.7</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>NH₄Cl</td>
<td>26.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MgCl₂-6H₂O</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>86.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MnCl₂-4H₂O</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CoCl₂-6H₂O</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H₃BO₃</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CuCl₂-2H₂O</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Na₂MoO₄-2H₂O</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>ZnCl₂</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>FeCl₂-4H₂O</td>
<td>370</td>
<td>18.0</td>
</tr>
<tr>
<td>S5</td>
<td>Na₂S-9H₂O</td>
<td>500</td>
<td>1.8</td>
</tr>
<tr>
<td>S6</td>
<td>Biotin</td>
<td>0.002</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Folic Acid</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyridoxine hydrochloride</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Riboflavin</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thiamin</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nicotinic Acid</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pantothenic acid</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B₁₂</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-aminobenzoic acid</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thiocetic acid</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1 - Concentrated Stock Solutions for BMP Test (Owen et al., 1979)**

**Procedure - Stock Solution**

1. Add 1 L of deionized water to 2L volumetric flask
2. Add 1.8 mL of solution 1 (S1), 5.4 mL of solution 2 (S2) and 27.0 mL of solution 3 (S3).
3. Add deionized water up to 1 800 mL mark.
4. Boil for 15 minutes while flushing with N₂ gas at approximately 1L/minute.
5. Cool to room temperature (continue flushing with N₂ gas).
6. Add 18 mL of solution 4 (S4), 1.8 mL of solution 5 (S5) and 1.8 mL of solution 6 (S6).
7. Change gas to 30% CO₂, 70% N₂ mixture and continuous flushing at 1 L/min
8. Add 8.4 g NaHCO₃ as powder.
9. Bubble 30% CO₂:70% N₂ mixture through porous diffuser until media pH stabilizes at approximately 7.1.
10. Carefully seal volumetric flask while minimizing introduction of air into container store in refrigerator.
<table>
<thead>
<tr>
<th>Volume of Solution</th>
<th>1L</th>
<th>2L</th>
<th>3L</th>
<th>4L</th>
<th>5L</th>
<th>6L</th>
<th>10L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water (mL)</td>
<td>500</td>
<td>1000</td>
<td>1500</td>
<td>2000</td>
<td>2500</td>
<td>3000</td>
<td>5000</td>
</tr>
<tr>
<td>S1 (mL)</td>
<td>0.9</td>
<td>1.8</td>
<td>2.7</td>
<td>3.6</td>
<td>4.5</td>
<td>5.4</td>
<td>9.0</td>
</tr>
<tr>
<td>S2 (mL)</td>
<td>2.7</td>
<td>5.4</td>
<td>8.1</td>
<td>10.8</td>
<td>13.5</td>
<td>16.2</td>
<td>27.0</td>
</tr>
<tr>
<td>S3 (mL)</td>
<td>13.5</td>
<td>27</td>
<td>40.5</td>
<td>54</td>
<td>67.5</td>
<td>81</td>
<td>135</td>
</tr>
<tr>
<td>S4 (mL)</td>
<td>0.9</td>
<td>1.8</td>
<td>2.7</td>
<td>3.6</td>
<td>4.5</td>
<td>5.4</td>
<td>9.0</td>
</tr>
<tr>
<td>S5 (mL)</td>
<td>0.9</td>
<td>1.8</td>
<td>2.7</td>
<td>3.6</td>
<td>4.5</td>
<td>5.4</td>
<td>9.0</td>
</tr>
<tr>
<td>S6 (mL)</td>
<td>9.0</td>
<td>18.0</td>
<td>27.0</td>
<td>36.0</td>
<td>45.0</td>
<td>54.0</td>
<td>9.0</td>
</tr>
<tr>
<td>NaHCO₃ (g)</td>
<td>4.2</td>
<td>8.4</td>
<td>12.6</td>
<td>16.8</td>
<td>21.0</td>
<td>25.2</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Table 2 - (Owen et al., 1979)

Procedure - Preparation of Assay

1. Remove stock solution at least 20 minutes before assay preparation, while mixing with 30% CO₂ and 70% N₂ gas mixture.
2. Place correct volume of stock solution into each 500 mL Wheaton bottle.
3. Place 20 mL of fresh Inoculum into each 500 mL Wheaton bottle (if applicable).
4. Place 0.50 g of 1.0 mm ground chicken manure sample into each assay (if applicable).
5. Purge each bottle with 30% CO₂, 70% N₂ gas mixture for 3 min in liquid portion.
6. Carefully seal 500 mL Wheaton bottle with a rubber seal while minimizing introduction of air into container. Crimp a metal lid onto top of bottle.
7. Carefully puncture rubber seal with a 20 mm gauge needle and attach a closed valve to the top of the needle.
8. Carefully shake bottle and place into pre-heated incubator.

