CHEMICAL ENGINEERING

EFFECTS OF ULTRASONIC TREATMENT ON LIGNOCELLULOSIC MATERIAL IN TERMS OF SOLUBILIZATION OF ORGANIC MATTER AND ANAEROBIC DIGESTION PROCESS YIELDS

EFECTOS DEL TRATAMIENTO DE ULTRASONIDO EN MATERIAL LIGNOCELULÓSICO EN TÉRMINOS DE SOLUBILIZACIÓN DE MATERIA ORGÁNICA Y SU FUNCIÓN EN EL PROCESO DE DIGESTIÓN ANAEROBIA

To be awarded the Degree in Chemical Engineering:

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A mi familia, todo lo que soy es por ellos
A mis amigos por su fe incondicional en mí,
y a Roberto por ser un apoyo constante que siempre
me ayuda a mantener el equilibrio
Gracias
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1. INTRODUCTION TO THE STUDY

Renewable energies currently represent a more profitable and beneficial alternative to traditional sources, due to their long lifespan, as they constitute a cycle of substrate and clean product with possibility of waste utilization, thus obtaining what is known as an energy source named "clean". The growing inherent need for energy in society, with increased consumption, has led to think of alternatives for obtaining and improving its production with pre or post treatments that will help in the final performance.

These problems associated with fossil fuels are more than known, as these may no longer meet the demand associated with economic growth, due to its limited life expectancy, their future exhaustion and because they are non-renewable energy sources and there for their waste produced constitutes a very high percentage of pollution in the planet.

Economic growth has always been accompanied by growth of energy consumption, and that even taking into account the progresses made in energy efficiency.

Many economic crises are due to the lack of these fossil fuels. It has been shown through empirical studies that increased oil prices reduce demand for economic factors, as companies increase their production costs, both capital and labor, as well as oil. This has a direct influence on the value added of production, which in turn can cause an economic slowdown.

Thus there has been an increasingly growing bet on renewable energy sources. In fact, there is an increasing trend to promote these alternatives to fossil fuels because of their more environmentally beneficial aspects. One of the candidates for the alternative fuel is hydrogen, or biomass. By definition, biomass represents the biological material of any living organism. Biomass has rich energy content and can be converted into heat and electricity in many ways: thermally, chemically, electrochemically, photo - chemically or biochemically.

By building biogas plants through anaerobic digestion processes, agriculture provides this sector with an important contribution to the distribution of energy produced from renewable resources and the elimination of organic waste. The by-product of the biogas production is the digested, which in turn can be used as high-quality fertilizer.
Currently, apart from trying to find new sources of sustainable energy with the environment, various methods are also studied in order to improve the efficiency of these treatments through pre and post treatments that have influence on a number of factors, such as kinetics of the organic substance degradation process, the characteristics of the substrate that is going to be used or the operating mode selected.

In this case an ultrasound pretreatment was used as a method of improving process efficiency, where the treated substrate is lignocellulosic, which represents one of the main challenges to solve, since it is hardly biodegradable material because of its lattice structure of lignin. Another challenge was the need to use solvents for degradation, which makes the process complicated with regard to environmentally friendly aspects as well as economically unsustainable.

The second challenge lies in the unknown chemical components and content in biomass. Biomass from different sources has varied content of cellulose, hemicellulose, lignin, free sugars, wax, proteins, alkaloids, organic and inorganic compounds, which are difficult to predict.

The third and most important challenge is the heterogeneous reaction systems of biomass. Biomass materials have a low thermal conductivity that creates a barrier to heat and mass transfer.

Conversion and product selectivity in biomass systems tend to be poor due to insufficient contact between catalyst and reactant.

Ultrasonic energy provides processing biomass systems with a special physic-chemical environment. The impact of high energy and ultrasonic corrosion contributes to the possibility of easier processing, and more fractionation of the particles that make biomass, as chemical reactions take place more easily, which means an increase in efficiency reaction and higher catalytic activity on thermochemical methods. Applying ultrasonic energy enhances mass and heat transfer in the reactions, and improves the contact separating heterogeneous reactants, intermediates and products, and thus accelerates the reaction rate or changes the kinetics.

In summary of the above, this project is based on experimental work, with the main objective to evaluate the effects of ultrasound treatment on the substrate used of biological nature, about how the kinetics of the process evolves and impacts the
effectiveness of biogas production. In the experimental setup an eight batch type reactor has been used, using as a feed a mixture of lignocellulosic residues, where a percentage of it corresponds to untreated material, while the remaining percentage has been treated with different ultrasound volumetric power input, and having four kinds of different conditions to be studied (the experiment is performed in duplicate).

Therefore, this mix of treated and untreated substrate is intended to reflect the effect of recirculation on the end product of an anaerobic digestion system, in which the resulting pre ultrasound treatment will be integrated, emulating what would be a configuration that tries to approach a semi continuous mode of operation, and analyses therefore will focus on the effect of such settings in terms of process efficiency and yield.

The analyses are focused on testing the treated ultrasound material to see the change in it. Secondly, background tests will be performed to materials subjected to anaerobic digestion,(using for it different mixtures of treated and untreated material),at the beginning, during and at the end of the test included, to assess in this case the evolution of the kinetics of the process, as well as the effectiveness of this new scheme of biogas production.

1.1. Principle of anaerobic digestion

The anaerobic digestion process is a biological process in which decomposition of organic matter and inorganic matter takes place in the absence of oxygen, in contrast to the aerobic biological process, where the process involves oxygen:

\[
\begin{align*}
\text{Aerobic:} & \quad \text{Glucose} + 6\text{O}_2 & = 6\text{CO}_2 + 6\text{H}_2\text{O} & \Delta G = -2826 \\
\text{Anaerobic:} & \quad \text{Glucose} & = 3\text{CO}_2 + 3\text{CH}_4 & \Delta G = -403
\end{align*}
\]

Financial incentives in many European countries have led to increased anaerobic digestion facilities (AD) to produce heat and / or electricity from biogas. The environmental impacts of the life cycle of a system of biogas production from agricultural residues that came from AD and cogeneration of heat and electricity in a combined heat and power production (CHP), suggest that it may lead to significant reductions in most of the environmental impacts in comparison to the alternatives that fossil fuels offer, including global warming potential (GWP) which can be reduced by up to 50% (Andrew Whiting et al, 2014).
Process efficiency depends inter alia on: the mixing regime, temperature, total solids, volatile solids, the hydraulic retention time, and mainly on the type of material introduced as feed rate, because it will make these parameters change. Numerous types of substrates have been used in the anaerobic digestion process, such as agricultural residues, manure, municipal solid waste, grasses (including wheat straw, rice and sorghum), which constitute an abundant supply of biomass, much of which is a product of waste food production such as waste of fruits and vegetables.

The advantages of anaerobic versus aerobic processes include lower biomass yield, lower energy requirements, fewer added nutrients, energy recovery from methane and higher volumetric capacity loads. Some of the disadvantages are the low rate of growth of microorganisms, the possible need for alkalinity pH control, production of odors and corrosive gases, and an additional treatment to meet discharge requirements (XJ Zhang 2014).

Anaerobic digestion as a definition is the process in which microorganisms break down biodegradable material in the absence of oxygen. This process generates several gases, including the most abundant: carbon dioxide and methane (depending on the degraded material as discussed in previous paragraphs). In bio-digesters, this release of gases is taken as an advantage for its use as fuel. The intensity and duration of the anaerobic process vary depending on various factors, which include the temperature and pH of biodegraded material.

**1.2. Main phases of anaerobic digestion**

Anaerobic digestion (AD) is a multistage process that can be summarized in four main stages as shown in the following diagram:
1.2.1. Hydrolysis

The first step is called hydrolysis. This is the transformation, mediated by enzymes, of the higher molecular weight compounds in simple monomers suitable for use as energy and cell carbon (for example, monosaccharides, amino acids, fatty acids, purines and pyrimidines, aromatics simple, etc.). These monomers can be easily absorbed by bacterium for fermentation. The main hydrolytic reactions are:

\[
\begin{align*}
(C_\text{n}H_{10}O_\text{y})_n + nH_2O &\to nC_\text{o}H_{12}O_\text{o} \\
(R-\text{CHNH}_2\text{COOH})_n + nH_2O &\to nR-\text{CHOH}_2\text{COOH} + nNH_3 \\
C_\text{a}H_\text{b}(\text{OCOR})_\text{c} + 3H_2O &\to C_\text{d}H_\text{e}(\text{OH})_\text{f} + 3\text{RCOOH}
\end{align*}
\]

Therefore, in this stage, there are three types of hydrolysis, depending on the hydrolyzed biopolymer:

1a hydrolysis of proteins
1b carbohydrate hydrolysis
1c lipid hydrolysis
Bacteria are usually not capable of assimilating particulate organic material, so this must be first degraded to soluble polymers or monomers. Therefore it is the hydrolysis process which involves the availability of substrates for anaerobic digestion and not, or only indirectly, the supply of particulate material to the reactor. The hydrolysis takes place by the action of cellulolytic enzymes, be they lipolytic, amylolytic, or proteolytic which can perform extracellularly; in this way, only lignin and some waxes are virtually inert to the process of anaerobic digestion (1988, Bermudez - Anaerobic Digestion JJ, LA).

Thus, these enzymes perform the role of catalysts in the reaction, being able to hydrolyze the cellulose by the cellulase, in cellobiose and glucose; or proteins by protease to polypeptides and amino acids; or finally lipids being hydrolyzed using lipase on fatty acids.

A catalyst is an entity that changes the chemical reaction rate, participating actively in it, but without becoming a product.
The main action is to reduce the potential energy barrier that the reactants must pass to form products. The introduction of the suitable catalyst in the system should favorably affect the values of A and E of the Arrhenius equation.

\[ k = A \cdot e^{-E/kT} \]

The presence of the catalyst is just to increase the reaction rate, but not to alter, in any way, the thermodynamic variables:

1. Cannot perform thermodynamically impossible reactions.
2. Do not alter the equilibrium constant.
3. Do not have influence on the heat of reaction, because it only depends on the reactants and products.

Because of this, hydrolysis is considered the limiting step of the kinetics of the process, and the factors influencing the lignocellulosic matrix biodegradability include therefore: lignin content, structure, cellulose and hemicellulose content, degree of cellulose crystallinity, particle size and specific surface area for the enzyme reaction and the pore volume (05-Mosier).

As mentioned in the introduction, one of the components that make difficult the process of obtaining biogas is the lignin from the lignocellulosic material which is used as substrate. The major components of the cell walls of plants are cellulose, hemicellulose, and lignin, which together form a complex and rigid structure. The cell walls of different plants vary greatly in appearance and properties. The complicated structure of the lignocellulosic
biomass contributes to their resistance to chemical and biological degradation. For example, in nature, the natural biodegradation of lignocellulosic biomass is slow because it requires the collective actions of many hydrolytic enzymes, including cellulase (endoglucanase, cellobiohydrolase, and beta-glucosidase), hemicellulase, and lignin-degrading enzymes.

Two main causes of the problem of lignocellulosic biomass in terms of enzymatic hydrolysis are thought to be: low accessibility of (micro)crystalline cellulose fibers, which significantly reduces the efficiency of cellulose, and (2) the presence of lignin (mainly) hemicellulose and cellulose surface, which prevents access to the cellulase substrate efficiently. Lignocellulosic biomass structured part consists of cellulose, hemicellulose and lignin. Cellulose (crystalline polymer of glucose) and hemicellulose (a complex amorphous polymer whose main component is a xylose monomer unit) constitute 60-90% by weight of the above ground biomass. Lignin, a large polyaromatic compound, is another important component of biomass.

As shown in image 3, the schematic location of these compounds in the lignocellulosic material and how the existence of a pretreatment would help the hydrolysis process are represented.
1.2.2. Acidogenesis

The second step is called acidogenesis, which converts the monomers resulting from the first stage such as sugars, amino acids, fatty acids, long-chain, organic monomers and oligomers. Simple organic substrates such as sugars and amino acids are oxidized to pyruvate, and subsequently become short chain fatty acids (C 1 -C 5) such as, mainly acetic, propionic, butyric, valeric, lactic acid and alcohols and ketones. These products form the starting substrate for the subsequent fermentation phase. Acetate and hydrogen produced in the early stages may be used directly by methane producing bacteria. Other molecules such as volatile fatty acids with a chain length greater than the acetate must go through a process of catabolism to be transformed as compounds that can be used by methane producing bacteria.

These volatile fatty acids are created along with ammonia, carbon dioxide, hydrogen sulfide and other byproducts:

*Fermentation of soluble carbohydrates:*

The major metabolic degradation pathway of organic acids to form glucose is the Embden-Meyerhof pathway, whose intermediary is the pyruvate.

Fermentation takes place by various microorganisms such as those associated with the degradation of glucose, which are Clostridium and convert glucose into butyric acid, acetic acid, CO2 and H2. Glucose is converted into pyruvate by the Embden-Meyerhof pathway, and pyruvate is unfolded to acetyl-CoA and CO2. Acetyl-CoA is reduced to fermentation products using the electron carrier as NADH derivative glycolytic reactions Embden-Meyerhof route.

*Aminoacid fermentation:*

The main products of the fermentation of amino acids and other hydrogenated molecules are short chain fatty acids, succinic, and N2 aminovaleric. Amino acid fermentation is considered a rapid process and, in general, does not limit the rate of degradation of protein compounds.

The final products of oxidation are NH3, CO2 and a carboxylic acid having one carbon atom less than the oxidized amino (n-butyric and isobutyric acid, isovaleric, caproic, hydrogen sulfide, metilcaptano, cadaverineetc ..)
Anaerobic oxidation of long chain fatty acids:

The long chain fatty acids are oxidized to short chain fatty acids by the beta-oxidation mechanism. Free fatty acids are introduced into the cell through the cell wall and once inside, are transformed into the corresponding CoA thioester. The β-oxidation is a spiraling cycle releasing an acetyl-CoA in each loop, producing acetic acid.

1.2.3. Acetogenesis

While some fermentation products can be metabolized directly by methanogenic organisms (H2 and acetic), others such as ethanol, volatile fatty acids and some aromatics must be transformed into simpler products such as acetate and H2, in order to bring to acetogenic bacteria.

From a thermodynamic standpoint, these reactions are not possible because under standard conditions (pH = 7, T = 25, P = 1 atm) they have positive free energies of reaction, as shown in the table below:

<table>
<thead>
<tr>
<th>Equation</th>
<th>ΔG° (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol + H₂O → Acetate + H⁺ + 2H₂</td>
<td>+5.6</td>
</tr>
<tr>
<td>Lactate⁻¹ + 2H₂O → Acetate⁻¹ + H⁺ + 2H₂ + HCO₃⁻</td>
<td>+4.2</td>
</tr>
<tr>
<td>Acetate⁻¹ + 4H₂O → H⁺ + 4H₂ + 2HCO₃⁻</td>
<td>+0.48</td>
</tr>
<tr>
<td>Propionate⁻¹ + 3H₂O → Acetate⁻¹ + HCO₃⁻ + H⁺ + 3H₂</td>
<td>+76.1</td>
</tr>
<tr>
<td>Butyrate⁻¹ + 2H₂O → 2Acetate⁻¹ + H⁺ + 2H₂</td>
<td>+48.1</td>
</tr>
<tr>
<td>Valerate⁻¹ + 3H₂O → 3Acetate⁻¹ + 2H⁺ + 3H₂</td>
<td>+96.2</td>
</tr>
<tr>
<td>Alanine + NH₄⁺ + H₂O → Acetate⁻¹ + HCO₃⁻ + NH₄⁺ + H⁺ + 2H₂</td>
<td>+7.3</td>
</tr>
<tr>
<td>Aspartate⁻¹ + 4H₂O → Acetate⁻¹ + 2HCO₃⁻ + NH₄⁺ + H⁺ + 2H₂</td>
<td>+4.9</td>
</tr>
<tr>
<td>Leucine + 3H₂O → transaminase⁻¹ + HCO₃⁻ + NH₄⁺ - H⁺ + 2H₂</td>
<td>+4.2</td>
</tr>
<tr>
<td>Glutamate⁻¹ + 4H₂O → propionate⁻¹ + 2HCO₃⁻ + NH₄⁺ + H⁺ + 2H₂</td>
<td>+5.8</td>
</tr>
<tr>
<td>Glutamine⁻¹ + 7H₂O → Acetate⁻¹ + 3HCO₃⁻ + NH₄⁺ + 2H⁺ + 5H₂</td>
<td>+70.3</td>
</tr>
</tbody>
</table>
But low H2 partial pressures of the order of $10^{-4} - 10^{-5}$ atm, in these reactions become thermodynamically favorable and the free energy change is sufficient to allow synthesis of ATP and bacterial growth. Therefore, the main inhibitor of the acetogenesis, whose accumulation causes the rapid one of substrates, is the accumulation of molecular hydrogen.

Some of the main chemical reactions that occur in this stage are:

$$
\begin{align*}
CH_3CHOHCOO^- + 2H_2O &\rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2 \\
CH_3CH_2OH + H_2O &\rightarrow CH_3COO^- + H^+ + 2H_2 \\
CH_3(CH_2)_2COO^- + 2H_2O &\rightarrow 2CH_4COO^- + H^+ + 2H_2 \\
CH_3CH_2COO^- + 3H_2O &\rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \\
4CH_3OH + 2CO_2 &\rightarrow 3CH_3COOH + 2H_2O \\
2CHCO_3^- + 4H_2 + H^+ &\rightarrow CH_3COO^- + 4H_2O
\end{align*}
$$

The oxidative capacity of bacteria involved is due to their ability to regenerate the coenzyme NADH which in its oxidized form NAD + is reduced as the end acceptor using H+ ions, according to the next reaction:

$$
\text{NADH} + H^+ \rightarrow \text{NAD}^+ + H_2
$$

1.2.4. Methanogenesis

The fourth step is called methanogenesis, converting the intermediates of the second stage in single end products, mainly CH4 and CO2. It is performed by two groups of organisms called: acetoclastic methanogens and methanogens using hydrogen. Acetoclastic methanogens use acetate as electron donor and electron acceptors, and acetate is divided into methane and carbon dioxide $(CO_2 + \text{CH}_4 \rightarrow \text{CH}_3\text{COOH})$.

The hydrogen used by the methanogens, using the latter as the electron donor and electron acceptor such as CO2, to produce methane: $4\text{H}_2 + 2\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{CH}_4$.

Approximately 72% of the methane produced from anaerobic digestion is acetate, and the remaining 28% is hydrogen (XJ Zhang, 2014).

The energy available in organic substances, except the one that is used for cell metabolism, is recovered by means of methane. The main reactions at this stage are:
1.3 Microbiology of anaerobic digestion

The microorganisms involved in the anaerobic digestion can be classified into two groups: (X and Z Yan Liu, 2011)

- **Nometanogens:**

  These fermentation bacteria deal with the first stage of AD (hydrolysis and acidogenesis). These bacteria are capable of excreting enzymes to hydrolyze macromolecular organic substances in small molecular products, which could be used as nutrients for personal growth, and used by other microorganisms.

- **Acetogenic bacteria producing hydrogen:**

  This group of bacteria metabolizes C3 or organic acids with higher molecular weight. In the AD system, methanogenic microorganisms that consume hydrogen can quickly clean and maintain the hydrogen partial pressure to an extremely low level. This leads to a thermodynamically favorable condition for hydrogen produced by acetogenic bacteria to decompose these organic compounds mentioned in acetate, H2 and CO2. This phenomenon is commonly known as interspecies hydrogen transfer.

- **Homoacetogens:**

  As mixotrophic bacteria capable of using both autotrophy and heterotrophy, homoacetogens can use both H2 and CO2 or saccharides to produce acetic acid, which not only increases the concentration of acetic acid for the production of methane but also keeps the low hydrogen partial pressure in the anaerobic system. However, the functions of these bacteria in the anaerobic process are currently being discussed. It is estimated that acetic acid is produced by these bacteria in the mesophilic digesters 1-4% and 3-4% by thermophilic digesters.
• Methanogens:

Methanogens are characterized by an extremely high physiological specialization and strict anaerobic behavior. They are able to convert organic and inorganic compounds into methane and carbon dioxide. Methanogens can be divided into two groups, consumers of acetate and hydrogen.

The microorganisms involved in the first and second steps grow relatively quickly because fermentation reactions give more efficient energy than those reactions leading to the formation of methane. Methanogens grow slowly and tend to be rate-limiting. The successful implementation and operation of an anaerobic system requires an appropriate balance between the hydrolytic and fermentative organisms involved in the first and second stages, and methanogenic organisms in the third step are maintained.

This balance is achieved by appropriate seeding with digested sludge or bio-solids from an active anaerobic treatment system as well as through control of the production of organic acid and pH during startup when the microbial populations are being established. Active planting is necessary because the doubling time (about 4 days to 35) of the critical microorganisms involved in Methanogenesis is slow (Rittman and McCarty, 2001).

The concentration of organic acid and pH of the reactor must be determined on a daily basis. Inhibition of biological reactions or system overload with organic waste can be often evidenced by a sudden increase in the concentration of organic acid. The main organic acids are a series of short chain fatty acids, which are generally termed volatile acids. Volatile acids that are usually found in the highest concentrations as intermediates during the onset of anaerobic system are acetic acid, propionic acid, butyric acid, and isobutyric acid. If the system is losing its buffer activity, a chemical base should be added quickly to avoid a fall in pH which would kill the critical methanogens.

Methane is only slightly soluble in water, its solubility in water is about 20 mg / L at 30 ° C and 1 atmosphere pressure which makes it easy to be picked up in the gas phase as a source .The energy conversion of COD to methane provides the mechanism for the stabilization of the biodegradable organic matter in the sludge .Without methane production, only a minimal reduction of COD occurs. As stabilizing COD anaerobic processes is directly related to the evolution of methane, methane production can be calculated from the COD removed in the process. The content of carbon dioxide gas produced in the anaerobic processes is between about 30% and 35%, and varies
PARTE I: INTRODUCTION TO THE STUDY

depending on the nature of the substrate. For example, the carbon dioxide content of carbohydrates is greater when being treated when proteins are treated.

