Synthesis of biotechnological schemes for biogas production from milk whey

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A mis padres y mi hermano: mi apoyo y felicidad.

“Y sin saberlo, pero pensándolo… el lactosuero con tallo de papa es un suculento plato energético en forma de biogás, casi comparado con la hamburguesa con queso cheddar y papas rústicas que comí esta mañana”. ¡Sweet Dreams!
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Abstract.

The implementation of projects focused on reducing dependence on fossil fuels has created the possibility of establishing new alternatives for energy generation. Industrial waste and lignocellulosic biomass generated in Latin American countries such as Colombia and Chile can be transformed into value-added energy products through anaerobic digestion. An interesting alternative for energy production is milk whey. Milk whey is one of the main residues of the dairy industry. This residue is not completely used and it is a source of contamination, given the high organic load. Similarly, the potato stem generated in the harvest stage is attractive for biogas production. This waste is non-treated, causing problems due to deficient use. In this sense, co-digestion of raw materials can improve the balance of nutrients in anaerobic digestion. Accordingly, the synthesis of new biotechnological schemes for the biogas production emerges as an alternative to use to the full potential of the milk whey, which can be combined in a co-digestion process with lignocellulosic wastes such as the potato stem. The present thesis evaluated the potential of energy production from co-digestion of milk whey and potato stem through experimental and simulation processes through different biotechnological scenarios. The main objective was to establish the economic and environmental prefeasibility of this process. Initially, the characterization of raw materials and biogas production an experimental level was analyzed using different relations of potato stem and milk whey. Subsequently, different scenarios were analyzed considering technical, economic and environmental analysis based on simulation processes. As a result, the co-digestion of milk whey and potato stem is a promising alternative for energy generation, where it generates a positive net economic profit but with limits in raw material costs and digestate selling prices.

Keywords: biogas, co-digestion, economic assessment, environmental assessment, milk whey, potato stem.
Resumen.

La implementación de proyectos enfocados en la reducción de la dependencia de combustibles fósiles ha creado la posibilidad de establecer nuevas alternativas para la generación de energía. Residuos industriales y biomasa lignocelulósica generada en países de América Latina como Colombia y Chile, pueden ser transformados en productos de valor a través de digestión anaerobia. Una alternativa interesante para la producción de energía es el lactosuero. El lactosuero es uno de los principales residuos en la industria láctea. Este es un residuo que no es usado en su totalidad y es una fuente de contaminación, el cual tiene una elevada carga orgánica. Del mismo modo, el tallo de papa generado en la etapa de cosecha es atractivo para la producción de biogás. Este residuo no es tratado, causando problemas de disposición. En este sentido, la co-digestión de materias primas puede mejorar el balance de nutrientes en la digestión anaerobia. En consecuencia, la síntesis de nuevos esquemas biotecnológicos para la producción de biogás surge como una alternativa para aprovechar todo el potencial del suero de leche, el cual se puede combinar en un proceso de co-digestión con residuos lignocelulósicos como el tallo de papa. La presente tesis evaluó el potencial de producción de energía a partir de la co-digestión de lactosuero y tallo de papa a través de procesos experimentales y de simulación considerando diferentes escenarios biotecnológicos. El objetivo principal fue establecer la prefactibilidad económica y ambiental de este proceso. Inicialmente, se analizó la caracterización de las materias primas y la producción de biogás a nivel experimental utilizando diferentes relaciones de tallo de papa y suero de leche. Posteriormente, se analizaron diferentes escenarios considerando análisis técnicos, económicos y ambientales basados en procesos de simulación. Como resultado, la co-digestión del suero de leche y el tallo de la papa es una alternativa prometedora para la generación de energía, donde se genera un beneficio económico neto positivo (41.05 M.USD) con limites en el costo de la materia prima y los precios de venta del digestato.

Palabras clave: biogás, co-digestión, evaluación económica, evaluación ambiental, lactosuero, tallo de papa.
Content.

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International conferences participation.


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Participation of this Thesis in Research Projects

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Internships Experiences.

Introduction.

The valorization of residues through different transformation processes allows obtaining chemical and energy products. Last years, the preference has been for energy production by thermochemical or biochemical ways [1]. Additionally first and third generation raw materials are also a subject of intensive research for these purposes [2]. All this tendency to establish biomass as a future substrate for energy production resulted in the implementation of several projects over the world focused on reducing fossil fuels dependence, creating the possibility for new alternatives in energy generation [3]. The midpoint between energy and other products processing is based on the concept of biorefineries that integrally can produce energy, chemicals, food and feed simultaneously avoiding discussions about energy and food competition [4]. Industrial waste and lignocellulosic biomass can be transformed into added-value energy products through anaerobic digestion (AD), a process where a group of specific microorganisms converts biomass to a methane-rich gas called biogas.