Procedure - Sampling

During the incubation, each assays is sampled for biogas production. Testing at regular intervals will produce more consistent data, but too frequent testing may cause disruptions to the BMP test. On average, 10 mL or more of biogas should be produced between each measurement.

1. Remove Wheaton bottle from incubator.
2. Connect valve to syringe and measure gas production volume.
3. Remove valve from syringe. **Record.**
4. Replace Wheaton bottle back into incubator.
# APPENDIX G – BMP Bottles Content Summary

<table>
<thead>
<tr>
<th>Bottle assay</th>
<th>Owen’s nutrient [ml]</th>
<th>Raw [ml]</th>
<th>Raw [g]</th>
<th>TS [g]</th>
<th>VS [g]</th>
<th>Feedstock Raw [g]</th>
<th>TS [g]</th>
<th>VS [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ground wheat straw</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>0,5g</td>
<td>0,45</td>
<td>0,43</td>
</tr>
<tr>
<td>Ground hay</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>0,5g</td>
<td>0,45</td>
<td>0,41</td>
</tr>
<tr>
<td>Ground switch grass</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>0,5g</td>
<td>0,43</td>
<td>0,40</td>
</tr>
<tr>
<td>Paper sludge</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>2g</td>
<td>0,43</td>
<td>0,27</td>
</tr>
<tr>
<td>FOG</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>0,25g</td>
<td>0,25</td>
<td>0,25</td>
</tr>
<tr>
<td>Cut wheat straw</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>0,5g</td>
<td>0,45</td>
<td>0,43</td>
</tr>
<tr>
<td>Ground wheat straw</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>2g</td>
<td>0,51</td>
<td>0,48</td>
</tr>
<tr>
<td>Ground hay</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>2g</td>
<td>0,48</td>
<td>0,44</td>
</tr>
<tr>
<td>Ground switch grass</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>2g</td>
<td>0,58</td>
<td>0,53</td>
</tr>
<tr>
<td>Paper sludge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOG</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>@2,3% TS</td>
<td>0,51</td>
<td>0,31</td>
</tr>
<tr>
<td>Cut wheat straw</td>
<td>50</td>
<td>50</td>
<td>48,4</td>
<td>0,774</td>
<td>0,465</td>
<td>8,6 ml @3% TS</td>
<td>0,25</td>
<td>0,25</td>
</tr>
</tbody>
</table>

Table G1 – Nominal mass introduced in BMP assay bottles
<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Unsonicated</th>
<th>Sonicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground wheat straw</td>
<td>90.8%</td>
<td>25.7%</td>
</tr>
<tr>
<td>Ground hay</td>
<td>89.8%</td>
<td>24.1%</td>
</tr>
<tr>
<td>Ground switch grass</td>
<td>85.5%</td>
<td>29.1%</td>
</tr>
<tr>
<td>Paper sludge</td>
<td>21.7%</td>
<td>3%</td>
</tr>
<tr>
<td>FOG</td>
<td>99.7%</td>
<td>3%</td>
</tr>
<tr>
<td>Cut wheat straw</td>
<td>90.8%</td>
<td>22.5%</td>
</tr>
</tbody>
</table>

**Table G2 – TS and VS concentration of sonicated and unsonicated feedstock**

**Calculus for Table G1**

**Ground wheat straw**
Unsonicated: 0.5 g raw * 90.8% = 0.45g TS; 0.45g TS * 94.9% = 0.43g VS  
Sonicated: 2g raw * 25.7% = 0.51g TS; 0.51g TS * 94.9% = 0.48g VS

**Ground hay**
Unsonicated: 0.5g raw * 89.8% = 0.45g TS; 0.45g TS * 91.5% = 0.41g VS  
Sonicated: 2g raw * 24.1% = 0.48g TS; 0.48g TS * 91.5% = 0.44g VS