Since methane is the only non-reactive compound, it represents the final product of the process. Methanogenic microorganisms can rise to reach the transformation of acetogenesis products through three ways:

- **Acetoclastic way:** $4\text{CH}_3\text{COOH} \rightarrow 4\text{CO}_2 + 4\text{CH}_4$
  
  As here named above, the beginning is the acetic acid from which thought anaerobic dismutation, methane and the corresponding carbon dioxide are formed.

- **Hydrogenotrophic Way:** $\text{CO}_2 + 2\text{H}_2\text{O} + 4\text{H}_2 \rightarrow \text{CH}_4$
  
  The hydrogenotrophic bacteria oxidize anaerobically hydrogen.

- **Methylotrophic Way:** $4\text{CH}_3\text{OH} \rightarrow 3\text{CH}_4 + \text{HCO}_3^- + \text{H}_2\text{O}$

Some methane microorganisms may use methanol and methylamines to generate methane.

Therefore, digestion stages can be summarized in these four stages, which are defined by six processes:

1. Hydrolysis of biopolymers
2. Fermentation of sugars and amino acids
3. Oxidation of anaerobic fatty acid and long chain alcohols
4. Anaerobic oxidation of intermediates such as volatile fatty acids except acetate.
5. Conversion of acetate to methane
6. Conversion of hydrogen to methane

Or again, if the attention is focused on reactions mediated by specific groups of microorganisms it can be distinguished in nine stages:

1. Hydrolysis of organic polymers to monomers such as sugars, organic acids and amino acids
2. Conversion of organic monomers to hydrogen bicarbonate and acetic, propionic and butyric acids and other organic products such as ethanol and lactic acid.
3. Oxidation of organic products reduced to hydrogen, bicarbonate and acetate by obligate hydrogen producing acetogenic bacteria (OHPA).
4. Acetogenic breathing of bicarbonate by homoacetogens (HOM).
5. Oxidation of organic products reduced to bicarbonate and acetate by nitrate-reducing bacteria (NRB) and sulfate-reducing bacteria (SRB).
6. Oxidation of acetate to bicarbonate SBR and NBR.
7. Oxidation of hydrogen by SBR and NBR.
8. Acetoclastic methanogenic fermentation.
9. Methanogenic breathing of bicarbonate.

Each of these steps must be maintained in dynamic equilibrium for methanogenesis to proceed at a maximum rate. Moreover, maintaining this balance is related to the nature of the substrates and the resulting hydrogen destination, since this must be removed continuously to ensure the production of acetic acid and not to become inhibitory.

Image 4: Redox processes in the microbial conversion of organic waste to methane
1.4. Reactor and its operating parameters

The efficiency of VFA degradation has been investigated in different reactor configurations. Some studies have shown that some VFA produced are influenced by design and operational parameters as the following:

1.4.1. HRT (Hydraulic Retention Time)

The mean hydraulic residence time is defined as the ratio between the reactor volume and the volumetric flow rate in the feed thereto.

\[
\text{TRH (days)} = \frac{V \,(m^3)}{Q \,(m^3/\text{day})}. 
\]

For the continuous mode of operation and assuming perfect mixing, it is the time during which the flow treated remains in the reactor.

In another series of studies, the production of gas per hour in digesters operating in 16, 20 and 24 days HRT, with a constant load of 40 kg m⁻³ TS day⁻¹ was recorded and plotted in Figure 2. It was observed that there was no uniformity in gas production over a period of 24 h. The maximum gas was produced in the fourth hour after the feed addition, and there were peaks on the gas production in more or less regular intervals. Moreover, more than 60% of gas was obtained in the first 12 hours of anaerobic digestion. Furthermore, comparisons of percentage of gas produced in regular time intervals in various operating digesters TRH indicated that during the first 12 hours, 74-5% of the total biogas produced was obtained operating at shorter hydraulic retention time (16 days), while only 59-03% of the gas is produced in the digesters operating at a HRT of 24 days for the same period. This may be due to variation in the domain of the microflora in the digesters operating at different retention times. Thus, increasing the HRT is not always beneficial in terms of improving the amount of biogas.

It was observed that using different microorganisms can contribute with HRT to improve the rate of biogas production. Using two examples of different microorganisms to evaluate the influence of the feed and how the biogas production with low HRT could be improved, which as has been seen in the previous paragraph is obtained at a higher percentage of biogas, it was observed that *Methanosarcina Barkeri* dominate in conditions where the dilution rate is high, while the *Methanothrix Soehngenii* predominate under
conditions of low dilution rates. Based on these observations, it is considered that in order to increase the efficiency of digestion, the paper feed volume may be introduced into the digesters twice daily, when operating in digesters with short hydraulic retention time.

Graph 2: Production of biogas in 24 h at different HRT

1.4.2. SRT (Sludge Retention Time)

The solids residence time is defined as the ratio between the total mass of volatile solids in the reactor and the flow rate of the extracted solids reactor, in this case recirculation is not considered biomass.

$$SRT = \frac{V \text{ (m3)} \times X \text{ (Kg microorganisms s/m3)}}{W \text{ (Kg microorganisms / m3)}}$$

Where X is the concentration of volatile solids in the reactor.
1.4.3. OLR (Organic Loading Rate)

Volumetric organic load is the amount of substrate to the reactor inlet, defined as:

\[ OLR = \frac{Q (m^3/day) \times S (Kg\,substrat/day)}{V (m^3)} \]

1.4.4. SGP (Specific Gas Production)

Specific biogas production is the amount of biogas produced per amount of biodegradable organic substrate, expressed as volatile solids content in the reactor feed:

\[ SGP = \frac{Q_{biogas} (m^3/day)}{Q ((m^3/day) \times S (Kg\,substrat/day))} \]

1.4.5. GPR (Gas Production Rate)

This acronym is defined as the amount of biogas produced relative to the reactor volume.

\[ GPR = \frac{Q_{biogas} (m^3/day)}{V (m^3)} \]

1.4.6. Evolution of substrate

The type of substrate used influences the kinetics of the process and therefore the efficiency.

\[ \eta(\%) = \frac{Q \left( \frac{m^3}{day} \right) S (Kg/m^3) - Q \left( \frac{m^3}{day} \right) S_e (Kg/m^3)}{Q \left( \frac{m^3}{day} \right) S (Kg/m^3)} \]

This equation can also be expressed in terms of the volatile solids:

\[ \eta(\%) = \frac{vS_{in} - vS_{out}}{vS_{in} - (vS_{in} - vS_{out})} \times 100 \]

\[ vS \] is the concentration of volatile solids in the effluent, both the input (in) and output (out).

It is also possible to squeeze in terms of soluble TOC, as it is one of the operating parameters characterizing the substrate as expressed in section 1.3.5.
TOC Elimination sol(%) = \frac{TOC_{sol \ in} - TOC_{sol \ out}}{TOC_{tot \ in} - TOC_{sol \ in}} \times 100

1.5. Biological process

From the perspective of waste treatment and resource recovery, it is important to evaluate the main factors governing anaerobic bioconversion processes in a system of AD. Suitable conditions that are essential for efficient and stable biogas production and the variation thereof which would cause a change in the biological equilibrium of reactions that make up the system, are detailed in the following sections.

1.5.1. pH, alkalinity, volatile acids

The pH range favorable for anaerobic microbes is 6.8 to 7.5. Methanogenic microorganisms are very sensitive to pH. Biogas production is generally inhibited or fails at pH values below 6 or above 8. However, recently it has been reported that some isolated methanogens still work well in a wider pH range of 5.5 to 9.5 (2011, X and Z Liu Yan).

The growth rate of methanogens is considerably reduced below pH 6.6, whereas an alkaline medium pH can lead to excessive disintegration of the granules and subsequent microbial process failure. Although the optimum pH of methanogenesis is around pH 7.0, the pH optimum for the hydrolysis acidogenesis has been reported as 5.5 to 6.5 pH. This is a major reason why some designers prefer the separation of the hydrolysis / acidification and acetogenesis / Metanogenesis in two-stage processes.

In this section it is interesting to name the alkalinity or buffering capacity, as an alternative method to control pH.

Alkalinity is the ability to neutralize acids that provide significant resistance to rapid changes in pH. It is also known as "buffer capacity". It is the result of the presence of various compounds (mainly bicarbonate, carbonate and hydroxide). A value of 2500mg CaCO3 L⁻¹ is considered normal for sewage sludge; however, a more desirable range CaCO3 2500-5000mg • L⁻¹ provides greater damping capacity for increased VFA, that can be accommodated with minimal fall in pH (F. Raposo et al, 2011).

The buffer capacity is often referred to as alkalinity in anaerobic digestion, which is the equilibrium carbon dioxide and bicarbonate ions, which provides resistance to the
significant and rapid changes in pH, and the buffering capacity is therefore proportional to the concentration of bicarbonate. The buffer capacity is a more reliable method of measuring the digester imbalance than the direct measurements of pH, such an accumulation of short-chain fatty acids that would significantly reduce the damping capacity before the pH decreases. It has also been shown that the proportion of inoculum for consumption can be modified to maintain a constant pH (Gunaseelan, 1997). (J. Ward Alastair.et all, 2008).

Inside the reactor, the pH is determined by the concentration of volatile fatty acids, ammonia concentration or mainly by the amount of CO2 in the liquid medium, which depends on its partial pressure in the biogas according to Henry's law, which states that, at constant temperature, solubility is directly proportional to the gas pressure exerted on the liquid phase, mathematically formulated as follows:

\[ S = k_s \times P \]

Where:

- \( P \) = is the partial pressure of gas (atm)
- \( S \) = is the gas concentration (solubility) (mol/l)
- \( k_s \) = is Henry's constant, which depends on the nature of the gas, and the liquid temperature (29.4 l*atm/mol de CO2)

According to the experiment conducted by Tong Zhang.et al in 2014, "Influence of initial pH on thermophilic anaerobic co-digestion of swine manure and maize stalk" in the digesters where the materials were mixed, and where all substrates and the inoculum were previously and individually homogenized, the study was carried out with three different proportions of total solids (TS) of content of SM (swine manure) of the total material (30%, 50%, and 70%) and every connection was conducted in five initial pH (6.0, 6.5, 7.0, 7.5, and 8.0) with sodium hydroxide and hydrochloric acid to change its pH.
After 35 days of experiment, the following figures were obtained:

Graph3: Representation of change of pH with different solids

It is observed that when the process enters the acidification step, the biogas production is gradually reduced as the pH decreases below 6. The biogas yield increased with increasing pH, gradually after the acid phase. This study supported by others such as shows that the main products of organic acids changed by changing the pH value in the reactor. Therefore, the tendency of change in pH affects the production of biogas. With the same initial pH, the resilience of the pH in the last stage of fermentation improved, and achieved an increase in the proportion of SM.

As can also be seen in the graphs, the treatments with a ratio of 30% of SM, the pH of each treatment was less than 6, which seriously affected the methane bacteria. Since methane bacteria cannot survive in a pH below 6, the buffering capacity of the substrate unbalanced, showing high alkalinity values / VFA.

In the case of treatment with a value of 70% of SM, the pH of each treatment was recovered gradually to above 7. The rate of recovery of pH 8 was the highest, followed by 6, 7.5, 6.5 and 7. It was also observed, as the low increase in the ratio alkalinity / VFA shows that there was an excessive accumulation of organic acids and that the substrate environment was suitable for bacterial activity.

With all this, it is concluded that the effects of pH on the potential of methane generally occurred at low levels of SM where the potential of methane increased significantly with
increasing the proportion of SM. Thus with proper adjustment of pH and the alkalinity of the medium, improved biogas production is achieved.

These results also match those obtained by (Zhen-Hu Hu et al, 2005):

In the graph shown, only a small amount of methane occurs at pH 4.5 to 5.5 (Figure 4), indicating that methane production was inhibited at pH 5.5, while at pH 6.0 to 7.5, approximately 480 ml of methanol were produced. Inhibition of methane production would not be remedied even when the medium pH is raised to the optimum range.
Cellulose degradation increased with pH in the range of test, but with the pH 5.5, there was a degradation of cellulose. This inhibition at low pH could not be overcome completely even when the medium pH is adjusted to neutral, so it is recommended to work with the rirmaining above pH 6.0.

PH sensitivity of cellulolytic bacteria may be explained by differences in the regulation of intracellular pH. When the extracellular pH of the acid-sensitive bacteria decreases, intracellular pH is relatively stable, but the increase in the transmembrane pH gradient results in a logarithmic accumulation of intracellular acid anions fermentation and thus leads to the inhibition of toxicity anion and products.

As for volatile fatty acids (VFA), which are named above, they also take their role in the production of methane. Its increase is matched by an increase of the organic load in the reactor, determining the acceleration of the hydrolytic activity and acidogenic activity with a negative effect on the overall balance of the process, and therefore the consequent change in pH, which as discussed above, leads to depletion of the buffer capacity. VFA concentration can be expressed in function of the concentration of acetic, which varies between 200-2000 mg ac/L. Typically the concentration of volatile fatty acids is no more than 2-3 g/L, expressed as acetic acid. If this level is exceeded, the formation of methane may decrease while continuing acid production and digestion cease within two to three days because methanogens acids cannot use at the same speed with which they occur.

It has been shown that if the concentration of these volatile acids exceed 4 g/L an inhibition of the substrate (glucose) will be produced in the fermentation process.

1.5.1.1. Relationship VFA and alkalinity

This is a parameter used to evaluate the production of VFA. Decreasing buffer capacity caused by the accumulation of VFA, predates pH decreases (Chen et al., 2014), so the VFA / alkalinity ratio is a more reliable parameter for the control of fermentation under imbalance condition.

Studies have shown that although the optimum ratio of this parameter is unique in each reactor, the ratio of volatile fatty acids (VFA) to alkalinity in the range of 0.3-0.5 was optimal for methanogens, and a value exceeding 0.6 is considered indicative of boost (Liew et al, 2012).
Once the digester is stabilized and sludge is well damped, that is, the proton concentration does not vary even when relatively large quantities of acid or alkali are added. If this buffering capacity is destroyed and the pH drops, the digester is "sour", that is, emission of unpleasant acid odors and metanogenesis ceases. CO₂ is water soluble and reacts with hydroxyl ions to form bicarbonate. HCO₃⁻ concentration is affected by temperature, pH and the presence of other materials in the liquid phase and the production conditions favoring their production increase the methane concentration in the gas phase.

1.5.2. Temperature

Temperature is an important extrinsic factor that significantly affects reproduction and the activities of anaerobic microbes. Different digesters temperature ranges from 8-65 °C. In normal cases, the gas production rate increases with increasing temperature. The digestion temperature can be divided into three subranges, low temperature below 25 °C (digestion psychotropic), moderate temperature from 25 to 45 °C (mesophilic digestion), and high temperature between 45 and 65 °C (thermophilic digestion). Most anaerobic digesters operated in mesophilic or thermophilic conditions. Increased production of methane is generally achieved in the temperature range 35 to 40 °C and mesophilic conditions approximately 55 °C for thermophilic conditions (2011, X and Z Yan Liu).

[Graph 6: temperature effect on biogas production volume expressed as a function of total volatile solids according to the retention time (HRT)]
Almost all digesters work into the limits of mesophilic temperatures and the optimal digestion is obtained at about 35 °C. The digestion rate at temperatures above 45 °C is greater than at lower temperatures. However, within this range of temperatures, bacteria are highly sensitive to environmental changes and maintaining these high temperatures is expensive and sometimes difficult.

For example, in a digester where the waste remains 12 days, the production of gas per unit of total volatile solids added daily is 20% higher at 45 °C than at 35 °C. Digestion does not suffer from an increase in temperature of a few degrees. But a sudden drop of only a few degrees can stop methane production without affecting acid-producing bacteria and this leads to an excessive accumulation of acids causing the failure of the digester.

In fact the major causes of excessive production of volatile acids are high charge rate, foaming and finally a low temperature. This provides an area that favors acetogens. Sedimentation of the foam and fibrous materials can prevent mixing the contents of the digester, which also contributes to the process and establishes uniform conditions.

The influence of temperature on other operating parameters is also demonstrated by studies such as those of (ChengliuGou, et al, 2014):

- **Effect on GPR:**

![Graph7: GPR variation according to different temperatures and ORLS](image-url)
These data indicated that the thermophilic methanogens acted more effectively and had a better ability to support high OLR than those in the other two lower temperatures.

- **Effect on system stability**

A significant increase in TVFA was reflected in graph 8, during the first days. It was in thermophilic condition, therefore this condition contributed to rapid hydrolysis/acidogenesis introducing the biggest quantity of TVFA generated in the reactor, which is then gradually used by the methanogens in the following days.

This study also revealed that OLRS low (<5 g VS L\(^{-1}\)d\(^{-1}\)), the system of mesophilic digestion had the best ability to keep stable, even with the lowest GPR.
The maximum OLR also confirmed that the thermophilic system could withstand a higher feedstock than with the ones that operate in lower temperatures.

- **Effect on the microbial community**

![PCR-DGGE profiles of 16S rDNA fragments to co-digestion samples. A-E: 35 °C for 1 to 5 g L-1d VS; F-K: 45 °C for 1 to 6 g VS·1d L-1; L-S: 55 °C from 1 to 7 g g·1 VS L-1d](image)

In image 5, genes bands are shown for methanogenic samples, that could be the reason for the relatively stable performance of CH\textsubscript{4} and the better removal efficiency of VS.

As seen in this image, the bands were almost at the same level for each temperature-only small changes are observed in the numbers of band as well as the intensity of some microbial species. However, when the OLR increased to the maximum value, a reduction of the band number was clearly detected in the third case (55 °C) reaching high levels and maintaining only five species of microorganism. This illustrates that thermophilic bacteria were more sensitive to environmental variation than the ones at 35 and 45 °C, which, as a result, caused faster decrease in yield of CH\textsubscript{4} and removal efficiency VS.

With all of this, it is concluded that differences in temperature had greater impact on the microbial community that increasing OLR and better process stability were found in mesophilic system with the greatest wealth of bacteria, while higher productivity and better loading capacity were observed in the thermophilic system.
1.5.3. Production and composition of biogas

The chemical composition of biogas indicates that the most abundant component is methane (CH₄); This is the first hydrocarbon series of alkanes and greenhouse gas. CH₄ mixing with air is combustible and burns with blue flame.

Table 2: Chemical composition of biogas (A. Hilkiahigon, 2008)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane (CH₄)</td>
<td>55-73%</td>
</tr>
<tr>
<td>Carbon dioxide (CO₂)</td>
<td>30-45%</td>
</tr>
<tr>
<td>Hydrogen sulphide (H₂S)</td>
<td>1-2%</td>
</tr>
<tr>
<td>Nitrogen (N₂)</td>
<td>0-1%</td>
</tr>
<tr>
<td>Hydrogen (H₂)</td>
<td>Traces</td>
</tr>
<tr>
<td>Carbon monoxide (CO)</td>
<td>Traces</td>
</tr>
<tr>
<td>Oxygen (O₂)</td>
<td>Traces</td>
</tr>
</tbody>
</table>

Production and composition are expressed as percentages of methane and carbon dioxide. Many parameters influence the biogas process, but nevertheless are difficult to measure, such as the sizing of the particles, the surface area or the nutrients needed to maintain the microbial metabolism.

1.5.4. Sized particles and specific surface area

The effects of the particles sizing can be significant and different parameters affect the process of anaerobic digestion:

*Cellulose degradation:*

As discussed in previous sections, cellulose is an important substrate for bacteria, which are responsible for process system reaction.

In graph 9, it is observed that the degradation efficiency achieved for a period of 216 h was 93% for cellulose with a particle size of 50 μm, and about 92% to a particle size of 100 microns, which indicates that the degradation efficiency was not substantially affected by the change of particle size up to 144 h, where 50 μm cellulose degradation curve was more pronounced than for 100 μm cellulose. This suggests that the particle size reduction was beneficial for the hydrolysis of cellulose. This difference can be partly attributed to the expansion of the surface area available that microorganisms could reach and adhere to.
Graph 9: Evolution of the substrate concentration during anaerobic digestion of cellulose by varying the particle size and the initial substrate concentration (4 g/l) and (b 8 g/l).

If the efficiencies achieved in Graph 9 (a) are compared, there is little difference between the different particle sizes, but instead of comparing them in the same graph, and if the comparison of the efficiencies is made between the graphs a and b where the initial state of the substrates is different, the efficiency of cellulose degradation 4 g L⁻¹ was seen to be slightly higher than 8 g L⁻¹ (Zhen-Hu Hu et al 2005).

VFA production and reducing sugars:

Table 3 VFA production example of two substrate level comparison

<table>
<thead>
<tr>
<th>Substrate level (g L⁻¹)</th>
<th>Particle size (µm)</th>
<th>VFAs (mg L⁻¹)</th>
<th>HAc (mg L⁻¹)</th>
<th>HPr (mg L⁻¹)</th>
<th>HBu (mg L⁻¹)</th>
<th>i-HBu (mg L⁻¹)</th>
<th>TOC (mg L⁻¹)</th>
<th>VFAs/TOC</th>
<th>Reducing sugars (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>50</td>
<td>2047</td>
<td>1064</td>
<td>881</td>
<td>121</td>
<td>36</td>
<td>1291</td>
<td>0.77</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2102</td>
<td>1334</td>
<td>529</td>
<td>153</td>
<td>31</td>
<td>1248</td>
<td>0.69</td>
<td>49</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>4598</td>
<td>2496</td>
<td>1750</td>
<td>305</td>
<td>47</td>
<td>2417</td>
<td>0.84</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3815</td>
<td>1944</td>
<td>1509</td>
<td>258</td>
<td>67</td>
<td>1711</td>
<td>0.78</td>
<td>62</td>
</tr>
</tbody>
</table>

Note: HAc = acetate; HPr = propionate; HBu = butyrate; i-HBu = iso-butyrate.
As shown in Table 3, more amounts of VFA were obtained with a particle size of 100 µm with a concentration of substrate of 4 g L\(^{-1}\) cellulose, but the reverse case occurs with sampling with a concentration of 8 g L\(^{-1}\) of cellulose.