**Ground switch grass**
Unsonicated: 0.5g raw * 85.5% = 0.43g TS; 0.43g TS * 91.9% = 0.40g VS  
Sonicated: 2g raw * 29.1% = 0.58g TS; 0.58g TS * 91.9% = 0.53g VS

**Paper sludge**
Unsonicated: 2g raw * 21.7% = 0.43g TS; 0.43g TS * 62.5% = 0.27g VS  
Sonicated:  

\[
2.3\% \text{ TS} = \frac{\text{mass of solid}}{\text{mass of water}} = \frac{21.7\% \text{TS} \times 35\text{g raw}}{300\text{g H}_2\text{O} + (78.3\% \times 35\text{g raw})}
\]

To get 2g raw from a solution of 35g raw in 300ml of water, you need 17.3 ml, if perfect emulsification:

\[
300\text{ml} = 34.7\text{g raw} \\
x = 2\text{g raw}
\]
Thus, to have 2 g @ 21,7% TS, we need 17,3 ml from a sonicated solution at 2,3% TS.

**FOG**

Unsonicated: 0,25g raw * 99,7% = 0,25g TS; 0,25g TS * 100% = 0,25g VS  
Sonicated:  

\[
3\%\ TS = \frac{\text{mass of oil}}{\text{mass of water}} = \frac{(300 - x) \times 0.92 \, \text{g/ml}}{x}
\]

Here, the mass (or volume) of water is 290,5g. Thus the volume of oil has to be 9,5 ml, which is 8,74 g oil. Now:

\[
300\, \text{ml} = 8,74 \, \text{g raw}  
\]
\[
x = 0,25\, \text{g raw}
\]

Thus, to have 0,25g of oil, we need 8,6 ml from a sonicated solution at 3% TS.

**Cut wheat straw**

Unsonicated: 0,5 g raw * 90,8% = 0,45g TS; 0,45g TS * 94,9% = 0,43g VS  
Sonicated: 2g raw * 22,5% = 0,45g TS; 0,45g TS * 94,9% = 0,43g VS
APPENDIX H – Biogas Composition Analysis Procedure, Calibration Curves and Standards.
Company: Agilent technologies
Type: Thermal conductivity detector (TCD)
Model: 6890N

Procedure:
Starting:
Open argon and air gas cylinder.
- Turn on GC by pushing the button located in the bottom left of the GC.
- Turn on the computer.
- Open the software by double clicking on the “Instrument 1 Online” icon.
- Load the right GC method. In the menu bar, click on “Method” and choose “load method” from the drop down menu. Choose “Methane Salsali.M” and click “OK”.

To analyse samples/standards:
- In the menu bar, click “Run Control”. Choose “Sample Info” from the drop down menu.
- Change the subdirectory to initials with current date (yyymmdd). Ex: MM121223
- Fill in the samples /standards name and fill any comments if desired.
- Click “OK”.
- Inject sample.
- Hit F5 on the computer keyboard.
- Press the START button on the GC. Wait approximately 7 minutes for the run to end.
For the next sample/standard, change the sample name, inject, hit F5 and press start on the GC.

Shutting down:
- Close the software
- Turn off the computer.
- Turn off the GC by depressing the button located in the bottom left of the GC.
- Close the air and argon gas cylinder
For calibration, different known fraction gas samples were injected to the GC. Each gas fraction test was injected three times.
APPENDIX I – Calculation of Ultrasonic Efficiency

The overall ultrasonic efficiency (Eff) is calculated using:

\[ \text{Eff} = \frac{E_{\text{out}} - E_{\text{in}}}{E_{\text{in}}} \times 100\% \]

Where \( E_{\text{out}} \) represent the extra methane production subsequent to the ultrasonic pretreatment and is defined as follow:

\[ E_{\text{out}} = \Delta M \times E_{\text{methane}} \]
\[ \Delta M = M_s - M_u \]

The output energy (\( E_{\text{out}} \)) thus depends on the product of the difference between the methane yield of the sonicated sample (\( M_s \), ml CH\(_4\)/g VS) and the unsonicated sample (\( M_u \), ml CH\(_4\)/g VS), and the energy content of methane (\( E_{\text{methane}} \)). The energy content used for the computation is 38.2 J/ml (Walsh et al., 1988). Also, \( E_{\text{in}} \) (J/g VS) is the energy used for each ultrasonication test:

\[ E_{\text{in}} = \frac{P \times t}{V \times VS} \]

Here, \( P \) is the power in Watt; \( t \), the ultrasonication time in second; \( V \), the volume of the sample in ml and; \( VS \), the volatile solid concentration of the sample (g VS/ml).
APPENDIX J – Solution Preparation Calculus

\[
TS\% = \left( \frac{\text{mass of solid}}{\text{mass of water}} \right) \times 100
\]

Wheat straw (2% TS)

\[
2\% = \frac{x \times 90.8\%}{300 + (x \times 9.2\%)}
\]

We thus need 6,63g of straw in 300ml of water to have a 2% TS wheat straw-water solution.