According to the same table, the concentration of reducing sugar was 49-74 mg L\(^{-1}\). As reducing sugars are easily used by anaerobic microorganisms, a low concentration of reducing sugars indicates that there was an accumulation of hydrolytic products in the anaerobic fermentation of cellulose. This suggests that the acidogenesis of hydrolytic products was much faster than that of crystalline cellulose hydrolysis, obviously these results according to a ruminal culture

*Methane production*

![Graph10: La Producción De metano a partir de celulosa Por los Microbios del rumen de cabra: nivel de sustrato (a: 4GL-1, b: 8gL-1)]

According to Graph10, there was a significant difference between the results of the two particle sizes in 8 g \(^{-1}\) of substrate, with 110 mL of methane for 50 µm particles and 310 mL in the case of 100 µm. Since methanogens are much more sensitive to the accumulation of VFA than the acidogenic agent, the reduction of methane production can be attributed
to increasing the adsorption sites available, i.e., with the particle size smaller and substrate concentration higher. This could result in an increase in hydrolytic rate and accumulation of VFA.

1.5.5. Nutrients

An organism needs different nutritional requirements to complete its vital functions, these requirements being carbon and energy for the synthesis of cellular material, or many others, such as nitrogen, phosphorus, potassium, sulfur, magnesium, calcium or iron.

Cell growth is therefore limited by the nutrients available to the cell. The C / N ratio determines the performance of the process because carbon is the energy source for the microorganisms while nitrogen is required for growth. If the nitrogen concentration is low, growth of bacterial communities will be limited, with the low kinetic of carbon consequent use, while on the other hand too high concentrations lead to a high production of ammonia, which would result in inhibition process. It has been shown that bacteria in digestion processes consume carbon at a speed corresponding to a value of 30: 1 in terms of the C / N (Hilkiahgoni A., 2008)

One advantage of the AD is the low need for nutrients derived from the small growth rate. The main nutrients are N and P. An acceptable theoretical relationship is COD: N: P: S 1000: 15: 3: 1.

In general, the higher hydraulic residence time, the greater is the tolerance to any fluctuation.

1.5.6. Inoculum

Inoculums with active methane-producing microorganisms are essential for rapid and successful implementation of an anaerobic digester. The presence of suitable microorganisms in high quality inoculum can facilitate implementation of the anaerobic digester. Otherwise, a long period of enrichment is required. Normally, the volume of inoculum must be above 10% of the total workload of the digester, and 20-30% of inoculum supplementation would favor the good start of digesters AD. (X and Z Yan Liu.et al, 2011).
The influence of inoculum in the batch test depends on six factors: origin / source, concentration, activity, preincubation, acclimatization / adaptation and storage (F. Raposo,. et al, 2011).

- **origin / source**

For a defined inoculum, the methane yield of an organic substrate is directly related to the degree of dissolution, whereas the degradation rate depends on the slowest of the four steps of anaerobic digestion process, ie hydrolysis (solubilization), acetogenesis, acidogenic and methanogenic.

Generally, it is used sludge from a biogas plant in operation. The digested sludge MWTP should offer the most appropriate and diverse source of active inoculum. This source is preferable for the following reasons: (i) wastewater plants treatment are found throughout the world, (ii) although treatment plants wastewater are different, they have common characteristics.

- **Concentration**

Practical experience has shown that the level of inoculum concentration affects the rate of biodegradation. Typically, the higher concentration of inoculum, the faster is the anaerobic conversion of the substrate, and the faster the tests of the probeare completed. Furthermore, the concentration affects the length of delay and the susceptibility of the degradation due to the inhibitory effects.

To study the anaerobic biodegradability of micro pollutants, a low concentration of inoculum (1-3 g TS • L-1) was suggested, as this also contributes to the formation of gas that can distort the results, if it is relatively high compared to the compound that is being analyzed.

VDI guideline 4630 suggested using a range between 15 and 20 g • L-1 VS from seed sludge.

- **Activity**

The inoculum activity has been limited to the assessment of the specific methanogenic activity, but for better identification of the quality of the inoculum used, it has recently been suggested that it should determine the activity of the different groups of microorganisms involved in the anaerobic process.
Different positive control substrates are used to measure the activity and also to check if the anaerobic biodegradation is operating correctly, for quality control purposes.

Considering the influence of the activity of the inoculum of anaerobic biodegradability, several studies such as (Moreno-Andrade et al, 2004), show how there is variety in biodegradability according to the use of different sources of inoculum, even for easy degrading substrates such as glucose, where the "lag time" of batch reactors are shorter using an inoculum acclimated to the environment. This can also be considered in the study by (Lucia Neves. Et al, 2010), where the influence of the adaptation of inoculum (graph 11) is shown.

- Pre-incubation

![Graph 11: accumulated methane production mesophilic temperature (a) Inoculum unsuitable lipids; (b) Inoculum adapted to lipids.](image)

The pre-incubation of sludge before feeding reduces the volume of gas produced in the blank controls, and has been postulated as a means of improving the accuracy with which the net production of gas can be measured.

Pre-incubation has been widely recommended for control of anaerobic biodegradability, since in such cases it is difficult to clearly distinguish the amount of biogas produced by the sludge itself.
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- **Acclimatization/ Adaptation**

The inoculum preculture with a substrate leads to induction of metabolic pathways for biodegradation, to increased affinity for the compound of microorganisms and also to an increase in the number of specific degraders.

- **Storage:**

Storage of sludge to be treated can affect the "lag time", and therefore the substrates can be degraded more slowly.

**1.5.7. Inhibitors**

DA processes can be inhibited to a greater or lesser extent by toxic substances present in the system. These substances can be:

- Intermediate products generated by metabolic reactions of bacteria digester (which can accumulate and exceed the buffering capacity of the reactor): \( \text{H}_2, \text{H}_2\text{S}, \text{NH}_3, \text{VFA} \).

- Substances that come regularly to food.

- Substances accidentally or in a timely manner introduced into the system

Based on their chemical nature, we can classify the inhibitory compounds into:

- Inorganic Toxins

- Natural Organic Compounds

- Xenobiotic compounds

*Volatile Fatty Acids*

The instability of an anaerobic reactor is usually shown by a rapid increase in the concentration of VFA, indicating a failure in methanogenic populations because of overload, influent pH variation, lack of nutrients, or infiltration of toxic substances.
**Sulphides**

Sulfates and sulfur amino acids are reduced to sulphides in the conditions prevailing in an anaerobic reactor. These sulfides are soluble or insoluble depending on the associated cations.

**Ammonia**

Although ammonium ion is an important buffer in anaerobic reactors, high concentrations thereof are a major cause of failure in wastewater containing a high concentration of proteins, amino acids or urea. The anaerobic biomass can adapt to their presence.

The toxicity of AGV, $\text{H}_2\text{S}$, $\text{NH}_3$ is associated with the non-dissociated forms. The amount of acid / free base depends on the degree of dissociation ($pK_a$), pH and total concentration in solution.

\[
\begin{align*}
\text{CH}_3\text{COOH} & \leftrightarrow \text{CH}_3\text{COO}^- + \text{H}^+ \\
\text{H}_2\text{S} & \leftrightarrow \text{HS}^- + \text{H}^+ \\
\text{NH}_4^+ & \leftrightarrow \text{NH}_3 + \text{H}^+
\end{align*}
\]

Ammonia is produced by biological degradation of the nitrogenous material, mostly in the form of proteins and urea. The amount of ammonia that is generated from anaerobic biodegradation of organic substrate can be estimated using the following stoichiometric ratio:

\[
\begin{align*}
\text{C}_a\text{H}_b\text{O}_c\text{N}_d + \frac{4a - b - 2c + 3d}{4} \text{H}_2\text{O} \\
\rightarrow \frac{4a + b - 2c - 3d}{8} \text{CH}_4 + \frac{4a - b + 2c + 3d}{8} \text{CO}_2 \\
+ d\text{NH}_3
\end{align*}
\]

Several mechanisms have been proposed for ammonia inhibition with regard to how it works, and therefore how to notice that such inhibition is occurring; these "leads" would be a change in intracellular pH, increased maintenance energy requirement, and inhibition of a specific enzymatic reaction. Ion ammonium $\text{NH}_4^+$ and free ammonia (FA) ($\text{NH}_3$) are the two main forms of inorganic ammonia nitrogen in aqueous solution. FA is suggested as
PARTE I: INTRODUCTION TO THE STUDY

the primary cause of the inhibition because of its permeable membrane. Ammonia hydrophobic molecule can passively diffuse into the cell, causing imbalance of protons, and / or potassium deficiency, which is one of the nutrients available for anaerobic bacteria. Among the four types of anaerobes, methanogens are less tolerant and more likely to stop growing because of this type of inhibition. (Ye Chen.et al.,2008).

Heavy metals

What is important is the soluble fraction and the effect of various factors (amount of biomass, reactor type, the substrate used, salt and oxidation state in which the metal is supplied, etc.). A relative toxicity value can be: Ni≈Cr> Cu>Cd≈Zn> Mo>Pb

The high adaptability and acclimatization of anaerobic systems must be noted, which means that bacteria can tolerate the presence of toxic after a period of exposure to sub-lethal concentrations thereof.

Factors affecting the toxicity and recovery

- Concentration of toxic
- Contact time
- Amount of biomass
- Structure of biomass
- Solids retention time

1.6. Types of substrates as feeding

Of the many different substrates that have been considered for the DA, most can be divided into the following five categories:

(a) the organic fraction of municipal solid waste (MSW), (b) organic waste from the food industry, (c) energy crops or agricultural crop residues, (d) manure, and (e) plant wastewater treatment plant (WWTP) residue. These substrates represent different types and levels of limitations of hydrolysis DA optimum performance; however, two main components can be identified between categories of substrate causing low bioavailability and / or biodegradability: microbial cells / floc.
(a) **Organic fraction of municipal solid waste (OFMSW)**

MSW are composed of different components among which the most important are: organic matter, paper and cardboard, plastic, glass, metal, fabric and wood.

When organic matter contained in the RSU is used as substrate for anaerobic fermentation, the process is called biomethane or biogas production.

In this process, organic matter is converted into biogas and poorer solid fraction in the compost, which can also be used as soil improver.

Before anaerobic digestion at plants of biomethanation, pretreatment is needed, consisting of the separation of the MSW and grinding to reduce the biodegradable fraction to an appropriate and uniform size, this facilitates biomethanation.

Moreover the OFMSW can also be utilized for the production of biogas in landfills. In this case, the waste is unloaded, extended and compacted to prevent air pockets therein, after covering with earth or other suitable materials, forming regular successive layers of varying thicknesses.

The OFMSW initially undergoes aerobic fermentation process until the oxygen is depleted and anaerobic conditions are reached, producing biogas.

The generated gas, as is diffused through the mass of waste, drags traces of organic compounds and other gaseous contaminants to the surface of the landfill, influencing emissions producing the greenhouse effect. However, the uptake of this biogas for energy use or use as a resource in processes of advanced technologies eliminates hazardous air pollutants.

(b) **Organic waste from the food industry**

Its use for the production of biogas is insignificant for the moment. Thus, for example, canning tomato wastes constitute 15 to 30% of the total amount of the processed product; in the case of peas and corn this proportion exceeds 75%. By isolation of solid waste, the concentration of soluble organic substances in the wastewater is reduced and those can be used more easily as by-products, food or fuel.

Considering the food industry wastes and wastes of fruits and vegetables, they tend to be low in total solids and high in volatile solids, and are easily degraded in an anaerobic
digester. The rapid hydrolysis of these raw materials can lead to acidification of a digester and the consequent inhibition of methanogenesis. (Alastair J. Ward, 2088)

(c) Agricultural and livestock waste

Agricultural residues can be of various types: pruning and branches of woody plants, green plants and stems of herbaceous crops, winter cereal straws, stalks and husks spring cereals, fruit and vegetable remains, waste plastic greenhouses, substrates, residues of plant protection products (pesticides and fertilizers), plant protection product containers, waste oil and containers, etc.

Livestock waste, meanwhile, are also varied: mix of animals (solid and liquid), remains of bed, food and water in varying amounts and fluid or pasty consistency, antibiotics and other medicines for veterinary use manure, detergents and packaging of veterinary medicines.

The intensification of livestock has led to the production of large volumes of organic waste with consequent management and disposal problems arising. Thus, the main environmental implications are deed to the production and manure management. The slurry is composed mainly of organic matter (65-75% dry basis), nitrogen (4-6% measured elemental nitrogen), phosphorus (3.5-5.5% in terms of P\textsubscript{2}O\textsubscript{5}), potassium (2.5 -4% as K\textsubscript{2}O), magnesium (0.5-1.5%) and calcium (3.5-4%). However, the composition of slurry is very heterogeneous depending on the species, age, type and feeding system, cleaning system, sanitary and physiological state of the animal, etc.

The biomass can be introduced in this section of substrate as it constitutes promising feedstock for anaerobic digestion. Grasses, including wheat straw, rice and sorghum are an abundant source of biomass, much of which is a waste product of food production. Its high proportion of recalcitrant materials is often required prior to fully realize the potential treatments. Harvest time can also significantly affect the performance of biogas plants. In the following table, different types of biomass and its contribution to the production of methane are shown.
Table 4: Types of biomass and methane production (Alastair J. Ward et al, 2008)

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Methane yield $m^3$ per kg volatile solids (SD in parentheses)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter rye</td>
<td>0.36</td>
<td>Petersson et al. (2007)</td>
</tr>
<tr>
<td>Oilseed rape</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Faba bean straw</td>
<td>0.441</td>
<td></td>
</tr>
<tr>
<td>Maize (whole crop silage)</td>
<td>0.390</td>
<td>Amon et al. (2007a,b)</td>
</tr>
<tr>
<td>Winter wheat straw</td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td>Summer barley straw</td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td>Sugar beet leaves</td>
<td>0.210</td>
<td></td>
</tr>
<tr>
<td>Sunflower (whole crop silage)</td>
<td>0.300</td>
<td></td>
</tr>
<tr>
<td>Maize (Tonale, early harvest)</td>
<td>0.334 (0.0057)</td>
<td>Amon et al. (2007a,b)</td>
</tr>
<tr>
<td>Maize (Tonale, mid harvest)</td>
<td>0.283 (0.0049)</td>
<td></td>
</tr>
<tr>
<td>Maize (Tonale, late harvest)</td>
<td>0.280 (0.0114)</td>
<td></td>
</tr>
<tr>
<td>Maize (LZM 600, early harvest)</td>
<td>0.313 (0.0214)</td>
<td></td>
</tr>
<tr>
<td>Maize (LZM 600, mid harvest)</td>
<td>0.326 (0.0161)</td>
<td></td>
</tr>
<tr>
<td>Maize (LZM 600, late harvest)</td>
<td>0.287 (0.0078)</td>
<td></td>
</tr>
<tr>
<td>Maize (PR34G13, early harvest)</td>
<td>0.366 (0.0252)</td>
<td></td>
</tr>
<tr>
<td>Maize (PR34G13, mid harvest)</td>
<td>0.302 (0.0070)</td>
<td></td>
</tr>
<tr>
<td>Maize (PR34G13, late harvest)</td>
<td>0.268 (0.0042)</td>
<td></td>
</tr>
</tbody>
</table>

(d) Manure

The use of manure for biogas production also reduces the amount of greenhouse gases released during normal storage. Some test results of BMP for fertilizers are shown in the Table below; illustrating the potential of methane, which varies widely among livestock. Factors contributing to the potential of methane from manure are the species, breed and stage of growth of the animals, feed, amount and type of bedding and all processes of degradation that can occur during storage (Alastair J. Ward, 2008).

Agricultural fertilizers contain ammonia concentrations that are greater than necessary for microbial growth and can produce inhibition in anaerobic digestion (as seen in previous chapters of inhibition by ammonium). A high concentration of ammonia may be advantageous when used with other materials that have low concentrations of nitrogen, and which serve as a source of substrate.
Table 5: Types of fertilizers and methane production (Alastair J. Ward et al, 2008)

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Methane yield m$^3$ per kg volatile solids (SD in parentheses)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>0.356</td>
<td>Møller et al. (2004a,b)</td>
</tr>
<tr>
<td>Sow</td>
<td>0.275</td>
<td></td>
</tr>
<tr>
<td>Dairy cattle</td>
<td>0.148</td>
<td></td>
</tr>
<tr>
<td>Beef cattle</td>
<td>0.328</td>
<td>Hashimoto et al. (1981)</td>
</tr>
</tbody>
</table>

(e) Wastewater treatment plant (WWTP)

The sludge or sewage sludge is the resulting semi-solid residue from the treatment of urban wastewater, whereby most of the dissolved contaminants and suspended content are removed in these waters.

Generally, the sludge are formed by the settled solids from the wastewater, by the excess of microorganisms produced during biological treatment, products sedimented by natural coagulation or caused by suspended or colloidal nature particles and chemical precipitates formed by the reaction of the coagulants with dissolved particles. Sewage sludge rich in nutrients (N, P and K), are constituted, in some cases, by more than 60% organic matter.

Waste sludge corresponds to those with major contaminant capacity, and therefore, their treatment is also required, their transfer being necessary in many cases.

1.7. Reactors

1.7.1. Anaerobic digesters

Large bioreactor volumes are required to provide the high retention time necessary to sustain the slow growing anaerobic microbial mass inside the bioreactor, which raises the cost of the process. Therefore, for an efficient anaerobic system with relatively small bioreactor volume, the design of anaerobic digesters should aim at providing an optimum environment for the growth of the anaerobic microorganisms given the complexity of their physiology and the syntrophic and/or antagonistic interactions among them. There are some specified certain criteria for these types of digesters:

- High retention of the active biomass (microorganisms) inside the bioreactor.
- Sufficient contact between the biomass and the substrate.
- High reaction rates and elimination of the limiting transport phenomena.
- Suitable environment for the adaptation of the biomass to various types of feedstocks.
- Suitable environment for all organisms under the operating conditions.

Depending on the solid content of the feedstocks, different bioreactor configurations can be used:

- Low solid content feedstocks (e.g. secondary wastewater treatment, wastewater from food industry, hydraulic flush manure systems; swine)
  - Anaerobic lagoons – fixed, floating, or submerged covers
  - Completely mixed reactors
  - Anaerobic filter reactors
  - Fluidised bed reactors
  - Upflow anaerobic sludge blanket reactors (UASBR)
  - Anaerobic baffled reactors (ABRs)

- Medium solid content feedstocks (e.g. dairy manure, ‘scraped’ swine manure, municipal or food industries sludge)
  - Plug flow reactors
  - Completely mixed reactors
  - Contact reactors.

- High solid content feedstocks (e.g. organic fraction of municipal solid wastes, agricultural residues, food processing waste; food residuals; pulp paper sludge)
  - Plug flow
  - Completely mixed
  - Leach-bed.
A brief description of the main bioreactor types follows:

• **Fixed-bed anaerobic reactor (anaerobic filter):**

  The wastewater is introduced from the bottom or the top of a column which is filled with inert material (rocks, cinder, plastic or gravel). The filling material provides the surface upon which microorganisms are attached forming a biofilm. The microorganisms can also be retained through entrapment in the microporous structure of the filling material. Clogging is a typical problem with this type of digester. The organic load of the wastewater must be low to medium. Recirculation must be applied so that the organic load in the entrance is maintained between 8 and 12 g/L. Wastewaters containing significant amounts of suspended solids or constituents that cause precipitation of organic and inorganic compounds are not suitable for this bioreactor type. The filling material must provide large void space to avoid clogging (95%) and have large specific surface (100–200 m²/m³).

• **Expanded and fluidized bed anaerobic digester:**

  This type of configuration allows a more effective mass transfer from the liquid phase to the membrane, because fine filling material is used (0.2–0.5 mm). The upflow velocity must be high enough (through recirculation) to maintain the expansion of the bed between 15% and 30%, while if the expansion raises up to 300%, the bed is characterised as fluidized. Energy consumption required to provide recirculation is the main disadvantage of this bioreactor. The wastewater must contain low suspended solids as in the case of the fixed bed bioreactors.
• UASBR:

The UASBR was designed as an alternative to wastewater treatment without the operating problems of bioreactors with filling materials but incorporating the concept of biomass immobilization.

In this bioreactor type, the microorganisms are agglomerated to form a dense structure (granule) with excellent settling properties and strength under adverse conditions. The granular sludge blanket remains in the bottom of the bioreactor. The feed is introduced from the bottom and the motion of the flow is upwards. The upflow velocity is very important since it influences the formation of the granules. Typical upflow velocities range between 0.5 and 3 m/h. The biogas produced is often entrapped in the granules making them lighter and buoyant with their potential wash out. An effective three phase separator on the top of the bioreactor results in the retention of the granule and their return to the sludge blanket. UASBR is a reliably tested technology for the treatment of a wide range of wastewaters (from municipal wastewater to high strength agro industrial wastewater) with low solid content. It has low installation, operation and maintenance costs. More than 900 full-scale units are currently being operated all over the world (Garcia et al., 2008). Hybrid systems have been developed to combine the characteristics of a UASBR and an anaerobic filter, expanded or fluidised bed reactor.

• ABR:

It is a rectangular tank with baffles. The wastewater flows above and below a series of baffles successively coming into contact with the biomass which is accumulated in the bottom of the bioreactor. This bioreactor type is
simple in structure, with no moving parts or mixers. The biomass is not necessary to have good settling properties as in the UASB, in order to be retained in the bioreactor. It is an efficient system at low retention times and its operation is stable under sudden changes in the organic loading rate. A modification of this bioreactor type led to the periodic anaerobic baffled reactor (PABR) which is based on the periodic feeding mode to all compartments. In PABR, the switching frequency of the feed allows flexibility in operation; the PABR can be operated as a simple ABR, if the switching frequency is set to zero, and, in the Production of biogas extreme case of very high switching frequency, as a single-compartment up flow bioreactor.