Hay (2% TS)

\[
2\% = \frac{x \times 89.8\%}{300 + (x \times 10.2\%)}
\]

We thus need 6,74g of hay in 300ml of water to have a 2% TS hay-water solution.

Switch grass (2% TS)

\[
2\% = \frac{x \times 85.5\%}{300 + (x \times 14.5\%)}
\]

We thus need 7,06g of switch grass in 300ml of water to have a 2% TS switch grass-water solution.

Paper sludge (2,3% TS)

\[
2.3\% = \frac{x \times 21.7\%}{300 + (x \times 78.3\%)}
\]

We thus need 34,7g of paper sludge in 300ml of water to have a 2% TS paper sludge-water solution.

FOG (3% TS) \((\rho_{oil} = 0.92)\)

\[
3\% = \frac{(300 - x) \times 0.92}{x}
\]

We thus need 8,74g (9,5ml) of oil in 300ml of water to have a 3% TS oil-water solution.
APPENDIX K – SAS code for unsonicated vs. sonicated biogas mean differentiation

Title "CH4 content (%) for unsonicated & sonicated cut and ground wheat straw biogas";
/*1 = Unsonicated and Ground*/
/*2 = Sonicated and Ground*/
/*3 = Unsonicated and Cut*/
/*4 = Sonicated and Cut*/
data Fraction;
input group time pourcentage @@;
datalines;
1 1 13.33 1 2 27.81 1 3 35.27 1 4 41.57 1 5 37.19 1 6 27.15 1 8 4.63 1 9 39.59 1 10 15.41 1 11 47.65 1 13 58.57 1 15 62.0 1 17 64.59 1 19 61.23 1 21 57.11 1 24 42.11 1 27 63.79 1 33 65.23 1 41 65.93 1 49 65.93 1 2 1 15.95 2 2 16.25 2 3 25.93 2 4 23.81 2 5 12.41 2 6 24.83 2 8 13.21 2 9 54.21 2 10 57.97 2 11 59.95 2 13 62.69 2 15 62.0 2 17 62.49 2 19 61.99 2 21 61.39 2 24 63.23 2 27 64.19 2 33 65.67 2 41 69.01 2 49 65.11 3 1 13.07 3 2 10.99 3 3 18.85 3 4 22.45 3 5 22.43 3 6 21.67 3 8 42.35 3 9 36.95 3 10 19.25 3 11 24.19 3 13 54.01 3 15 62.57 3 17 59.19 3 19 62.15 3 21 61.57 3 24 64.11 3 27 64.33 3 33 65.85 3 41 64.71 3 49 65.47 4 1 14.75 4 2 24.43 4 3 32.01 4 4 38.47 4 5 38.77 4 6 43.93 4 8 39.95 4 9 45.39 4 10 37.07 4 11 28.63 4 13 55.77 4 15 61.91 4 17 61.91 4 19 63.57 4 21 61.87 4 24 66.41 4 27 63.21 4 33 64.53 4 41 67.83 4 49 66.23
run;
proc print;
proc mixed plots=all;
class time group;
model pourcentage = time group;
repeated time/type=cs subject = time*group;
lmeans time/pdiff cl adjust=tukey;
run;
******************************************************************************

This code is the one used for the biogas statistical analysis. Similar codes were used to measure the potential difference in the means for biogas production, yield, and digestion rate.
APPENDIX L – Laboratory standards for TOC determination

Those procedural blanks measurement are used to know the already present organic carbon in deionised water; the water used for all the solution preparation.

A KHP solution of 103.1 mg/L of NPOC had been prepared to measure the sensibility of the analyzer through the experimental time period.
The zero of the carbon analyzer had been assessed with Milli-Q water.

Some measurement had been done in duplicate to include this error in the standard deviation calculus.