• Plug flow:

It is a long narrow insulated and heated tank. The digested material flows from one end of the tank to the other as fresh feedstock enters the bioreactor. The bioreactor can be placed horizontally or vertically. It is used in the case of solid feedstocks. In order to provide mixing, various practices are applied. In the Dranco process (vertical, downflow plug flow digester), the fresh feedstock is mixed with a portion of the digested material and is introduced from the top of the bioreactor. The same concept can be applied while the plug flow reactor is placed horizontally. In this case slowly rotating impellers inside the reactor can aid the horizontal movement of the mixture, also serving for mixing, degassing and suspension of the heavier particles. In another plug-flow type configuration (Valorga process), the horizontal flow is circular and biogas injection at intervals under pressure through a network of nozzles provides mixing.

• Leach bed:

The feedstock is loaded in a vertical bioreactor to form a bed through which a liquid stream percolates as a leachate and is recirculated to the top of the same reactor where it is produced.
• Complete mixed anaerobic digester – anaerobic contact process.

It usually consists of a round insulated tank, above or below ground. Heating is provided through coils with hot water inside the tank or an external heat exchanger. Mixing is achieved through a motor driven mixer, recirculation of the mixed liquor or biogas. The cover can be floating or fixed. In the case of low solid content feedstocks and in order to enhance the biomass concentration in the bioreactor, a modification of the complete mixed anaerobic digester led to the anaerobic contact process. In this configuration, the bioreactor is followed by a settling tank (or inclined parallel plates, membranes, etc.); to separate the sludge from the supernatant. The sludge is recycled to the bioreactor increasing the biomass concentration.

• Covered anaerobic lagoon:

It is a large earthen impoundment, lined with appropriate geomembranes and covered with a flexible or floating gas tight cover. They are used mostly for manure treatment. No heat and mixing are provided; therefore the ambient temperature is prevailed making this type of digester unsuitable in cold climatic conditions.

1.8. Process optimization

One of the forms to have an optimization in the anaerobic digestion system are:

- By anaerobic microbiological fermentation characterization key stages, using molecular biology techniques: Polymerase chain reaction (PCR), Gradient Gel Electrophoresis of denaturation (DGGE), etc.

- Validation of new substrates of high potential for biogas as glycerin and other byproducts of the production of liquid biofuels, for the citrus and vegetable production, specific energy crops for biogas, microalgae, etc.
- Development of highly efficient processes in terms of sanitation or reduction of biological (pathogen removal, etc.).

- Monitoring and modeling of anaerobic fermentation process: standardization and process improvement, continuous monitoring of intermediate products (VFA), etc.

-Co-digestion: The treatment of two or more waste by anaerobic digestion is called anaerobic co-digestion. Against digestion processes that employ a single substrate, this approach has significant technical, environmental and economic advantages. Anaerobic co-digestion can take advantage of the complimentarily of the waste composition. The best example is the co-digestion of manure livestock and food waste. Livestock waste has reduced concentration of organic matter and a low C / N, but has a high concentration of micro and macronutrients (basic for the growth of critical anaerobic microorganisms) as well as buffer capacity (alkalinity) to prevent acidification processes. Food waste rich in carbohydrates, proteins and fats usually have a high proportion of biodegradable organic matter and a high C / N, but the anaerobic digestion is negatively affected by a lack of micronutrients and by acidification problems. Thus, the mixture of both types of waste results in more stable processes and in a notable increase in the biogas production process. For example, producing biogas 10-20 m3 / t in a mono-substrate anaerobic digestion with cattle manure could double incorporating 20-30% of food waste.

The co-digestion can integrate the recovery of organic waste in a given geographical area. Thus we obtained, on the one hand, a source of energy from renewable sources in the form of biogas, and on the other hand, a product resulting from digestion called digestate with characteristics of organic fertilizer in agriculture and applied under controlled conditions.

Economically, the increase in biogas production translates into higher revenues from electricity sale and / or use of the heat produced. In addition, some waste management resources used as co-substrates can also generate revenue. Additionally, the fact of integrating all processes into a single facility which treats all types of waste in an area would save investment and operating costs when compared with the separate treatment of each waste managed.
1.8.1. Operating parameters

1.8.1.1. Hydraulic retention time

Boullagui et al. (2003) have derived the relationship between the residence time, and reduction of volatile solids, ie methane production.

The decrease of the hydraulic retention time decreases the reduction of volatile solids and the specific production of biogas (l / kgVS added) while there is an increase of the speed of production of biogas (\( \frac{\text{biogas}}{\text{reactor} \cdot \text{d}} \)) and a decrease of the content of methane. It is suggested that the low efficiencies of degradation at low values of HRT may result in the decrease of microbial activity.

![Graph 12: effect of the HRT on the methane production](image)

The magnitude of methane generation was significantly affected by HRT as shown in Graph 17. Differences in methane generation became remarkable between the control and pretreated substrate, as the HRT was decreased, except when HRT was 3 days. When HRT was 4 days, the control digestion was still unstable and later resulted in failure. Therefore, the maximum organic load of the control fed in the digester was approximately 5.0 kg-VS/m3 day, when the HRT was extended to 6 days. (Q. Wang, 1997)

1.8.1.2. Organic loading rate

Organic loading rate (OLR) is an important operation parameter for AD process. On the premise of the system stability, higher OLRs means higher waste treatment capacity and
biogas production, so the suitable operating OLR is always the hot topic in research of AD. It has repercussions on different parameters as pH, VFA or production of methane.

A feeding cycle covers the period from the feeding point up to the next feeding of the following day. Figure 17 shows the variations in pH value during a 24 h feeding cycle with changing OLRs. pH values decreased rapidly from the moment of feeding. After 1–2 h, pH values began to revert back to normal levels. A more significant decrease in pH value was observed with increased OLRs.

Accordingly, VFA increased right after the substrate was added, reaching the highest value at 1–2 h after feeding. The VFA then began to reduce to constant concentration. Along with increasing OLRs, the VFA concentration also showed remarkable increase.
Regarding the methane production, the results are displayed in the following graph:

Methane content decreased from the moment of feeding, and after 1–4 h the methane content reverted to its original levels. The biogas produced during AD was mainly composed of CH4 and CO2. Three metabolic pathways related to CH4 and CO2 were found as follows: (1) acidification which produced CO2; (2) acetoclastic methanogenesis which produced equal volumes of CH4 and CO2; and (3) hydrogenotrophic methanogenesis which produced CH4 and consumed CO2. At the start of the feeding cycle, CO2 was produced during the acidification of the organics. The increasing acetate promoted the reaction of acetoclastic methanogenesis which in turn resulted in a decrease in CH4 concentration. Hydrogenotrophic methanogenesis was then promoted by the increasing CO2. At this point, CO2 was consumed and CH4 concentration was recovered.

So the only thing that is missing to compare at this point is the relation between VF / CH4 production at different ORLs.
This graph presents the variations in methane production and VS removal rate with OLR added to the reactor.

With the increasing OLRs, the VS removal rate and methane concentration decreased accordingly. Under OLR of 8.0 kg VS (m3 d)\(^{-1}\), although pH value, methane production, and VS removal rate appeared normal, the anaerobic system displayed VFA inhibition at the outset of feeding cycle, and had higher risk of acidification.

1.8.1.3. Use of additives

(K. Stamatelatou. etal (2011))

– The addition of powdered leaves, crop residues, etc. seems to increase the biogas production; the additives create a more favorable environment for the microorganisms and offer sites for the substrate local concentration. Production of biogas through adsorption seems to have positive impact on biogas production.

– The addition of microbial strains (such as cellulolytic bacteria and fungi or cell lysate) increases the substrate digestibility.

– The addition of inorganic elements, adsorbents or chelating agents seems to help through various ways, by: (1) increasing the density of bacterial flocs, (2) contributing to the formation of vital metal containing enzymes, (3) solubilizing trace elements via combining a chelating agent with a metal, and (4) increasing stability via adsorption.
1.8.1.4. Temperature

Temperature of operation: All digesters usually operate within two temperature ranges, either at 35–40°C (mesophilic) or 50–60°C (thermophilic). Mesophilic anaerobic digestion is applied for digesting rumens of animals and feedstock from industrial and farm activities, while thermophilic anaerobic digestion is more suitable for sanitation of pathogen-bearing feedstocks. Another advantage of thermophilic anaerobic digestion is the fast conversion rates of the feedstock (induced by the fast metabolism of the microorganisms due to the high temperature) and, consequently, the lower retention time (and reactor volume) required. However, the psychrophilic range of temperatures (<20°C) has also been studied, especially in lagoons and swamps. Thorough studies on reactor design and in-depth parametric analysis for psychrophilic consortia are lacking. It has been acknowledged, however, that, in psychrophilic conditions, systems favoring biomass accumulation are required to secure high efficiency. Another possibility has been to apply genetic engineering in the attempt to introduce stable enzymes, active in cold temperatures to provide with improved catalysts in the biomethanation.

1.8.1.5 Solid content of digesting mixture

When the solid content of the digesting mixture is less than 3–4 % (little or no suspended solids), then the digesters are usually a single phase liquid system. Digesters treating solids are characterized as wet or dry depending on whether the solid content is up to 12–15% or more.

Wet anaerobic systems are in a slurry form and can still be mixed through agitation, while for the dry anaerobic systems the plug-flow type digesters are most suitable.

1.9.2. Parameters depending on the substrate used

1.9.2.1. Pre-treatment and post-treatment

They are applicable mainly when high solid feedstock is involved. In general, pretreatment methods can be divided into three main types according to the means used for altering its structural features: mechanical, physicochemical and biological. Mechanical pretreatment is always mostly applied before any other kind of pretreatment, and actually refers to milling, through which reduction of particle size of solids is achieved. The reduction in particle size leads to an increase of available specific surface. Both physicochemical and
biological pretreatment methods may enhance biodegradability, but physicochemical methods yield in general higher efficiencies. During physicochemical pretreatment, the feedstock is exposed to acid, alkaline or oxidative conditions, at ambient or high temperature. The use of high temperatures without the addition of some chemical agent, called thermal pretreatment, can also be used. Combinations of two or more physical and chemical pretreatment methods are also possible, such as acid-catalysed steam explosion, ammonia fiber explosion (AFEX) and CO2 explosion. For lignocellulosic feedstocks, steam pretreatment, lime pretreatment, liquid hot water and ammonia based pretreatments seem to have high potential (Hendriks and Zeeman, 2009). The main effect of these methods is to dissolve the hemicellulose and alter the lignin structure, improving the accessibility of the cellulose to hydrolytic enzymes. In the case of municipal activated sludge, the goal of pretreatment is to rupture the cell wall and to facilitate the release of intracellular matter in the aqueous phase for subsequent degradation and enhance dewaterability. Various pretreatment methods have also been studied. Ultrasonic pretreatment seems to be promising, since full-scale studies have showed an improvement in sludge dewaterability.
PARTE II: PRE-TREATMENT AND ULTRASOUND TECHNIQUES

2. PRE-TREATMENT AND ULTRASOUND TECHNIQUES

The main aim of this study is to find out how a pre-treatment technique included in the process of anaerobic digestion would contribute to an improvement in the obtainment of biogas production.

Due to the lack of fossil energy sources, techniques that efficiently circulate renewable carbon are an urgent priority, and if the use of fuel-based devices is continued, efficient methods to produce biofuels need to be found. (Jia Luo. Et al, 2014)

As one of the most important biomass sources of biofuels production are mainly sugars, polysaccharides, lignocelluloses, chitosan, lipids, algae and polyols, the properties of these sources and even more in the case of this study, lignocellulosic biomass, which makes it resistant to biodegradation. Due to the complexity and variability of biomass chemical structures, the optimal pre-treatment method and conditions depend on the types of lignocellulose present in it. Several structural and compositional properties were found to have impact on the biodegradability of lignocellulosic biomass, including cellulose crystallinity, accessible surface area, degree of cellulose polymerization, presence of lignin and hemicellulose, and degree of hemicellulose acetylation. The goal of pre-treatment techniques is to alter such properties to improve biomass amenity to enzymes and microbes. In general, different pre-treatment methods affected these properties to different degrees; however, all methods had a major effect on the accessible area of lignocellulosic biomass. The effects of different pre-treatment techniques on the chemical composition and physical characteristics of lignocellulosic biomass are summarized in the following table. (Yi Zheng. Et al, 2014)

All in all, because of the complexity and interactions of each component and between them, an effective pretreatment method is needed not only to alleviate these problems but

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Increase of accessible surface area</th>
<th>Decrystalization of cellulose</th>
<th>Solubilization of hemicellulose</th>
<th>Solubilization of lignin</th>
<th>Alteration of lignin structure</th>
<th>Formation of fufural/ hydroxymethylfurfural (HMF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Irradiation</td>
<td>●</td>
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<td>●</td>
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<td>Steam explosion</td>
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<td>●</td>
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<tr>
<td>Liquid hot water</td>
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<td>Catalyzed steam-explosion</td>
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<td>Ionic liquids</td>
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<td>Thermal acid</td>
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<td>Thermal alkaline</td>
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<td>Thermal oxidative</td>
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<td>Ammonia fiber explosion</td>
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<tr>
<td>Biological pretreatment</td>
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</tbody>
</table>

* ● = major effect, ○ = minor effect, ND = not determined, and blank = no effect.
also to produce multiple desirable effects (e.g. lignin removal and decrease of cellulose crystallinity), so that biomass can be efficiently degraded for high biogas yield.

In order to improve biogas production from lignocellulosic biomass, a pre-treatment process is necessary to disrupt the naturally recalcitrant carbohydrate lignin shields that impair accessibility of enzymes and microbes to cellulose and hemicelluloses.

Although the function of pre-treatment for both bioethanol and AD is to overcome the limiting step of substrate hydrolysis by improving the enzymatic degradability of lignocellulosic biomass, the selection and integration of pretreatment methods, parameters, and strategies may differ depending on downstream processes, that is to say, that the property conditions in the production of biogas might also be profitable to the process of AD.

2.1. Types of Pre-treatment Techniques

Different types of pre-treatment techniques have been studied along these years, as well as their effects on the process of improving the efficiency of the AD process, and so the biogas obtained.

In conclusion, all pre-treatment techniques significantly increase the accessible surface area of cellulose via various mechanisms such as lignin and hemicellulose removal/redistribute, particle size reduction, and pore expansion.(XianzhiMeng, 2014).

2.1.1. Chemical Pre-treatment Techniques

Chemical processes rely on oxidative reactions. The most chemical pre-treatment techniques used are ozonation, as well as chemicals such as acids, bases, and ionic liquids, to alter the chemical characteristics of biomass. Some of the most chemical treatments studied in the case of lignocellulosic material are as follows:(Yi Zheng. Et al, 2014)
In the table above some of the most used chemical pre-treatment techniques are listed, such as the alkaline pretreatment where bases are used to remove lignin, hemicelluloses, and/or cellulose, rendering lignocellulosic biomass more degradable to microbes and enzymes. The acid pre-treatment is also a remarkable method, where concentrate acid and low temperature or even dilute acid and high temperature are used to help with cellulose hydrolysis. Dilute acid pretreatment primarily hydrolyzes up to 100% of the hemicellulose into its component sugars (e.g. xylose, arabinose and galactose),
depending on the pretreatment conditions. It can also disrupt lignin to a high degree, but is not effective in dissolving lignin in most cases. The main result of dilute acid pretreatment is to significantly increase the susceptibility of cellulose to microbial degradation and enzymatic hydrolysis.

In this case there are some disadvantages, due to the use of acids, such as their recovery because of their toxicity, their high price, the restructuration of the reactor and all the process using non metallic materials, as well as the energy costs derived from the process.

*Catalyzed steam-explosion*

Steam pre-treatment can be carried out with the addition of catalysts such as H2SO4, SO2, and NaOH. The presence of a catalyst can improves biodegradability of lignocellulosic biomass, reduce the production of inhibitory compounds, and result in more complete solubilization/recovery of hemicelluloses.

*Wet oxidation*

Wet oxidation is an oxidative pre-treatment method that consists of the addition of water and an oxidizing agent (e.g. air, oxygen and hydrogen peroxide [H2O2]) to feedstocks, prior to pretreatment.

Wet oxidation can effectively increase the biological accessibility of the cellulose fraction to microorganisms and enzymes through removal of lignin and hemicelluloses.

*Ozonolysis pre-treatment*

Ozone is a powerful oxidant which can be used as a pre-treatment (ozonolysis) for lignocellulosic biomass. Ozonolysis pre-treatment results in more degradable biomass primarily via lignin-degradation, with a slight alteration of hemicellulose; however, it has very little effect on cellulose. Main pretreatment parameters are: water content in the reactor, particle size, and ozone concentration in the gas stream. This pre-treatment technique is generally carried out at ambient temperature and pressure, and does not generate the inhibitory compounds associated with other thermo-oxidative pre-treatments, such as wet oxidation and wet explosion.
Mechanistically, ozone reacts with polysaccharides, proteins and lipids, transforming them into smaller molecular-weight compounds, so that the cellular membrane is ruptured, spilling the cell cytoplasm.

Oxidative pre-treatment with peroxides

Oxidation pre-treatment with peroxides is a common method for enhancing biological conversion of lignocellulosic biomass to bioethanol.

Ionic liquids pre-treatment

This is a new way of chemical pre-treatment with good effectiveness on cellulose dissolution for bioethanol production, due to the fact that high amounts of cellulose can be dissolved at mild conditions, which are 90-130 °C and ambient pressure, and its efficiency of recovery is about 100%, so the residues left in the downstream of the process are minimum. There are also other advantages such as low toxicity, low hydrophobicity, low viscosity, thermal stability, broad selection of anion and cation combinations, enhanced electrochemical stability, high reaction rates or non-flammable properties.

What these liquids are capable of doing involves oxygen and hydrogen atoms of cellulose hydroxyl groups, which form electron donor electron acceptor complexes that interact with ILs. Upon interaction between cellulose’s hydroxyl groups and ILs, hydrogen bonds are broken, leading to opening of the hydrogen bonds between molecular chains of cellulose, resulting in cellulose dissolution

2.1.2. Biological Pre-treatments

As have been shown in the first part of this study, the hydrolysis step is the main stage talking in terms of kinetic of the AD system. As seen in previous paragraphs, not only chemical treatments are used to improve this sense of effectiveness. Indeed, biological treatments have the advantage of not leaving residues in the process. On the other hand, there are also other disadvantages, in that it is conducted under much milder environmental conditions, so that few inhibitors, which could negatively affect anaerobic digestion, are generated. However, long pre-treatment time has limited the use of these processes in commercial applications. In addition, there is competition for carbohydrates between pretreatment and downstream biogas production, because certain levels of carbohydrates are required by microbes during biological pre-treatment.( Yi Zheng. Et al, 2014).
Biological pre-treatments include both the use of microorganisms with high ability to degrade a substrate and the addition of enzymes that support biological reactions within anaerobic digesters. (Alessandra Cesaro. Et al, 2014).

In general it has been found that the pre-composting substrate is the best biological pre-treatment. Composting is a continuous process of reducing organic substances into smaller volumes under natural or controlled conditions it involves a decomposition process till the ratio of carbon to other elements are equal in the same time providing plants with nutrients in absorbable conditions (Saili Nur Shafawati. et al, 2013).

Some other works like that carried out by (Jun Wei Lim. Et al, 2012) studied the effect of microaeration as pre-treatment. The term “microaeration” was defined as the introduction of small amounts of oxygen into an anaerobic biochemical process to enable both anaerobic and aerobic biological activities to occur within a single bioreactor. Other studies such as that by (Jun AnChiam. etal, 2014) corroborate what was stated in previous studies, where different bacterial species were compared, and many of them possessed the ability to consume oxygen and maintain a reducing environment so that fermentation could occur despite higher amounts of oxygen introduced. In comparison to anaerobic conditions, microaeration led to a significantly more diverse bacterial community.

There was a greater distribution of the bacteria, which enabled the acidogenic reactor to metabolize a wider variety of substrates, giving rise to enhanced COD solubilization and VFA production under microaeration conditions.

Other recent studies focus on genetic manipulation of biomass feedstock, where the main aim is to change the cell wall components and structures to improve cellulose accessibility. (XianzhiMeng. etal, 2014)

Other authors (Carrere H.et al, 2010; Carballa M.et al, 2007; Goel R.et al, 2003; Yoem I.et al, 2002; Javkhlan Ariunbaatar.et al, 2014)consider the configuration of the reactors as a way of including a biological pre-treatment in the system. If physically separate, the acidogens from the methanogens can result in a higher methane production and COD removal efficiency at shorter hydraulic retention time (HRT) in contrast to conventional single-stage digesters (Parawira W.et all, 2005).
2.1.3. Physical Pre-treatment Techniques

Physical pre-treatment refers to methods that do not use chemicals or microorganisms during the pre-treatment processes. Included in this paragraph, both mechanical and thermal ones have to be listed, but other emerging technologies are being implemented at both research and industrial level, such as high pressuring machines, microwave and ultrasound devices, which constitute the purpose of this study.

*Mechanical pre-treatments*

Mechanical pre-treatment is used to reduce both the particle size and crystallinity of lignocellulosic materials, in order to increase the specific surface area and reduce the degree of polymerization. This effect can be obtained by a combination of chipping, grinding or milling, depending on the final particle size of the material. It is proved by studies such as “Effect of particle size on biogas yield from sisal fibre waste”, from (Anthony Mshandete. et al, 2006), that smaller particles provide a large surface area available for the microorganisms, resulting in increased microbial activity; thus, the anaerobic biodegradability increases, is shown in Graph X below:

![Graph 17: Effect of pre-treatment, by size reduction of sisal fibre waste, on anaerobic biodegradability: (a) NDF(neutral detergent fibres) degradation(%), after 65 of digestion and (b) improvement in biogas production as potential increase in methane yield (%) for different sizes of sisal fibre waste in comparison with non treated material.](image-url)
However, this particle reduction can accelerate the hydrolysis and acidogenesis steps as well as the production of soluble organic material like volatile fatty acids (VFAs), resulting in excessively high organic loading in the anaerobic digestion reactor. It was found that excessive size reduction caused VFA accumulation, which brought a decreased solubility and decreased methane production from the anaerobic digestion process.

What is more, the power requirement of this type of pre-treatment technique is usually high depending on the final particle size that is wanted as well as the type of the substrate used.

*Thermal pre-treatments*

Thermal treatment has been used as a conditioning process for raw or digested sludge as it improves the dewaterability properties of such waste. Heat treatment alters the structure of the insoluble fraction to make it more liable to biodegradability. Often thermal pre-treatments are coupled with alkaline ones to test the combined effects on specific organic substrates, with the main advantage of reducing both thermal energy consumption and reagent doses. Under proper conditions, the thermo-chemical pre-treatment can significantly improve organic substrate solubilisation, but it is evident that the enhanced solubilisation does not necessarily result in improved methane production, due to the occurrence of some problems, such as:

– The possibility of producing compounds which can inhibit subsequent anaerobic digestion processes;

– Solubilisation of molecules characterised by a structure which is difficult to degrade;

– Use of chemicals, which can lead to toxicity problems.

Another disadvantage in this type of pretreatment is that, as in mechanical pre-treatments, high energy consumption is needed, which makes the process unprofitable.

*Microwave irradiation*

Microwave uses the ability of direct interaction between a heated object and an applied electromagnetic field to increase heat, thus combining both thermal and non-thermal effects generated in aqueous environment. The movement of ions and the vibration of polar molecules give rise to heat and extensive intermolecular collisions which accelerate chemical, physical, and biological processes.
Ultrasound

As in this study, ultrasound has been used more frequently to pretreat organic matter and use it in a digestion system. This system is based on monolithic cavitations, which have physical effects, generated by the collapse of cavitational bubbles, as well as chemical effects, because of the formation of free radicals due to the breaking of these bubbles, in liquid solutions. (Alessandra Cesaro et al, 2014)

If compared with other pre-treatment techniques (thermal, base, acid and bacterial product), US process proved to be the most versatile one, as it was effective with different kinds of fat-predominant solid substrates. (S. Luste et al, 2009).

Bearing in mind all these types of pre-treatments, in this table a summary of the major mode of action is shown for different pre-treatments in terms of cellulose accessibility increase, as the case of study, in which lignocellulosic material is used as substrate.

Table 8: Major mode of action for different pre-treatments (Xianzhi Meng et al, 2014)
2.1.4. Combination of Pre-treatments

Pretreatment methods have also been studied in combination to obtain further enhancement of biogas production and faster AD process kinetics.

*Thermo-chemical pre-treatment*

The combination of microwaves with chemical pre-treatments, as well as with microwave irradiation at temperatures higher than 145 °C, resulted in a larger component of refractory material per gCOD, causing a decrease in the biogas production. This could be explained by increased hydrolyses of proteins and carbohydrates due to chemical pre-treatment. In the presence of heat the produced amino acids and sugars reacted together forming complex polymers such as melanoidins. However, alkaline pre-treatment coupled with thermal methods at lower temperature (70ºC) could result in higher (78%) biogas production with higher (60%) methane content.(Rafique R.et all, 2010), (Carrere H.et all, 2009)

*Thermo-mechanical pre-treatment*

In this case there have been some studies that have proved that combining ultrasonic and alkaline pretreatment (Elliot A.et al, 2012) resulted in the best rate in the hydrolysis stage, but on the other hand the concentration of ammonia in the reactor increased, which means instability in the system.

2.2. Ultrasound

One of the purposes of this study is to attempt to understand how ultrasonic techniques work, as well as their effects, in the process of anaerobic digestion.

disintegration. Ultrasound is known to disintegrate sludge flocs and disrupt microbial cell walls, and it causes the release of soluble substances. (A. Tiehm et al., 2005) showed that applying ultrasound (3.6 kW, 31 kHz, 64 s) to sludge disintegration can release the organic substances into the sludge, so that the soluble chemical oxygen demand (SCOD) in the supernatant increases from 630 to 2270 mg/L. (Fen Wang et al., 2005)

2.2.1. General Principle

A sound wave is a lengthwise wave transmitting which is associated with sound. If it spreads into an elastic continuum, it generates a local variation of pressure or density, which is transmitted in the form of periodic or quasi-periodic spherical wave. Mechanically sound waves are a type of elastic wave.

Pressure variations, humidity or temperature in atmosphere, lead to the displacement of the molecules that are formed. Each molecule transmits the vibration to those molecules near it, causing a chain movement.

Most acoustic waves are three-dimensional, as is shown in the following image, and would spread out spherically from a point source (The Acoustic Bubble, T Leighton, 1994)

Image 6: How a sound wave spreads

The characteristics of a wave are important in terms of ultrasound, because these might be used to understand the way ultrasound affects the material utilization:
Amplitude: is the major distance from the position of equilibrium. Bigger amplitude is translated into greater transportation of energy.

Wavelength: is the distance between two successive crests.

Frequency: is the number of oscillations of the wave per unit time.

Propagation speediness

When sonicated at frequencies higher than 1 MHz, the liquid starts to stream at molecular level. (Technical University of Hamburg-Harburg, Ultrawaves.UK S.A 2011)

At different acoustic frequencies the following remediation effects can be obtained:

- Frequency range between 20 kHz - 100 kHz: disintegration of cells, disinfection, destruction of polymers, release of enzymes.

- Frequency range between 100 kHz - 1 MHz: breakup of the structure of chloro-organic compounds such as chlorophenol, TBT, MTBE, release of enzymes.

- Frequency range between 1 MHz - 10 MHz: desorption of absorbed organic molecules from solid surfaces, biologically available organic matter, and simultaneous biological degradation.
Some studies like the one carried out by (Juan de la Cierva, 2010), which focused on the effects of US in food, established that ultrasound can be divided into different frequency ranges. Until recently, most applications of ultrasound in food technology involved a non-destructive analysis which referred particularly to the quality assessment; such applications use high frequency (100 kHz to 1 MHz), low power (typically <1 W cm⁻²) US. Low-intensity ultrasound is most commonly applied as an analytical technique to provide information on the physicochemical properties of food such as firmness, ripeness, sugar content, acidity, etc (Demirdöven & Baysal, 2009).

Ultrasound effects on liquid systems are mainly related to the cavitation phenomenon. Ultrasound is propagated via a series of compression and rarefaction waves induced on the molecules of the medium passed through image showed below (Mason, Riera, Vercet, & Lopez-Buesa, 2005). At a power sufficiently high, the rarefaction cycle may exceed the attractive forces of the liquid molecules and cavitation bubbles may form from gas nuclei existing within the fluid. These bubbles, distributed throughout the liquid, grow over the period of a few cycles to a critical size until they become unstable and violently collapse (Shukla, 1992; Mason, 1998; Barbosa-Cánovas & Rodríguez, 2002). The implosion of cavitation bubbles leads to energy accumulations in hot spots, generating extreme temperatures (5000 K) and pressures (1000 atm), which produce, in turn, very high shear energy waves and turbulence in the cavitation zone.
2.2.2. Cavitation

Ultrasonic disintegration is a well-known method for the breakup of microbial cells to extract intracellular material (Harrison, 1991), which is the purpose in this study. The impact of ultrasound waves on a liquid causes the periodical compression and rarefaction of the medium. Cavitation occurs above a certain intensity threshold, when gas bubbles are created, which first grow in size before violently collapsing in a few microseconds (A. Tiehm.et al, 2003). The violent collapse produces very powerful hydromechanical shear forces in the bulk liquid surrounding the bubble. It has been shown that macromolecules with a molar mass above 40,000 are disrupted by the hydromechanical shear forces produced by ultrasonic cavitation. The mechanical forces are most effective at frequencies below 100 kHz (Portenlänger, 1999).
This really means that cavitation consists of formation, growing and explosion of bubbles in a liquid, which has previously been treated with an acoustic camp (Kardos, 2001).

It may be considered that the time necessary for the increase in size of the bubble, \( \tau_g \), can be expressed as (Abramov, 1998) with:

\[
\tau_g = 0.75T + \frac{i - 1}{T}
\]

\( i \) = number of cycles

\( T = \frac{1}{f} \) = ultrasonic wave period equal to the inverse of the frequency

When the bubbles are formed there are different ways in which these can behave. In the case in which the peak acoustic pressure in the rarefaction cycle is not intense enough to allow expansion of the bubble and collapse, it is referred to as stable cavitation. However, if cavitation takes place in the case in which the peak sound pressure, during the cycle of rarefaction, appears to allow the growth of the bubbles in the middle or more acoustic cycles with subsequent rapid collapse, it is known as transient cavitation.

2.2.3. Formation and Evolution of Bubbles

The question of what formation of a bubble really involves, lies in the fact that the bubble activated by a sound field to undergo an energetic motion which is detected by pertinent observations (e.g. because of its shape and size, acoustic emissions, sonoluminescence, erosive properties, etc...), is not simply formed from the bulk liquid, but is seeded from some pre-existing gas pocket in the liquid. Therefore in some situations this new surface is created by expansion of existing surfaces. Because of this (Apfe, 1981) generalizes his definition of acoustic cavitation as “being the formation of a vapour cavity or bubble in response to acoustic field” to “encompass any observable activity involving a bubble or population of bubbles stimulated into motion by an acoustic field”.

As pointed out above, ultrasonic waves create pressure differences within a solution for the enhancement of physical (mechanoacoustic) and chemical (sonochemical) processes. This occurs at frequencies beyond the audible range, typically between 20 and 1000 kHz. Ultrasound is generated by either piezoelectric or magnetostrictive transducers. Piezoelectric transducers are more commonly used today and they manipulate the piezoelectric property of some ceramics. The piezoelectric material will respond to alternating current with mechanical vibrations to produce ultrasound of a characteristic
Some researchers (Robert Mettin et al, 2014) have studied the influence of these bubbles in the process of sonication, finding that larger bubbles show faster motion than smaller ones, which is due to an interaction driven by secondary Bjerknes forces (T.G. Leighton, 1994; H.N. McMurray et al, 1999).

If the bubble of radius R is considered as shown in the following image, there is internal pressure $p_i$ within the bubble as a result of the pressure of gas ($P_g$) and the pressure of liquid vapour ($P_v$), so that:

$$p_i = P_g + P_v$$
The pressure within a bubble at rest is greater than the pressure in the liquid immediately outside the bubble as a result of surface tension forces. If the pressure in the liquid outside the bubble, at the bubble wall, is \( p_l \), within the bubble it is:

\[
P_i = p_l + p_v = p_l + \frac{2\pi}{\rho}
\]

Due to the acoustic field, all the bubbles with a radius greater than the critical radius and with internal pressure higher than the outside, become mechanically unstable and tend to grow. During this process there is not much gas, which has time to diffuse into the surrounding liquid, starting from the bubble. When the phase of the acoustic field is inverted and the liquid is invested by a compressive force, the external pressure becomes higher than the inside area, the bubble ceases to grow in size and begins to implode at very high speeds that can exceed that of sound in the liquid. The vapor in the bubble tends to condense and the implosion process, adiabatic, is arrested by the gas and the remaining steam that can reach extreme conditions of temperature and pressure.

The motion of the bubble in the liquid is not linear as is the gas; it behaves linearly only for small displacements, that is, for small variations of pressure.
2.2.4. General Effects of Ultrasonic Technologies

**Mechanoacoustic Effects**

These are caused by the pressure differentials which augment mixing processes and the microjets from the bubble collapse. The formation of microjets (Suslick, K. S, 2002) occurs when the cavitation takes place near a solid boundary of larger size than the bubble. The normally spherical cavitation is distorted by the asymmetrical liquid motion near the boundary. The microjets produced are responsible for cleaning applications of ultrasound due to the erosion capabilities. Additionally, microjets have the ability to break cell walls and increase the available surface area in heterogeneous systems.

![Image 12: Formation of microjets near a solid boundary (Suslick, K. S, 2002)](image)

**Sonochemical Effects:**

Ultrasound is also used to enhance chemical reactions or to choose a certain reaction pathway. These pathways are normally radical driven processes. Reaction augmentation via ultrasound often results in faster reactions at lower temperatures than otherwise possible and can reduce the amount of chemicals required in a process (Mason, T. J, 2003).

2.2.5. Influencing Factors

Some factors that are used in US pretreatment affect the severity and incidence of cavitation and hence the degree of sonochemical and mechanoacoustic influence. Generally an increase in viscosity, decrease in temperature and increase in external pressure applied will increase the lower cavitation threshold. The operating parameters affect the severity and incidence of cavitation and hence the degree of sonochemical and mechanoacoustic influence. Generally an increase in viscosity, decrease in temperature and increase in external pressure applied will increase the lower cavitation threshold.

Other parameters that must be controlled are the temperature applied, which must be optimal for the ultrasonic effect, as well as the pressure applied. If there is an increase in
the pressure of the system an increase in power will be needed to achieve the same amount of cavitation, so higher amplitude is also needed in the rarefaction of the cycle to create a transient bubble.

Taking all these aspects into account, the effects of the parameters such as frequency, solvent, gas saturation, geometry, and reactor configuration need to be studied.

### 2.2.5.1. Intensity

Ultrasound is propagated through longitudinal waves that comprise rarefactions (negative pressures) and compressions (positive pressures). When the acoustic pressure at the rarefaction cycle is greater than the local cavitation threshold pressure, any minute cavity available will grow in size and become stable or transient bubbles (Laborde et al., 1998). It follows that the process of bubble formation could be regarded as a competition between liquid strength $P_{cv}$ and acoustic pressure $P_A$:

$$P_{cv} = P_0 - P_v + \frac{2}{3\sqrt{3}} \sqrt{\frac{(2\sigma_L/R_0)^3}{P_0 - P_v + 2\sigma_L/R_0}},$$

$$P_A = \sqrt{2I\rho C},$$

where $P_{cv}$ is the cavitation threshold pressure, $P_v$ is the saturation vapour pressure, $R_0$ is the initial bubble/cavity radius, $\sigma_L$ is the surface tension, $P_A$ is the acoustic pressure, $I$ is the ultrasound intensity, $\rho$ is the density of the medium and $C$ is the velocity of sound in that medium.

The formation of cavitation bubbles will be realized only if $P_A$ is greater than $P_{cv}$ (Abramov, 1998; Tatake and Pandit, 2002). It can be noted from the equations above that $P_{cv}$ is dependent on the nature of the medium, while $P_A$ depends on both properties of the medium and ultrasound. Hence, given a similar test sample, where $P_{cv}$, $\rho$ and $C$ are constant, cavitation bubble evolution will be solely dependent on sonication intensity. As expressed by the last equation above, the acoustic pressure $P_A$ is proportional to the square root of sonication intensity.

As the study developed by (Kuan-Yeow Show, 2007) shows, the acoustic pressure increased with the sonication intensity applied (Graph below). Thus, it can be deduced
from such theoretical consideration that the higher the sonication intensity, the more powerful the acoustic pressure exerted to overcome liquid strength $P_c$, which leads to more extensive particle disruption.

Graph 18: Acoustic pressure as a function of sonication

### 2.2.5.2. Frequency

The normal frequencies used for mass transfer improvement are the lower ones, and the higher ones are used for increased sonochemical activity (Kanthale, P. et al, 2008; Adewuyi, Y. G, 2001).

At lower frequencies (under 100 kHz) the bubbles have more time to grow, and, therefore, the cavitational collapses are more violent. At higher frequencies more bubbles are produced which eventually collapse, producing more radicals.

There is general agreement that radically driven processes are maximized at high frequencies and physical effects are maximized at low frequencies. When considering a heterogeneous system such as biomass in solution, it is important to consider which frequency effects are more desirable and effective, be the mechanical erosion or be the chemical attack (Madeleine J. Bussemaker et al, 2013).

The volume change of the bubbles of high intensity is, however, lower than that obtained at low intensity, and for this reason a lower amount of energy will be released, and it will collapse at lower temperatures.
The radius of the resonance of the bubbles, which influence collapse and thus the intensity of the phenomena of disintegration and solubilization, is pariah (Kuan-Yeow Show, 2007).

\[ R_{\text{max}} \approx \frac{3.28}{f}. \]

Where \( R_{\text{max}} \) is the resonant or maximum radius (cm) and \( f \) is frequency.

### 2.2.5.3. Solvent

**Types**

Solvents which turned out to be successful at pretreatment of lignocellulose coupled with ultrasound were organized in three categories: aqueous, organic liquid, and ionic liquid.

Chemicals within the ultrasonic solution will change the viscosity of the solution which in turn will affect the cavitational threshold and physical properties such as augmentation of mass transfer and presence of shear forces of the solution. The chemical species also have the potential to partake in the bubble collapse and produce different radicals and species available to interact with the lignocellulose in the solution. Because of this, it is usually found that the US is combined with other types of pretreatments mentioned in section 2.1.

**Effects**

The solvent will affect the physical properties of the solution, such as surface tension and viscosity, which govern the severity of the cavitational collapse and subsequent ultrasonic effects. Furthermore the chemical behavior of the solution will influence the overall outcomes of ultrasonic irradiation (Madeleine J. Bussemaker. et al, 2013)

In an aqueous solution, hydrophobic solutes influence the ultrasonic effects. Solutes which are hydrophobic will gather at the bubble interface and can either act as radical scavengers in the hot region surrounding the bubble or reduce the maximum temperature reached during bubble collapse, quenching sonoluminescence (Ashokkumar, M.et al, 1997; Ashokkumar, M.et al, 2000). The mechanisms of quenching occur through evaporation of the solutes into the oscillating bubble and subsequent degradation reactions within the bubble, which means that the degradation products remain in the
bubble and over several oscillations they will accumulate, quenching sonoluminescence and maximum temperatures. The more volatile the solute, the more it is able to quench sonoluminescence due to the increased ability of evaporation into the bubble.

Thus, quenching is believed to be more influential on stable cavitation, where the solutes are able to reach near equilibrium absorption conditions, rather than intransient cavitation fields. These observations highlight the potential effects of solutes within an aqueous system.

In an organic solvent, degradation products were produced from the solvent. In organic solvents the volatility of the solvent can influence the effects since the volatility is governed by vapor pressure which determines the ease of evaporation into the cavity. An increase in volatility decreases the intensity of the cavitational collapse, which in turn decreases the maximum temperatures and reaction rates (Suslick, K. S. et al, 1984).

Ionic liquids have very little vapor pressure; however, sonochemical effects are still observed within them, as are the degradation products of the liquid.

Studying the solvent is necessary to mention their physical properties, as this explains the interaction with the cavitation and with the acoustic propagation. These are viscosity, vapor pressure, temperature, surface tension, or the concentration of solids.

### 2.2.5.4. Dissolved Gas

The gas present within the solution can influence the physical and chemical processes brought about by ultrasound. Ultrasound leads to oxidizing radicals, and the presence of different gases will affect not only the rate of the formation of these radicals but also which reactive species are produced. The effects of the gas dissolved are influenced by shockwave intensity, polytropic ratio, and thermal conductivity. The intensity of a shockwave will be reduced by a cushioning effect of dissolved bubbles; however, an increase of gas in the cavitational bubble can lead to an increase in the intensity of the shockwave.

The ideal environment to work with would be with the use of noble gases, as these are able to increase the temperature reached during the collapse of a bubble and thus can enhance sonochemical reactions. However, the use of noble gas is an expensive option, and the enhanced yields would need to be high enough to justify the additional cost.
2.2.5.5. Geometry

A study by (Gogate, P. R. et al, 2011) pointed out that geometry affected power dissipation, ultrasonic flow, and mass transfer within a sonicated solution. The sonochemical effects were attributed to the migration of active bubbles to the outer solution, outside of the sonication zone. The active bubbles would then dissolve, decreasing the yield of radicals in solution. The consideration of geometry, liquid height and wave attenuation is also important when considering a heterogeneous system such as biomass, as biomass may act as a reflector of the ultrasonic wave and effectively change the sonication region.

2.2.5.6. External Pressure

The increase of the external pressure increases the value of pressure rarefaction; therefore it will be more difficult to obtain cavitation. Breaking the bonds of the liquid medium will require a greater intensity of sonication; however, upon reaching the threshold of cavitation, the phenomenon of collapse of the bubbles will cause most intense effects (Pilli, 2011).

2.2.5.7. Reactor Configuration and Design

Reactor design such as geometry, batch versus flow, and the method of ultrasound delivery will influence the efficacy and viability of the treatment.

Alteration of the reactor geometry via liquid height and reactor diameter was found to influence the sonochemical yield and thus the ultrasonic pressure wave properties, which are most likely to be responsible for the observed differences.

Scale-up of ultrasonic treatment would need to consider the effect of flow in a reactor. Stirring combined with ultrasound was successful in improving enzymatic hydrolysis, (Yachmenev, V.et al, 2009) delignification, (Baxi, P. B.et al, 2012) and liquefaction, (Kunaver, M. et al, 2012) at low frequencies.

The various types of reactor configurations differ mainly on the basis of geometry of transducers, location of transducers and operating frequencies. Broadly speaking, sonochemical reactors can be classified into direct immersion type reactors (ultrasonic horn), indirect irradiation type (ultrasonic bath, flow cells, etc.)
Ultrasonic horn is typically an immersion transducer type reactor generating very high intensities near to the tip of horn. The intensity decreases with an increase in the distance from the tip of horn, and generally the active cavitation zone is restricted over the range of 3–5 cm, depending on the power input and operating frequency. Ultrasound horn is suitable for small scale operation and where vigorous stirring is important. Acoustic streaming occurs generally away from the transducer surface towards the free surface and undergoes reflections at the reactor wall or the liquid surface generating a mixed recirculation.

In the case of ultrasonic bath, transducers are placed at the bottom of the tank and the ultrasonic generator is usually a separate unit. The most active zone is observed just above the plane of transducers with maximum intensity at the center of the transducer. The area of irradiating surface is much higher as compared to the single transducer based reactor and thus there is a better distribution of the incident energy into the reactor volume. The arrangement of transducers can be positioned in triangular, hexagonal or any other arrangement to maximize the active cavitation zone in the reactor (P.R. Gogate, 2008).

A flow cell configuration offers flexibility for either batch or continuous operation. Furthermore, as the multiple sides of the flow cell are equipped with transducers, multiple frequency operation is also possible in the given sonochemical reactor configuration. Multiple frequency transducers in the reactor give uniform cavitation activity as well as higher cavitation intensity at similar levels of power dissipation (Vitthal L. Gole. et al, 2012).

2.2.5.8. Feedstock Characteristics

The initial feedstock is an important consideration as the physical properties as well as the chemical composition will determine the efficacy of ultrasonic treatment.

*Type of Biomass:*

There are different types of lignocellulosic biomass, which will respond in a different way to ultrasonic treatment under the same conditions.

Ideally an ultrasonic pre-treatment process would be useful for all types of lignocellulosic biomass. However the literature indicates that for a variety of goals, such as polysaccharide extraction, delignification, glucose hydrolysis, and liquefaction, optimal
conditions vary significantly among biomass types. Therefore ultrasonic treatment of lignocellulose could only be practical for a single biomass type or for a homogenized sample.

**Loading:**

Biomass loading was found to be the least influential parameter as compared to lime loading and ultrasonic treatment time (Velmurugan, R. et al., 2011), although there must be a control of this parameter to increase the efficiency of the process, so delignification, reducing sugar yield, could be more effective under ultrasonic pretreatment.

### 2.2.6. Effects on the Environment

The cavitational effects can be classified as chemical (generation of free radicals) and physical (liquid streaming associated with intense turbulence). In some cases, such as the production of biodiesel, are limited by mass transfer, so physical effects are likely to be controlling. In heterogeneous liquid/liquid reactions, cavitation collapse at or near the interface will cause disruption and mixing, resulting in the formation of very fine emulsions. When very fine emulsions are formed, the surface area available for the reaction between the two phases is significantly increased, which result in increased rates of reaction (P.R. Gogate *et al.*, 2011). The emulsion formed using cavitation is usually smaller in size and more stable (Vitthal L. Gole *et al.*, 2012).

For obtaining effective intensification of the synthesis process, maximizing the physical effects of acoustic streaming is needed. These effects are dependent on the bubble size, life of the cavity and the collapse time, which in turn depend on power dissipation, operating frequency, physicochemical properties of the solvent, and geometry of equipment.

#### 2.2.6.1. Physical Characteristics

One of the indicators useful at the time of describing the effects of these pre-treatment points at physical characteristics are.
**Particle size:**

One of the effects of using ultrasound as pre-treatment is the disintegration ability that makes particles reduce their size, which also means a release of carbohydrates and proteins into the milieu.

The study by (Kuan-Yeow Show, 2007), with sludge from a wastewater plant, noticed that the particle disruption was apparently related to the exposure time in the ultrasonic field. Drastic particle size reduction was noted in the initial 1 min of sonication, and the disruption subsided subsequently. The results obtained suggested that sonication could be set for less than a minute for energy cost optimization.

Graph 19: Effects of sonication time on particle size (Kuan-Yeow Show, 2007)

In the following graph 25, it is possible to see in more detail what is explained in the graph above. The particle size distributions were analyzed with respect to sonication time. The graph illustrates that particles, which are larger than 4.4 mm exhibited the most intense disruption in the initial 1 min, and the disruption became marginal on further sonication. It turned out that micro-flocs (<4.4 mm) were less susceptible to ultrasonic disruption than macro-flocs (44.4 mm). It could be interpreted that the binding forces in micro-flocs, such as cells, are much stronger than those in macro-flocs that are made up of a more loosely bound aggregation. It is also likely that macro-flocs have larger surface areas exposed to the sonication, causing a greater extent of disruption; these results are also supported by the study carried out by (C. P. Chu. *et al*, 2001)
The goal of this study is to find out how ultrasonic pre-treatment acts with lignocellulosic substrate. The structure of lignocelluloses is complex and recalcitrant, with cellulose, hemicellulose and lignin as the three most abundant components (Jia Luo. et al, 2013). One of the primary requirements for the thermochemical and biochemical conversion of lignocelluloses is the pre-fractionation of raw lignocelluloses before reactions; therefore, for that aim ultrasound is used for this study. Lignin and hemicellulose should be firstly removed from cellulosic materials, as the raw lignocellulosic complex hinders access of enzyme molecules and chemical catalysts. The critical issue is how to improve the efficiency and economics in the destruction of lignocellulosic structure and the separation of the connected components at mild condition. The reduction in the reaction severity also means less production of by-products, as well as less alteration of the glucosidic structure of cellulose.

Another important parameter to take into account is the need for opening the crystalline structure of raw cellulose at mild conditions. Compared with lignin and hemicellulose, the regularity in the chemical and crystalline structure is the most prominent characteristic of cellulose. Hundreds to tens of thousands of D-glucopyranoses constitute the molecular chains through β (1→4) glucosidic linkages with unified orientation, while most chains participate in the recalcitrant crystalline region of raw cellulose by intra and intermolecular hydrogen bonds, which make cellulose strongly hydrophobic, and difficult to be dissolved.
or degraded into common solvents, which is one of the main problems of lignocellulosic material. In the degradation dynamics of cellulose, the lowering and destruction of its crystalline structure requires extremely high activation energy.

The last requirement is related to the two first requirements above mentioned, and lies in the intensification of mass transfer in heterogeneous reactions containing solid lignocellulosics. The surface of lignocellulosics needs to be increased through pre-treatment for better contact with solvents for reactions.

Ultrasonic pre-treatment can address these requirements, because ultrasonic treatment features high local strength with its intense cavitation and overall moderate effects.

As can be seen in this image, ultrasonic energy applied as pre-treatment has special mechanistic effects on the structural integrity of lignocelluloses. Ultrasonic treatment can destroy wax layers and silica bodies deposited onto the surface of lignocellulosic structures and can aid in their removal (Jia Luo et al, 2013). The result of this is that the size of biomass particles can be reduced when subjected to high power ultrasound.
Indeed studies such as that by (Samin Rezania et al, 2009) used ultrasound technology for reducing the particle size using lignocellulosic material, obtaining these results:

![Graphs showing particle size distribution over time](image)

Image 14: Average particle sizes following sonication (initial particle sizes 23 ≤ x ≤ 560 μm). b Average particle sizes following sonication (initial particle sizes ≤ 295 μm). c Average particle sizes following sonication (initial particle sizes ≤ 75 μm). (Samin Rezania, 2009)

All these results clearly show effectiveness of ultrasonic irradiation in reducing the particle size. It was also noticed that the average particle size appears to increase after reaching some minimum value. This is attributed to agglomeration of particles that pack together from the impact in the high energy sonication environment. Agglomeration occurs often for micron (<10 μm) or nano-sized particles, even if they are dry. In a wet environment, wet agglomerates are bonded by the effects of surface tension and capillary forces of the liquid binder (Pietsch, W, 2007).
The same results were founded by (Bishnu Karki, et al, 2010), which shows that the particle size reduction was directly related to sonication amplitude and time.

As well as these authors, (DipakVitthal Pinjari, et al, 2010) explains that the change on particle size is due to two possibilities. The first possibility is that when the cavity collapses asymmetrically on the surface of the solid surface, it produces a high velocity liquid jet pointed towards the particle surface, which results into an action similar to liquid jet cutting. The second one is that when the cavity collapse occurs, the shock wave generated travels through liquid media generating local pressure gradient and fluid shear causing attrition of the solid materials (particles), and the particle size reduction takes place.

The relation between the intensity of ultrasonication and the reduction on particle size has been studied (as the following graph shows, there appears to be only modest
enhancement as ultrasonic intensity increases from 20% to 60% (40 to 110 W) and mostly in terms of the amount of time to reach the minimum size rather than in obtaining smaller particles (SaminRezania. etal, 2009).

Image 15: Average particle sizes throughout 12 h of sonication using 20% intensity. b Average particle sizes throughout 12 h of sonication using 40% intensity. c Average particle sizes throughout 12 h of sonication using 60% intensity(SaminRezania. Et al, 2009)

To see in a more detailed way what a reduction on particle size means, the study carried out by (SaminRezania. Et al, 2009) shows, with help of SEM images, the sewage prior to sonication and after 4 h of sonication, both at ×400 and ×5,000 magnification. The unsonicated sample shows a more rigid and connected structure with larger particles, while the sonicated sample appears to have smaller, disconnected particles. The surfaces of the sonicated particles also appear to be smoother.
PARTE II: PRE-TREATMENT AND ULTRASOUND TECHNIQUES

Image 16: SEM images of a) un-sonicated sawdust compared to b) sonicated sawdust. (SaminRezania. Et al, 2009)

**Viscosity:**

The aim of explaining the changes on viscosity is due to its relation with the particle size. An increase in the viscosity often brings inconvenient consequences to the process as it is more difficult to establish conditions of mixing and so the homogenization of the system.

(Dasari, R. K.et al, 2007) showed an unexpected benefit of processing materials with smaller particles in that the viscosity of the biomass slurries dropped significantly as particle sizes in the slurry were reduced. This carries an important advantage, especially when a system is used in a large scale. Following these points, ultrasound would help at the time of reducing the viscosity as it can reduce the particle size. Unexpectedly though, the viscosity increased as the particle size decreased in the range tested here. The viscosity changes due to morphological changes of the particles.
Graph 22: Viscosity vs. shear rate (10% initial solids concentration, un-sonicated particles size range=10≤x≤75 μm; 4-h sonicated particle size range=0.05≤x≤12 μm; 12-h sonicated particle size range=1.5≤x≤25 μm) (SaminRezania. Et al, 2009)

This is attributed to the differences in the surface features of the particles between the two sets of slurries; larger particles have more and longer surface fibers that become entangled, thereby increasing the viscosity, whereas smaller particles that have had the fibers sheared off behave more like particles in slurries with smoother surfaces, where increased surface area and friction are the controlling phenomena.

With this image it is easier to understand what is stated in lines above:

Image 16: differences in the surface features of the particles between the two sets of slurries
PARTE II: PRE-TREATMENT AND ULTRASOUND TECHNIQUES

In case a, larger biomass particles have larger fibers on the surface, which are more easily entangled as they move past other particles. In case b, smaller particles have smaller fibers on the surface and become less entangled. This explains why the viscosity decreased when particle size decreased. Cases c, d show how the dominating effect on viscosity for particles without surface fibers, such as coal slurries, is the increased surface area per unit volume of particle, which leads to more friction, and thus greater viscosity.

2.2.6.2. Chemical Characteristics

Studying the chemical changes in biomass treated with ultrasound means talking about how the chemical structure (in this case, lignocellulose biomass: cellulose, hemicellulose and lignin) changes, and at the end, how these changes will help at the time of increasing the volume of biogas.

*Solubilization of organic matter:*

One of the effects that can show these changes is the increase of solubilization of the organic matter.

This parameter can be quantified from three parameters:

VDSs/Vs, SCODs/TCOD and water content of the sludge cake. Along with these, it can be measured DDCOD as defined by (Tiehm et al, 2001) comparing the ultrasonic process and the maximum soluble chemical demand COD NaOH obtained by alkaline hydrolysis.

\[
DDCOD = \frac{COD_{Ult} - COD_U}{COD_{NaOH} - COD_U} \times 100(\%)
\]

where DDCOD is the degree of disintegration; CODUlt is the COD of the ultrasonically treated sludge sample (mg/l); CODU is the COD of the non-treated sludge sample (mg/l); CODNaOH is for alkaline hydrolysis; COD of a reference sample in a 1 M NaOH solution at 20 _°_C for 24 h (mg/l).

From the study by (Seungmin Na. et al, 2007), it was found that the ultrasonic process did not change total organic matter (TS) or TCOD quantity. Therefore, in terms of specific supplied energy (Ev) input, the ultrasonic treatment did not induce evaporation or mineralization phenomena. VDSs/Vs and SCODs/TCOD were increased, as well as DDCOD increase. All of these changes are reported in the following graph:
Graph 23: DDCOD, water contents and solid distribution (VDSs, VS, SCODs, TCOD) variation as function of volumetric supplied energy (kJ/L). (Seungmin Na et al, 2007)

Other studies carried out by (C. P. Chu et al, 2001) showed the changes in SCOD/TCOD and BOD/TCOD ratios after ultrasonic treatment.

Graph 24: SCOD/TCOD and BOD/TCOD vs. sonication time
Where BOD/TCOD shows the percentage of the total COD that is biodegradable, and the SCOD/TCOD ratio shows the relation between the COD soluble and the COD in a solid phase. This graph above shows an increase in these parameters.

In lignocellulosic biomass, the main goal is to convert lignocellulose to saccharides, which serve as substrate to the fermentation bacterium, in the phase of hydrolysis. In this point it is important to mention firstly the sonochemistry of carbohydrates. The mechanical effect of the acoustic waves improves heterogeneous reactions, e.g., of simple or polymeric sugars, in terms of smoother experimental conditions reducing the need of expensive or polluting solvents. Secondly, due to the easy formation of transient reactive species (radicals), new transformations can be designed (Nathalie Kardos. et al, 2001).

As the study by (Jia Luo. et al, 2013) shows, sonication improves hydrolysis of lignocellulosic materials into sugars. Degradation of starch from different sources such as waxy rice, corn meal, maize, potato and cassava chip is promoted by ultrasonic pretreatment(Kardos N. et al, 2001; Nikolic S. et al,2010;Montalbo-Lomboy M. et al, 2010; Isono Y. et al, 1994; Nitayavardhana S. et al, 2010; Hernoux A. et al, 2013). By sonication pre-treatment, an increase of glucose concentration was shown.

In the study by (Bishnu Karki. et al, 2010), the effect of ultrasonic pretreatment on protein release is shown, and in the graph below it can be seen that protein released is generally proportional to sonication time.

Graph 25: Protein yield at various sonication conditions. (Karki.et al, 2010)
But what efficiency of providing substrate really shows is the total sugar release, because these sugars are the bacteria substrate for biogas production. In the study by (Karki.et al, 2010), it was determined that total sugar release was generally proportional to sonication time.

Other studies such those by (Michael W. Easson.et all, 2011) founded that the introduction of ultrasound to treat switch grass improved the amount of reducing sugars:

Graph 26: Total sugar yield at various sonication conditions. (Karki.et all, 2010)

Graph 27: Untreated switch grass with/without ultrasound (Michael W. Easson.et all, 2011)
The synergistic effect results in the lowering of the barrier to diffusion - limiting enzyme / substrate binding and increases the overall reaction rate.

One of these sugars is the glucose, the main sugar used as substrate in lignocellulosic material. As (Samin Rezania.et al, 2009) show, there was more glucose release with the ultrasound pretreatment, existing a relation between the time of sonication with the glucose obtained. It also pointed out that there is a limit in the improvement to sugar release that may be realized when particle sizes are reduced below at least 33 μm. This appears to be counterintuitive, since smaller particles have larger surface area per unit volume and, therefore, more cellulose may be accessible for the enzyme to reach and at a faster rate. The higher amount of amorphous fibers should also tend to increase the rate and extent of glucose release.

An explanation of this phenomenon may be attributed to the effect that the lignin that is likely to be sheared off during sonication would have. Results by (Sun et al, 2004) showed that ultrasonic treatment of bagasse led to a release of over 90% of the original hemicelluloses and lignin. If lignin is releasing during sonication and floating free in the solution, then lignin adsorption of enzyme is higher than the substrate since it has higher affinity to enzyme than the substrate (Converse, A. et al, 1990), accounting for the loss in activity.
Crystallinity:

Another important parameter when analyzing how ultrasound interacts with lignocellulosic material is crystallinity, since it shows how cellulose is attacked into the medium.

Spiridon et al. (2011) observed that a decrease in LOI (lateral order index), which serves to indicate the crystallinity of a polymer, means that treated cellulose material showed an increase in cellulose surface accessibility and would theoretically enable more efficient hydrolysis of cellulose chains. Their study revealed that LOI and HBI (hydrogen bond intensity) values decreased for treated cellulosic materials in comparison with untreated ones, because the cellulosic materials lost their crystalline structure and restructured themselves into mostly amorphous forms instead.

Filson and Dawson-Andoh (2009) had results that showed an increased crystallinity index after hydrolysis, for residues (of Avicel and recycled pulp). They suggested that this was an indication of reduced amorphous cellulose domains in both starting lignocellulosic materials. (Dimitrios Koutsianitis et al., 2014).

Some other studies, such as that carried out by SaminRezania et al., 2009, showed that results indicated that the degree of crystallinity rises with time. If all or some of the amorphous volume is removed, the percentage of crystallinity would increase. These results suggest that the amorphous fibers at the surface, as well as amorphous lignin, have been sheared off during sonication.

![Graph 29: Changes in degree of crystallinity over time (SaminRezania. Et al, 2009)](image-url)
2.2.7. Effects of Ultrasonic on Lignocellulosic Biomass

In order to evaluate the potential of ultrasound as a pre-treatment option, for lignocellulose, it is important to know the effects of hydrodynamic and ultrasonic cavitation on lignocellulosic biomass.

2.2.7.1. Mechanisms of US pre-treatment of lignocellulose

As already pointed out in the environment section, ultrasound is a good pre-treatment method to increase hydrolysis. Enzymatic hydrolysis of sugar cane bagasse was improved by ultrasonic pretreatment, with an increase in glucose yield by 21.3% (Yachmenev, V. et al, 2009). The increase in glucose yield was almost linear with an increase in sonication time, linking the ultrasonic pretreatment to improved hydrolysis. In conclusion, hydrolysis of lignocellulosic biomass was improved by ultrasonic pretreatment for a variety of materials. Ultrasound was able to increase yields of glucose, xylose, and ethanol in downstream processing gas well as reduce the treatment times required, attributed to enhanced accessibility and delignification.

**Enhanced Accessibility and Delignification of Lignocellulose**

The efficacy of ultrasonic pre-treatment for improved processing was attributed partly to mechanoacoustic effects. Mechanoacoustic effects improved mass transfer and enhanced accessibility of biomass for subsequent processing. Further evidence of mechanoacoustic effects in the treatment of lignocellulose were observed from an increase in pore size and improved liquefaction (Sul'man, E. M. et al, 2011).

Improved liquefaction times demonstrated that mass transfer was improved under the influence of ultrasound. The observed increases of surface erosion, solubilization, and accessibility under the influence of ultrasound contribute all to the improvement of subsequent processing of lignocellulose and delignification of lignocellulose enhances hydrolysis yields of cellulose. Ultrasonic pretreatment tended to augment delignification, which coupled with improved hydrolysis yields, which was demonstrated by studies such as those by (Sul'man, E. M. et al, 2011; Csoka, L. et al, 2008).
2.2.7.2. Mechanisms for Extraction of Lignin from lignocellulose with Ultrasound

Ultrasound improved the yields of the lignin extract and increased the purity while retaining the molecular structure. However, treatment had varied effects on the molecular weight of the extracted lignin, and longer times were not always optimal (Sun, J. X.et al, 2004; Garcia, A.et al, 2012). The structure of lignin extracted with ultrasound tended to be the same as non-ultrasonic extractions, despite improved yields and purity. While the trend was for lignin to retain the molecular structure, differences in molecular weight were observed between ultrasonic and non ultrasonic extractions (Sun, X.et al, 2002; Sun, R.et al, 2002; Yuan, T.et al, 2011). The different responses of molecular weight to ultrasound are likely to be due to competing depolymerization and condensation reactions.

_Ultrasonic Depolymerization, Separation, Degradation, and Condensation of Lignin_

Depolymerization and separation of lignin will contribute to the increased lignin extraction yields with ultrasound; however, ultrasound also degrades lignin components. Separation of lignin with ultrasound occurs via cleavage of the lignin-hemicellulose linkages. Cleavage of the linkages was evidenced in the increased purity of lignin and hemicellulose extractions with ultrasonic treatment (Sun, X.et al, 2002; Sun, R.et al, 2002; Sun, J.et al, 2004). Depolymerization of lignin occurs through hemolytic cleavages of the phenyl ether β-O-4 and α-O-4 bonds, and degradation of ultrasound can occur through hydroxyl attack on the lignin structure which mostly occur on the aromatic ring (Pranovich, A. V.et al, 1998); this led to hydroxylated, demethoxylated, and side chain eliminated products.

The ability of ultrasound to enhance cleavage of linkages between hemicellulose and lignin increased the separation and purity of the extracts. Furthermore, ultrasound influenced the molecular weight of the lignin extracted. The influence on molecular weight was not uniform attributed to competing separation, degradation, and recondensation reactions. The condensation reactions of lignin are theorized to occur in the interfacial region around the collapsing bubble, thus depending on the nature of the treatment solution (Madeleine J. Bussemaker.et al, 2013).
2.2.7.3. Mechanisms for Extraction of Carbohydrates from Lignocellulose with Ultrasound

Ultrasound was also utilized for hemicellulose and cellulose extraction and modification (Sun, R.et al, 2002; Sun, J. X.et al, 2002; Sun, J. X.et al, 2004; Hromadkova, Z.et al, 2003; Hromadkova, Z.et al, 1999).

_Ultrasonic Depolymerization, Separation, Degradation, and Condensation of Carbohydrates_

Ultrasound causes depolymerization, and degradation of cellulose and hemicellulose from mechanoacoustic and sonochemical effects. This occurred via a cumulative effect of the hydroxyl radicals, shear forces, and pyrolytic degradation of hydrophobic polymers in the hot region around the collapsing bubble. The separation of lignin and carbohydrates was enhanced by cleavage of the hemicellulose lignin linkages under ultrasound.

Evidence of degradation and repolymerization under ultrasound was also found. The formation of oligomers was observed to occur from ultrasonic treatment of water-soluble corn hull xylan (Ebringerova, A.et al, 1997). This was attributed to radical recombination as new unsaturated structures with enolic and/or unsaturated α/β-carbonyl groups were formed. The degradation of cellulose in solution was also observed. Cellulose extracted from wheat straw and sugar cane bagasse produced a lower viscosity solution when ultrasound was used. The viscosity was lowered from the destruction of cellulose (Sun, J. X.et al, 2004; Sun, R. C.et al, 2004) from either depolymerization or degradation.
2.2.8. Operating Parameter

Table 9: Summary of the operating parameters on US treatment

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>UNITS</th>
<th>MATHEMATICAL EXPRESSION</th>
<th>TERMS</th>
<th>MEANING</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_s$: Specific Energy</td>
<td>[KJ/kgTS]</td>
<td>$E_s = \frac{P \cdot t}{V \cdot TS}$</td>
<td>$P = $ power supplied [kW] [ t = $ sonication time [s] [ V = $ volume of the sample sonicated [l] [ TS = $ concentration of total solids in the sample [KGTS / l]</td>
<td>is the ratio between the energy supplied and the amount of total solids present in the treated medium</td>
</tr>
<tr>
<td>$E_o$: Specific volumetric energy</td>
<td>[J/l]</td>
<td>$E_o = \frac{P \cdot t}{V}$</td>
<td>$P = $ power supplied [kW] [ t = $ sonication time [s] [ V = $ volume of the sample sonicated [l]</td>
<td>The dose of US is defined as the ratio between the energy supplied and the volume of the treated sample</td>
</tr>
<tr>
<td>$P_v$: Volumetric power</td>
<td>[W/l]</td>
<td>$P_v = \frac{P}{V}$</td>
<td>$P = $ power supplied [kW] [ V = $ volume of the sample sonicated [l]</td>
<td>The density of the US is defined as the ratio between the power supplied and the volume of the treated sample</td>
</tr>
<tr>
<td>$P_s$: Surface power</td>
<td>[W/cm²]</td>
<td>$P_s = \frac{P}{A}$</td>
<td>$P = $ power supplied [kW] [ A = $ area of the surface of the probe used [cm²]</td>
<td>The intensity of the US is defined as the ratio between the power delivered and the surface of the probe used for their generation</td>
</tr>
</tbody>
</table>

In the table 12, are shown the different operating parameters when talking about the ultrasonic treatment. All of them have their importance at the time of making the probe in function, bearing in mind parameters as the volume of the sample, the power applied the time of sonication, the surface area or the content of total solids.
3. MATERIALS AND METHODS

3.1. Substrate description used in the test

In this study, it is aimed to evaluate the effects of ultrasound pretreatment in terms of substrate characteristics, kinetics and methane production on the anaerobic digestion process. The experiment was carried out by sonication of lignocellulosic material from an anaerobic digestion, i.e. material already digested which will serve to supply as feeding to a series of reactors in discontinuous mode (batch). Each of these reactors has different mixtures composed by a percentage of treated material with ultrasound and the rest of raw material.

With this work system it is attempted to simulate conditions of recirculation sonicated digested material with the aim of evaluating the best operating conditions for further work system in semi-continuous or continuous.

The experimental campaign was carried out on digestate samples collected at the exit of a full-scale anaerobic digestion plant treating a mixture of organic wastes of food industry, silage energy crops, olive pomace and manure.

3.2. Material characterization

The material used for the experiment was characterized by analyzing the following parameters:

Table 10: Summary of the parameters and the methodologies used

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>METHODOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>(Standard Methods, 2005)</td>
</tr>
<tr>
<td>Total solids (TS)</td>
<td>Volatile solids (VTS) and fixed solids (FS)</td>
</tr>
<tr>
<td>Suspended solids (TSS) and filterable solids (FS)</td>
<td>(Standard Methods, 2005)</td>
</tr>
<tr>
<td>Total organic carbon (TOC)</td>
<td>Soluble and total fraction</td>
</tr>
<tr>
<td>Chemical oxygen demand (COD)</td>
<td>Soluble and total fraction</td>
</tr>
<tr>
<td>Carbohydrates, Soluble and total fraction</td>
<td>(Debois Methods, 1954)</td>
</tr>
<tr>
<td>Total nitrogen variation (TKN) and ammonia nitrogen (NH₃)</td>
<td>(Methods of analysis of compost Piedmont region, 1998)</td>
</tr>
<tr>
<td>Composition of the biogas produced during AD</td>
<td>(Standard Methods, 2005)</td>
</tr>
</tbody>
</table>
3.3. Preparation of the soluble fraction

Untreated material is subjected to centrifugation for 10 minutes at 4000 rpm. Thus the separation of precipitate and supernatant is obtained. This last one, is filtered by a vacuum pump using a glass fiber filter with a pore size of 1.2 microns.

3.3.1. Material characterization methodology

This section is, for describing the methodologies followed for the chemical-physical characterization of the material used in the experiments described.

3.3.1.1. Ph

The pH was measured by a pH meter previously calibrated with standard buffer solutions at pH = 4 and pH = 7.

3.3.1.2. Total solids (TS), volatile solids(VS) and fixed solids(FiS)

Total Solids

Total solids represent dry residue obtained after evaporation at 105 ± 5 ° C of a sample to constant weight. Thus is obtained the sum of the organic fraction and inert fraction present in the substrate.

The samples are left at that temperature for approximately 24 hours. Then they are inserted into a vacuum chamber to prevent contamination of the mixture, and also this let to cool themselves for the next step that is to be weighed.

Calculation:

The total solids content was calculated as a percentage by weight by the following formula:

$$TS (%) = \frac{\text{weight} \, 105^\circ C - \text{tare}}{\text{initial weight}} \times 100$$

Volatile solids and fixed solids

Volatile solids represent the amount of organic matter present in the sample. These samples are subjected to a temperature of 550 ° C for two hours and then there must be a time waited to cool the samples and to be weighed.
Fixed solids represent the fraction of solids constituting the residue after the sample, previously dried, is introduced into a furnace at a temperature of 550 ° C to constant weight.

Calculation:

Volatile solids and settled solids are calculated as follows:

\[ VS(\%) = \frac{\text{weight } 105^\circ C - \text{weight } 550^\circ C}{\text{weight } 105^\circ C} \times 100 = 100 - FS_i(\%) \]

\[ FS_i (\%) = \frac{\text{initial weight } - \text{weight } 550^\circ C}{\text{weight } 105^\circ C} \times 100 \]

3.3.1.3. Suspended solids ( TSS ) and Filterable solids ( FS )

Suspended solids represent the amount of solids remaining on the filter during the filtration process.

Filterable solids are those which form part of the permeate during the filtration process. Represent the fraction of the total solids to less than a diameters of 1.2 \( \mu m \).

For determination thereof, has the following two methods; First, it is obtained the weight of the watch glass and filter, then they are introduced into an oven at 105 ° C to remove the moisture and to do the sterilization. Then, it is weighed (approximately) 2 grams of the sample and filtered with the aforementioned filter, trying to eliminate all possible humidity without breaking it. Once the filtration is done, the sample is allowed to dry with the glass filter for 24 hours in an oven at 105 ° C.

Calculation:

\[ TSS \left( \frac{mg}{L} \right) = \frac{P_1 - P_2}{\text{initial weight}} \times 10^6 \]

Being

\[ P_1 = \text{Weight of the watch glass + Filter Weight + Weight sample after 105^\circ C (g)} \]

\[ P_2 = \text{Weight watch glass filter + Weight (g)} \]

\[ FS \left( \frac{mg}{L} \right) = TS - TSS \]
3.3.1.4. Total Organic Carbon (TOC); total and soluble fraction

For the determination of TOC, it has been used an instrument of organic carbon, Model TOC-V Total organic carbon analyzer CSNE 200 (Shimadzu), in which the determination of total carbon (TC) and inorganic carbon (IC) is achieved. The organic carbon (TOC) was calculated by the difference between TC and IC, which are determined analytically by assessing the CO2 generated by combustion at high temperature, of the total and inorganic carbonic substance present in the sample using an infrared analyzer.

TOC content was assessed for both the soluble fraction (STOC) and for the total fraction (tTOC).

From the operational point of view, in the determination of the TC and IC analyzes, were performed in double for each test, to ensure homogeneity.

The Shimadzu module for solid samples, consists of two ovens to measure total and inorganic carbon, and a system of not dispersive infrared rays (NDIR) for analyzing the spectrum of CO2 released by the sample. CO2 production from the furnace combustion tube passes and is sent to NDIR through a carrier gas (air). The device shows a peak in the area which is proportional to the concentration of the carbon present. This concentration is given in percentage.

The TC reading, expressed as a percentage, is determined by the combustion of the sample at 900 ° C.

IC reading, expressed as a percentage, is determined by releasing the CO2 through the reaction of the carbonate with phosphoric acid (5 ml) at a temperature of 200 ° C which not allows the decomposition of this organic substance.

Once is determined the milligrams of total and inorganic carbon using the calibration curve, the value of TC and IC in percentage is determined using the weight of the sample.

\[
TC (\%) = \frac{TC \text{ weight of the sample}}{weight \text{ of the sample}} \times 100
\]

\[
IC (\%) = \frac{IC \text{ weight of the sample}}{weight \text{ of the sample}} \times 100
\]
PARTE III: MATERIALS AND METHODS

Image17: SSM-5000A module for determining the solid TOC

Reading the TOC of the liquid sample in mg/L is obtained as in the case of the solid module, using the difference between the values of TC and IC.

TC is determined by introducing in the instrument, through an automatic injector, 5 µl of the sample, the ones which have been oxidized in an oven at 680 ° C. Carrier gas flows in the first combustion tube removing CO2 and other gases produced during combustion. Then flows in the electronic dehumidifier where it is cooled and dewatered and finally passes through a halogen cage, reaching the detector cell where CO2 is read.

Moreover, the IC values are determined by acidification of the samples by dosing phosphoric acid so a pH below 3 is achieved and thus vary the chemical balance of the carbonate and bicarbonate according to the following reactions:

\[
\text{Me}_2\text{CO}_3 + 2\text{HCl} \rightarrow \text{CO}_2 + 2\text{MeCl} + \text{H}_2\text{O}
\]

\[
\text{MeHCO}_3 + \text{HCl} \rightarrow \text{CO}_2 + \text{MeCl} + \text{H}_2\text{O}
\]

Where:

Me = generic Cation (Ca or Mg) present as carbonate or bicarbonate
3.3.1.5. Chemical Oxygen Demand (COD); total and soluble fraction

The COD measurement represents the oxygen required to chemically oxidize the substance in a sample through a strong oxidizing agent in acid medium.

As oxidizing agent is used potassium dichromate (K2Cr2O7) because its ionic form is a strong oxidant in acid medium.

The reaction that takes place by contacting the sample with dichromate solution acidified with sulfuric acid and maintaining an elevated temperature is:

$$C_{12}H_{9}O_{6} + cCr_2O_7^- + 8cH^+ \rightarrow nCO_3^2+ (a+8c/2)H_2O + 2cCr_2^+$$

Where:

$$c = 2n/3 + a/6 - b/3$$

As a result dichromate reduction to trivalent chromium is obtained. To facilitate the reaction silver sulfate (Ag2SO4) is used as catalyst.

It must be controlled the concentration of chlorides because they interfere with the dichromate ions causing an oxidizing agent consumption.
This interference is eliminated by adding mercury sulfate (HgSO4), as mercury ion combines with the chloride ion to form the complex mercuric chloride hardly ionizable.

\[
\text{Hg}_2^{+} + 2\text{Cl}^{-} \rightarrow \text{HgCl}_2
\]

The analytical procedure indicates that it is compulsory to operate with excess of oxidant to have the security that the organic substance is completely oxidized. Therefore, the measurement of residual dichromate is performed by titration with ferrous ammonium sulfate, as the ferrous ion can give the following reaction:

\[
6\text{Fe}^{2+} + \text{Cr}_2\text{O}_7^{2-} + 14\text{H}^{+} \rightarrow 6\text{Fe}^{3+} + 2\text{Cr}^{3+} + 7\text{H}_2\text{O}
\]

The end of the titration, ie, the disappearance of dichromate ion, is given by the color variation caused by the presence of an indicator, ferroin.

For calculating COD is necessary the analysis of a blank control using the same method, substituting the sample with deionized water sample.

Through the difference between the reagent consumption from the titration, that is verified with white, and the one that is verified by analyzing the sample, it is obtained the effective oxidant consumption expressed in terms of equivalent oxygen. The formula for calculating the COD is:

\[
\text{COD} = \frac{A - B}{V} \cdot N \cdot 8 \cdot 1000
\]
Where:

A = mL consumed by the target of titrant
B = mL consumed by the sample of titrant
V = volume of the sample (mL)
N = normality of the solution of ammonium iron sulfate
8 = equivalent weight of oxygen
1000 = dimensional Factor
COD = mg O₂ / L

COD content was assessed for both the soluble fraction (SCOD) as well as to the total fraction (TCOD).

### 3.3.1.6. Carbohydrates, soluble and total fraction

Carbohydrate analysis is performed by reading a spectrophotometer (Perkin Elmer model Lambda-3 UV / VIS), Figure X, with a wavelength of 488 µm by phenol-sulfuric colorimetric method of Dubois (1954). Analyses were performed in double for each test.

Calculation:

\[ y = 113.07 \times x + 1 \]

Where:

x = Absorbance of the sample – Absorbance of the white

y = Concentration (mg / L)

### 3.3.1.7. Calculation of the solubilization increments

Increased solubilization of organic compounds can be calculated by analyzing the parameters COD, TOC and carbohydrates. It can be expressed in response to particulate and soluble substances under.
PARTE III: MATERIALS AND METHODS

If referring to particulate substance has the following expression:

\[ IP_x[\%] = \frac{S_{x_{us}} - S_x}{T_x} \cdot 100 \]

If reference is made soluble substance has the following expression:

\[ IS_x[\%] = \frac{S_{x_{us}} - S_x}{S_x} \cdot 100 \]

With:

Ip, Is increased solubilization parameter to consider depending on the particulate and soluble substance, respectevely (%) 

Tx0 = total substrate concentration before sonication (mg / l) 

Sx0 = concentration of the soluble substrate in the sample before sonication (mg / l) 

Sxus = substrate concentration in the sonicated sample (mg / l) 

X = parameter characterization of substrate includes, COD, TOC or carbohydrates 

3.3.1.8. Variation of total nitrogen (TKN) and ammonia nitrogen (NH3)

This analysis is performed by reading a spectrophotometer (FullTech), Figure X, with a wavelength of 410 µm. TKN method implies a first phase of digestion and a second one of distillation; ammonia analysis are done just with a phase of distillation.
Analyses were performed in double for each test.

Calculation:

\[ y = 4.91703x + 0.21665 \]

Where:

\( x = \text{Absorbance of the sample} - \text{Absorbance of the white} \)

\( y = \text{Concentration (mg/L} \text{ N-NH}_4^+\) \)

### 3.4. Ultrasound treatment

Ultrasound tests were performed using a volume of 150 mL material, using a beaker of 200 mL. Before you begin this process, a cooling bath surrounds said vessel to prevent overheating of the sample, and prevent evaporation phenomena or the formation of toxic compounds or inhibitors for next anaerobic digestion process, which the treated material is used. Monitoring temperature during sonication was performed using a thermometer ensuring that 37°C are not exceeded.

The instrument allows the regulation of the duration of the work cycles of the probe to prevent overheating phenomena.

### 3.4.1. Description of ultrasound device

The ultrasound machine Sonics VCZ 750 (Figure X) consists of:

- Feeder

<table>
<thead>
<tr>
<th>Table 11: Characteristics of the feeder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potencia</td>
</tr>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>Height</td>
</tr>
<tr>
<td>Width</td>
</tr>
<tr>
<td>Length</td>
</tr>
<tr>
<td>Weight</td>
</tr>
</tbody>
</table>

- Booster: attached to the converter and the sonotrode, which allows the amplification of the vibration amplitude in the emitting surface of the sonotrode.
- Converter: Model CV 33, consisting of piezoelectric crystals.

Table 12: characteristic of a converter

<table>
<thead>
<tr>
<th>Diameter</th>
<th>63.5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>183 mm</td>
</tr>
<tr>
<td>Weight</td>
<td>900 g</td>
</tr>
<tr>
<td>Cable length</td>
<td>1.8 m</td>
</tr>
</tbody>
</table>

- Sonotrode: Instrument permitting mechanical amplification of the vibrations of the amplitude generated by the drive.

Table 13: Characteristics of the sonotrode

<table>
<thead>
<tr>
<th>Diameter</th>
<th>63.5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>183 mm</td>
</tr>
<tr>
<td>Weight</td>
<td>900 g</td>
</tr>
<tr>
<td>Cable length</td>
<td>1.8 m</td>
</tr>
</tbody>
</table>

- Acoustic hood

Image 21: Sonication equipment

3.4.2. Treatments performed

Experiments are classified based on the specific energy used. This is defined as:

$$E_s = \frac{P \cdot t}{V \cdot T_s}$$
Where:

\[ ES = \text{Specific Energy (KJ / kgTS)} \]
\[ P = \text{Power (W)} \]
\[ t = \text{time of sonication (s)} \]
\[ V = \text{Work volume (L)} \]
\[ TS = \text{total solids concentration of the sample (kg / L)} \]

Specific energies studied in this study vary between 500 and 50000 KJ /kgTS, studying the effect of ultrasound on the lignocellulosic material to the following energies: 500, 3000, 6000, 10000, 20000, 30000 and 50000 KJ / kgTS. The treatments were carried out for each test in double in order to carry out a statistical evaluation of the effects of sonication on the sample.

At the beginning and the end of each treatment was measured the pH to control optimum working range. This control parameter is required since it is important factor for the bacteria responsible for the hydrolysis in the anaerobic digestion.

Before starting the sonication process there must be followed these steps; the sonotrode is inserted about 2 mm into the sample, start the compressor (helps to cool the system), and adjust the wave amplitude at which it is wanted to work. This study used three wavelengths: 20%, 50% and 80% of the maximum amplitude (35 µm). These wave amplitude values correspond to values of 0.1, 0.2 and 0.4 W / ml.

In Table X the corresponding specific energy values are given for each of the treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specific Energy(KJ/Kg TS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 500</td>
<td>6000</td>
</tr>
<tr>
<td>US 3000</td>
<td>36000</td>
</tr>
<tr>
<td>US 6000</td>
<td>75510</td>
</tr>
<tr>
<td>US 10000</td>
<td>120000</td>
</tr>
<tr>
<td>US 20000</td>
<td>240000</td>
</tr>
<tr>
<td>US 30000</td>
<td>370800</td>
</tr>
<tr>
<td>US 50000</td>
<td>618000</td>
</tr>
</tbody>
</table>
3.5. Anaerobic digestion in batch tests

3.5.1. Description of the instrumental equipment

The tests were conducted by using Pyrex glass bottles of 1L of capacity. Agitation and temperature thereof are controlled by using magnetic heating plates. To ensure the maintenance of the temperature of the batch constant, they have been coated with insulating material.

Each of the bottles is constituted by two outputs: one located in the bottom of the bottle, in which has been introduced a portion of a plastic pipe where has been added a clip of Mohr, this mechanism will serve to take samples throughout the entire test. The other output is at the top of the bottle which is connected via Tygon tubing to a eudiometer, which has a volume of approximately 5.5 liters.

Eudiometers constitute the hydraulic system to perform the measurement of produced biogas. They consist of an acidified saturated saline solution, which is obtained with a solution of 200 g / l of NaCl and a few drops of 96% HCl to obtain the acid solution. Each eudiometer consists of two outputs provided with two valves for opening and closing. The first output is used for the binding by Tygon tubing, with the batch test. However, the second is used for sample extraction biogas analyzed.

Once all the samples are inside the batch, and are connected to the eudiometers and all the outputs are closed, one proceeds is to introduce nitrogen at a pressure of 0.5 atmospheres in order to ensure anaerobic conditions necessary for the process of digestion.

![Image 22: Experimental equipment used for testing anaerobic digestion](image-url)
3.5.2. Description of the batch tests performed

Two tests of anaerobic digestion were performed, each using different sonication energy on the material and different percentage of mixing treated and untreated materials with ultrasound treatment.

The duration of the first test has been 56 days and the workload used for each batch was 500 ml. The percentage of mixture of treated material and untreated material was 75% treated and 25% untreated for energies of 20000 and 50000 kJ / kg TS and 25% treated and 75% untreated for an energy of 50000 kJ / kg TS.

The second test was running when this thesis was written, so the duration which is taken into account is 38 days, as well as the first probe it have been used 500ml of sample for each test. In this case the mixing ratio between material treated and untreated material was 25% treated and 75% untreated for energies of 3000, 10000 and 20000 kJ / kg TS.

For both two tests have been studied in parallel the evolution of a blank control, ie 100% of untreated material, in order to observe what the ultrasound effect was in the material and the consequences that this entails in the anaerobic digestion.

The different mixture percentages above have been made in order to evaluate the influence of substrate / biomass rate on the anaerobic digestion process.

In order to understand the changes that produce ultrasound in the anaerobic digestion process, it have been studied the evolution of the following parameters:

• Daily: production of biogas

• Every three days: composition of biogas

• Weekly: pH, STOC

• At the beginning and at the end of each test:sCOD, TCOD, STOC, tTOC, soluble and total carbohydrates, total and volatile solids and pH.
3.5.3. Composition of the biogas produced during the anaerobic digestion process

Determining the composition of the biogas is carried out by gas chromatography analysis by using the chromatograph Varian 3600 CX (Figure X) consisting of the following parts:

- Micro-packed column Resteck of length 2m inner diameter of 1 mm
- Capillary column Teknokroma length 30 m and internal diameter 0.53 mm
- Electric heat conductivity detector (TCD)
- Termoionizing Flame Detector (FID)

The analytical method used parts from an initial temperature of 80 ° C and an initial duration of two minutes until it reaches a temperature of 100 ° C with a gradient of 2.5 ° C / minute.

This test is done for each different batch, obtaining with each chromatogram the composition of CH4 in the first stay, followed by the composition of CO2, lasting each test about 10 minutes.
### Table 15: Summary of Activities

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Analized characteristics</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate characterization</td>
<td>Analized parameters</td>
<td>TS; VS; TOC; COD; carbohydrates; pH, NH₃, TKN, SST, SF; SFi</td>
</tr>
<tr>
<td>Ultrasound treatment</td>
<td>Specific energies (KJ/Kgₑ₉)</td>
<td>500; 3000; 6000; 10000; 20000; 30000; 50000</td>
</tr>
<tr>
<td>Characterization of the treated material</td>
<td>Analized parameters</td>
<td>TS; VS; TOC; COD; carbohydrates; pH, NH₃, TKN, SST, SF; SFi</td>
</tr>
</tbody>
</table>
| Mixtures utilized                        | Percentage of the mixture       | 100% blank control  
25% NS – 75% US  
25% US – 75% NS x          |
| Biogas probe                             | Energies used to the US treatment (KJ/Kgₑ₉) | 3000; 10000; 20000; 50000                                                  |
|                                          | Analized parameters            | Volume and biogas composition; pH and sTOC                                |

*NS= Non sonicated material

US= Sonicated material
4. RESULTS AND DISCUSSION

In this part it is going to be represented the effects of the ultrasound on the lignocellulosic material that had been used in this experiment and also see the paper of the percentage of treated material with ultrasound to see what is more competent in terms of effectiveness of the process.

4.1 Effects of Ultrasonics treatment on digestate solubilization

In the following tables and graphs it is represented the evolutions of the substrate operating parameters when ultrasound has been applied at different volumetric amplitudes.

As cited Ward (2008), the COD is a good indicator of the performance of the biodegradation process because the undigested material (AD) requires oxygen in aerobic environment to complete degradation. The only COD degradation pathway appears to be the production of gas with low solubility, is hydrogen and methane. COD, however, indicates the amount of organic carbon which is linked to an organic compound and so carbohydrates analysis.

Table 16: Experimental results for ultrasound pretreatment at different energies (KJ/Kg ST) and using amplitude of 20%

<table>
<thead>
<tr>
<th>Energy</th>
<th>500</th>
<th>3000</th>
<th>6000</th>
<th>10000</th>
<th>20000</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS [g/l]</td>
<td>84.0</td>
<td>80.6</td>
<td>82.4</td>
<td>84.7</td>
<td></td>
</tr>
<tr>
<td>VS [g/l]</td>
<td>59.5</td>
<td>56.5</td>
<td>58.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tTOC [g/l]</td>
<td>29.5</td>
<td>28.8</td>
<td>28.9</td>
<td>36.6</td>
<td></td>
</tr>
<tr>
<td>sTOC [g/l]</td>
<td>5.2</td>
<td>7.1</td>
<td>8.9</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>tCOD [g/l]</td>
<td>86.7</td>
<td>80.0</td>
<td>88.8</td>
<td>88.9</td>
<td>105.0</td>
</tr>
<tr>
<td>sCOD [g/l]</td>
<td>11.2</td>
<td>17.8</td>
<td>19.9</td>
<td>19.2</td>
<td>29.3</td>
</tr>
<tr>
<td>Carb. tot [g/l]</td>
<td>19.2</td>
<td>14.8</td>
<td>15.7</td>
<td>15.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Carb. sol [g/l]</td>
<td>1.6</td>
<td>1.7</td>
<td>2.0</td>
<td>1.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

In the table 19 are represented the results from the different probes obtained by using the ultrasound treatment with a wave amplitude of 20%. It have been studied the total and volatile solids as well as parameter of COD, TOC and carbohydrates to the solid and soluble fraction, in terms to observe the effect of ultrasound in the organic matter.
As well as the first table 19, mentioned in this chapter, this one is referred to the results obtained for using amplitude of 50%.

**Table 17: Experimental results for ultrasound pretreatment at different energies (KJ/Kg ST) and using amplitude of 50%**

<table>
<thead>
<tr>
<th>Energy</th>
<th>500</th>
<th>3000</th>
<th>6000</th>
<th>10000</th>
<th>20000</th>
<th>30000</th>
<th>50000</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS [g/l]</td>
<td>84,1</td>
<td>75,3</td>
<td>80,7</td>
<td>79,3</td>
<td>82,2</td>
<td>82,2</td>
<td></td>
</tr>
<tr>
<td>VS [g/l]</td>
<td>59,5</td>
<td>55,6</td>
<td>57,1</td>
<td>56,1</td>
<td>57,9</td>
<td>58,1</td>
<td></td>
</tr>
<tr>
<td>iTOC [g/l]</td>
<td>25,7</td>
<td>32,4</td>
<td>28,3</td>
<td>32,5</td>
<td>31,6</td>
<td>30,4</td>
<td></td>
</tr>
<tr>
<td>sTOC [g/l]</td>
<td>5,7</td>
<td>10,4</td>
<td>8,7</td>
<td>11,2</td>
<td>11,3</td>
<td>15,2</td>
<td></td>
</tr>
<tr>
<td>tcOD [g/l]</td>
<td>76,8</td>
<td>81,3</td>
<td>75,8</td>
<td>82,5</td>
<td>86,5</td>
<td>79,3</td>
<td></td>
</tr>
<tr>
<td>scOD [g/l]</td>
<td>16,1</td>
<td>25,1</td>
<td>19,8</td>
<td>30,0</td>
<td>33,2</td>
<td>37,4</td>
<td></td>
</tr>
<tr>
<td>Carb. tot [g/l]</td>
<td>13,3</td>
<td>16,7</td>
<td>15,5</td>
<td>16,3</td>
<td>19,7</td>
<td>16,1</td>
<td></td>
</tr>
<tr>
<td>Carb. sol [g/l]</td>
<td>1,4</td>
<td>2,7</td>
<td>2,1</td>
<td>3,6</td>
<td>2,8</td>
<td>4,3</td>
<td></td>
</tr>
</tbody>
</table>

For the last experiment used for studying the ultrasound effects on lignocellulosic material, it was chosen wave amplitude of 80% (table above), so in this way a great range of wave amplitudes is bear in mind.

**Table 18: Experimental results for ultrasound pretreatment at different energies (KJ/Kg ST) and using amplitude of 80%**

<table>
<thead>
<tr>
<th>Energy</th>
<th>500</th>
<th>3000</th>
<th>6000</th>
<th>10000</th>
<th>20000</th>
<th>30000</th>
<th>50000</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS [g/l]</td>
<td>82,2</td>
<td>80,9</td>
<td>81,8</td>
<td>86,0</td>
<td>84,6</td>
<td>87,5</td>
<td></td>
</tr>
<tr>
<td>VS [g/l]</td>
<td>57,8</td>
<td>56,7</td>
<td>58,3</td>
<td>62,0</td>
<td>60,4</td>
<td>61,7</td>
<td></td>
</tr>
<tr>
<td>iTOC [g/l]</td>
<td>29,2</td>
<td>29,0</td>
<td>28,5</td>
<td>26,0</td>
<td>25,8</td>
<td>25,4</td>
<td></td>
</tr>
<tr>
<td>sTOC [g/l]</td>
<td>5,7</td>
<td>7,8</td>
<td>9,1</td>
<td>11,3</td>
<td>11,5</td>
<td>14,1</td>
<td></td>
</tr>
<tr>
<td>tcOD [g/l]</td>
<td>65,8</td>
<td>71,3</td>
<td>67,6</td>
<td>62,3</td>
<td>80,5</td>
<td>78,0</td>
<td></td>
</tr>
<tr>
<td>scOD [g/l]</td>
<td>15,6</td>
<td>20,3</td>
<td>21,3</td>
<td>32,0</td>
<td>30,9</td>
<td>39,2</td>
<td></td>
</tr>
<tr>
<td>Carb. tot [g/l]</td>
<td>15,0</td>
<td>12,0</td>
<td>14,4</td>
<td>14,1</td>
<td>16,0</td>
<td>17,7</td>
<td></td>
</tr>
<tr>
<td>Carb. sol [g/l]</td>
<td>1,3</td>
<td>1,9</td>
<td>2,8</td>
<td>2,8</td>
<td>3,3</td>
<td>4,2</td>
<td></td>
</tr>
</tbody>
</table>

For the three cases is important to notice that the total fractions of each parameter (TOC, COD and Carbohydrates) didn’t experimented a big variation. This is an important point to have into account, because this means that the total organic matter didn’t suffer phenomena of volatilization or mineralization during the probe, even when the specific energy was varied.

This also can be seen through the analysis of Total and volatile solids by varying the US energy, as is shown in the graph 36:
Graph 30: Representation of total and volatile solids (g/l) vs Specific energy (KJ/Kg ST) at different wave amplitudes: a) 20%, b) 50%, c) 80%

Both the TS and VS contents did not significantly vary after sonication, confirming the results attained by (Seungmin Na et al, 2007), checking that the absence of variation on TS means that there has not been phenomenon of volatilization or mineralization.

The following graph it is showed the evolution of soluble organic matter at different amplitudes.

Graph 31: Soluble organic matter (sCOD, STOC, Scarbohydrates) as a function of both Es and amplitudes

As shown in the above figures, an increase of organic carbon in solution, for the case of the sCOD as for STOC and carbohydrates, occurs for the three cases in which the volumetric amplitude varies, being the most pronounced values those with amplitude 50%. 

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It can therefore be concluded that ultrasound produces an increased of the substance in solution of organic matter.

The results obtained agree with the tendency of higher amplitude at higher energies, higher amount of solubilization of organic matter; but it is observed as in the case of using 80% of amplitude, lower values are obtained than with the amplitude of 50% in the case of using energy of 50000 KJ/Kg TS. This means that even using higher amplitude (80%) with higher energies the effect on lignocellulosic material is the same o lower than using a lower amplitude (50%).

Different studies relate a higher content of total solids with higher solubilization of organic substance (Jiang, 2014). This does not happen in this study, as for Show et al. (2007) since, comparing same energy(50000KJ/Kg TS) for a range of 50%, there is an amount of TS = 82.2 g / L while at an amplitude of 80%, the amount of TS is 87.5 g / L and the solubilization of organic substance (sCOD) is lower in the case of working with 80% of wave amplitude, which is the one with higher content of total solids.

The following graph 38 shows the variation of sCOD vs specific energy. As well as the graph of sCOD, to check the increase of solubilization of organic compounds, it has been used the following parameters: Ip, for the particulate substance, and the Is to soluble substance.

The information this graph shows is that specifying an energy, it can be seen the effect of amplitude in the solubilization action. The tendency of the curves is that the increase of soluble substance is bigger at higher amplitudes. But as well as in the previous graph, this
tendency is broken when the energy is up to 30000 KJ/Kg TS as the values are much more similar changing to be a higher solubilization for the case of 50% of amplitude and energy of 50000 KJ/Kg TS.

The following graph 39 shows the variation of sTOC vs specific energy, as well as the respective increments:

Graph 33: concentration of soluble organic matter, STOC (a), Ip (b) Is as a function of both Es and amplitudes

Comparing the results obtained in the graph 39 with the same amplitude varying the energy, it is observed that it is obtained an increase of sTOC increasing the energy. This happens when using an amplitude of 80% and 50%; in the case of an amplitude of 20%, it is seen that from an energy of 10000 KJ/Kg TS, there is no increase obtained. So varying the energy at low amplitudes doesn´t make any effect on the solubilization of the organic matter.

Rating with the same amplitude range, the dominant factor in terms of solubilizing is the energy applied. As specified in the study by Bougrier et al. (2005), for energy values lower than 6000 KJ / kgTS, not representative solubilization values are obtained as can be seen in this graph. However, it is observed that the largest increase is obtained for solubilization energies from 10000 kJ / kgTS, what can be checked from graph b, where from an amplitude of 50% the biggest change on the increment of soluble substance is when is applied and energy (10000-20000 KJ/Kg TS).
PARTE IV: RESULTS AND DISCUSSION

The last case in which is compared the specific energy, is the soluble carbohydrates, as well as the increments of the soluble and particulate substance, showed in the graph 40:

Graph 34: concentration of the soluble organic matter, Scarbohydrate in solution, Ip (a), and Is (b) as a function of both Es and amplitudes

In this graph 40, the content of soluble carbohydrates increases with applied energy of sonication. If it is compared the same energy of sonication, e.g. 20,000 KJ / KGTS at different amplitudes, greater solubilization is obtained at amplitude of 50%, than for 80%.

4.1.1. Total Kjeldahl Nitrogen and soluble organic nitrogen

In the following graph is represented the concentration of TKN and NH3 by varying the specific energy of sonication, for the soluble fraction as well as for the total one.

Graph 35: TKN and soluble NH3 as function of Es for: a) the total fraction of the material and b) for the soluble one
The conclusion of this graph evidence that concentration of TKN remained unchanged after US, suggesting that ultrasound did not induce mineralization phenomena of organic matter.

To understand the correlation of these parameters in terms of proteins, the following graph 42, shows the evolution of them by varying the specific energy of sonication:

Graph 36: Solubilization of proteins with the US energy

Treatment with US causes cell lysis, with the consequent release of intracellular constituents, proteins and carbohydrates in the surrounding medium. The sonolysis causes solubilization of proteins and thus the nitrogen present in the medium one of the principal substrates to the bacteria.

In the three following graphs 43 a, b, c, it is represented the results of the different concentrations of the soluble organic matter (TKN, sCarbohydrates, sTOC) vs COD:

Graph 37: Relation between: a) TKN with sCOD, b) sCarbohydrates with sCOD and c) sTOC with sCOD
In the graph 43 it is represented the relation between the different parameters that express the solubilization of the organic matter, obtained at different specific energies of sonication.

In the graph 43a) it is showed the relation between the evolution of sCOD, a parameter that indicates the evolution of the organic matter that is going to be used in the process of anaerobic digestion(sCOD), and the solubilization of the TKN, which express the amount of ammonia and organic nitrogen. This comparison shows that exits a lineal correlation between the increment of both parameters, proving that if there is an increase in sCOD means an increase in TKN and vice versa. At the end, this means that the nitrogen in solubilization (because of the ultrasound treatment) forms part of the organic matter that is going to be used in the AD, so the ultrasound is capable of releasing the nitrogen associated to organic compounds.

The same purpose have the graphs 43 b) and c), trying to establish a relation between sCOD with the carbohydrates (b) and with the sTOC (c). Both graphs also show the same lineal tendency, showing what is mentioned above: an increase of soluble carbohydrates and TOC goes hand in hand with an increase of sCOD. So the ultrasound treatment allows the solubilization of the organic matter that is going to be used in the AD process.

**4.1.2. Total Suspended Solids and Filterable Solids**

This study is supplemented with total suspended solids and filterable solids: the first gives an idea of the US effect in terms of their role in particle size and so reducing the dimensions of the particles letting the organic matter in contact with the solvent; the others indicate those solids in solution, i.e., the available organic matter in the medium.

The following graph 44 represents the concentration of TS and FS in terms of mg/L when different specific energies of sonication are used, with amplitude of 50% of the maximum one.
The highest value of energy for obtaining the highest concentration of FS is 20000 KJ/Kg TS with a percentage of FS around 30% followed in a second place by 50000KJ/Kg TS with similar results (25% of FS), when the initial percentage was 11%, showing that increasing the specific energy to increase the filterable solids is only beneficial to an applied energy of 20000KJ/Kg TS. This results agree with the sCOD data, where the values at these two energies where the highest ones, having a bigger quantity of sCOD at an energy of 20000KJ/Kg TS, justified by the high value obtained with the FS analysis.

4.2. The effect of digestate sonication on the AD process

The next graph 45, shows the evolution of normal liters of methane obtained through the anaerobic digestion, using different energies of sonication and percentage of treated material.
The higher value of normal liters of methane is obtained for the mixing 50000-75%, so is the one which has obtained more methane. This agrees with the results in the part 4.1, where the highest values of solubilization were obtained for an energy of 50000 KJ/Kg TS.

But turning to compare therefore different energies of sonication and the same percentage, it appears that there are no noticeable changes. The conclusion therefore is that using the same percentage of material treated; when energy is increased not greater production of methane is obtained. So although in the results of ultrasound showed that the energy of 50000 KJ/Kg TS was the best operating option, anaerobic results have shown that the energy is not the limiting factor. This can be due to an excessive energy causes bacteria death or that there is big amount of substrate that is not used creating conditions in which the biomass remains in excess of the available substrate, which cause a stop in the production.

This information is useful in terms of the profitability of the process since, the more energy of sonication is applied, is more cost associated with the process.

In the following table is resumed the information of sTOC values obtained during the AD test for the different energies and percentages of material treated with US.

<table>
<thead>
<tr>
<th>Energy (KJ/Kg TS)</th>
<th>Percentage</th>
<th>sTOC Values (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20000</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>50000</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>50000</td>
<td>25%</td>
<td></td>
</tr>
</tbody>
</table>

Graph 39: Representation of the normal litre of methane regarding with the volatile solids (VS) and total organic carbon (tTOC) vs the days of the anaerobic digestion probe
Table 19: Results from the anaerobic digestion of the total organic carbon in solution (sTOC) mg/L vs days bearing in mind the Specific energy of sonication and the grade of mixture

<table>
<thead>
<tr>
<th>Mixture/Days</th>
<th>0</th>
<th>4</th>
<th>11</th>
<th>18</th>
<th>29</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>50000-25%</td>
<td>6277</td>
<td>9785</td>
<td>10346</td>
<td>8774</td>
<td>6750</td>
<td>8235</td>
</tr>
<tr>
<td>50000-75%</td>
<td>12496</td>
<td>10522</td>
<td>12779</td>
<td>9023</td>
<td>8401</td>
<td>11387</td>
</tr>
<tr>
<td>20000-75%</td>
<td>9911</td>
<td>11623</td>
<td>11623</td>
<td>6869</td>
<td>9741</td>
<td>10184</td>
</tr>
</tbody>
</table>

In the following graphs are represented the values of the table 22, comparing concentration of sTOC trough the time, comparing energies, percentages of treated material and both.

Graph 40: Representation of total organic carbon on solution (sTOC, mg/l) vs days of anaerobic digestion probe; a) compares same energy with different mixture grades; B) compares different energies same mixture range and c) different energies and mixture ranges

The next step is to try to explain these results of normal liters of methane, using sTOC information collected during the AD probe, seeing the influence of the energy and percentage of treated mixture

Graph a: Comparing same energy and different percentage of treated material, it appears that lower values are obtained for the case of 50000-25% at the end of the process. This is related to greater consumption of the substrate by the bacteria and also that there is more production of sTOC in the case of 50000-75% all along the process so as at the end it is the one with more production of methane

Graph b: In this case different energies are compared with the same percentage of treated material. The result is that there is more sTOC for the case of 50000-75% so at the time of
working with the same percentage of material treated with ultrasound energy, it is better higher energy to increase the organic carbon in solution. But it is important to notice that there is not a very high difference between both cases (which agrees with the results of methane production graph X), so it is necessary to evaluate what is the difference of the methane obtained with 20000-75% and 50000-75% and if the cost of the energy utilizing energy of 50000KJ/Kg TS is beneficial in the economic balance.

Graph c: In the last case different energies and different percentages of treated material were compared to see the limiting factor in the solubilization of the organic carbon. As in graph a) the higher values are obtained with the higher percentage of material treated, but with the lowest energy (20000-75%), so in this case the conclusion is that, it is better to work with more percentage of material treated at lower energies than with a lower percentage and higher energy.
CONCLUSIONS

In the first part of the experimental phase of this study, the has been evaluated the effects of ultrasound in the material utilized, of lignocellulosic composition. There has been used specific energy values of 500, 3000, 6000, 10000, 20000, 30000, 50000 KJ/Kg TS, using for each value different amplitudes: 20%, 50% and 80% of the maximum operating amplitude, respectively. With these treated samples, are obtained different parameters (pH, TS, Vs, COD, TOC, Carbohydrates, NH3, TKN) that shows the evolution of the solubilization of the organic matter. From these results is concluded that: there has not been mineralization and volatilization phenomena because of the absence of a variation in the total organic substance. The next point observed is that, by comparing between different values of amplitudes, the solubilization of organic matter increases when higher specific energies are applied at higher values of amplitude. This tendency is followed till a certain point, where it is observed that for the higher energies (50000 KJ/Kg TS), the solubilization of organic matter is bigger when 50% of amplitude is used, instead of 80%. Comparing the results obtained when the same amplitude is applied, it is observed that for the lowest values of energy, there is not an important increase in solubilization terms, turning to have bigger percentage of increments in the range of (10000-20000 KJ/Kg/TS), following all the values an increasing tendency: more solubilization obtained when the specific energy applied, is higher, but showing no remarkable differences between the solubilization results obtained at the highest energies (20000-5000 KJ/Kg TS). And comparing between the values obtained at different amplitudes, the bigger amount of organic matter in solubilization results when is applied an amplitude of 50% for each value of energy.

The same tendency is observed in the analysis of proteins, when using the same amplitude, it is observed that the values of the TKN in the total fraction didn´t showed a variation indicating that there has not been phenomena of volatilization or mineralization.

Contrary in what is observed for the tendency followed by the solubilization of proteins, which increase when higher values of energy are applied.

Trying to explain why these solubilization parameters increase, there has been studied different correlations between them showing, that indeed they follow a lineal tendency,
demonstrating that when there is an increase in one of them, this tendency is followed by the other.

In the second phase of this study, anaerobic digestion takes part, by using different samples of material treated with ultrasound at two specific energies (20000, 50000 KJ/Kg TS) with the same amplitude (50%) and with different percentages of material treated and untreated.

What was observed was that in terms of normal liters of methane there was an increasing tendency followed by the samples in order of much volume of methane obtained: white control (non treated material), 20000-25% of treated material, 20000-75% of treated material and the highest volume obtained, 50000-75% of treated material. Although this tendency is followed, the difference between 20000-50000-75% of treated material is not very high, so the limiting operating factor is the percentage of material treated and not the energy used.

In term of solubilizations TOC analyses showed that the highest value was obtained for an energy of sonication of 50000 KJ/Kg TS and a percentage of treated material of 75%. But highlighting that the difference with the sample of 20000-75% is not pronounced, which agrees with the conclusion of the previous graph.

So bearing in mind all these points, ultrasound energy has beneficial results in terms of solubilization of the organic matter in the lignocellulosic material, having the best results for the highest energy applied (50000 KJ/Kg TS); taking into account that the best work option from all seen, is an amplitude of 50% using an energy of 20000 KJ/Kg TS, with the highest percentage of treated material.


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