Biogas is a source of clean and renewable energy, it has a high calorific value, it protects the environment from air pollutants and reduces greenhouse gases (GHG) after combustion [5]. Biogas is composed mainly of 50-70% methane (CH₄) and 30-50% carbon dioxide (CO₂); it may contain traces of other compounds such as hydrogen sulfide [6]. However, biogas composition depends on external factors, such as the type of raw material, the technology used and anaerobic digestion conditions, among others. Different raw materials are used for biogas production depending on the region where the technology is applied. The most commonly used raw materials are municipal solid wastes, lignocellulosic materials and animal manure, which it can be found the milk whey and the potato stem.

Milk is one of the main products for human consumption in the planet. Milk processing for the production of cheese generates a residue called “milk whey” in great amounts, with a proportion of almost nine kilograms of milk whey per kilogram of cheese [7]. This residue is
not widely used and it is a source of contamination, given the high organic load. Because of this, different productive systems have been proposed to obtain added-value products from this residue [8]–[10]. However, in countries as Colombia and Chile the lack of these technologies, the competition with other substrates and the limited demand for food products based on milk whey generate huge excess quantities of milk whey obtained in the dairy industry not exploited and representing serious environmental problems.

On the other hand, potato crop is a residue existing in most of the world without effective industrial valorization. The potato stem generated in the harvest stage is disposed in landfills, without exploiting its potential to produce biogas despite its lignocellulosic composition. In the literature, practically no information was found on the production of biogas from this residue, allowing the expansion of its use as an organic source for biofuels. Additionally, it can be observed that this crop and its residues is emerging with an interesting potential to be used for different purposes than food.

An alternative for the use of this residues is energy production using biogas as energy vector. This proposed can be developed by co-digestion processes. The co-digestion refers to anaerobic digestion processes where multiple substrates are used after a strategic selection based on their characteristics. Usually the first reason to use co-digestion is the fact that in some cases one substrate or raw material is not enough to make digestion cost-effective [11]. Anaerobic co-digestion can be considered one of the most relevant topics in anaerobic digestion research providing different challenges in phenomena understanding, modelling development and process implementation [12]. It should be noted that the strategic integration of raw materials in anaerobic co-digestion processes is an interesting alternative to reduce availability and characteristics problems of single substrate anaerobic digestions. Therefore, it could be promising the integration of potato stem and milk whey as an innovative alternative to recover energy with economic benefits as a result of the increase of the overall raw material availability for the anaerobic digestion. The real possibilities of using any biomass or residue through different energy conversion strategies (including the use of more than one residue simultaneously for the same energy purpose) should be analyzed seriously based on techno-economic analysis [13]. Last allows developing non-expensive calculations and simulations based on the composition of the raw material or its combination with others as conceptual basis for following steps in developing this type of anaerobic digestion technologies.
In order to evaluate the potential of milk whey and potato stem for energy production, the objective of the present thesis is to develop a techno-economic and environmental assessment of different biotechnological schemes for the biogas production through anaerobic co-digestion using milk whey and potato stem as substrates.

References.


Thesis Hypothesis.

The evaluation of different technological routes for biogas production from milk whey using process engineering allows proposing a more efficient process from a technical, economic and environmental point of view.

Thesis objectives.

General objective

- Propose biotechnological schemes for biogas production from milk whey considering the co-digestion of the raw material and the final application of the product.

Specific objectives

- To perform the physical-chemical characterization of the milk whey, potato stem and anaerobic sludge.

- To evaluate experimentally the effect of co-digestion for biogas production from milk whey and potato stem.

- To propose and evaluate biotechnological schemes for biogas production from milk whey as raw material.

- To select the best technological route for biogas production comparing the biotechnological schemes proposed from a technical, economic and environmental point of view.
1. Principles of anaerobic digestion.

Anaerobic digestion (AD) is a process by which a group of specific bacteria and archaea metabolically transform biodegradable organic matter into biogas [1]. Biogas is a source of clean and renewable energy, it has a high calorific value, it protects the environment from air pollutants and reduces greenhouse gases (GHG) after combustion [2]. Biogas is composed mainly of 50-70% methane (CH₄) and 30-50% carbon dioxide (CO₂); it may contain traces of other compounds such as hydrogen sulfide [3]. However, biogas composition depends on external factors, such as the type of raw material, the digester used and anaerobic digestion conditions, among others.

1.1. Degradation pathway during anaerobic digestion.

Biogas production from organic raw materials considers a series of successive steps, where the product of one step acts as a substrate in the subsequent step. This process is complex, where a synergetic interaction of enzymes and microorganisms act to degrade the substrate. However, the degradation can generally be divided into 4 steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. A schematic overview of the degradation process is presented in Figure 1-1.

1.1.1. Hydrolysis.

Hydrolysis is the first step of anaerobic digestion (AD) processes. In this step, substrates composed mainly of complex molecules such as carbohydrates, proteins and lipids are hydrolyzed to simple molecules (e.g. amino acids, sugars and fatty acids). In general, large carbon-chains compounds can be transform to smaller molecules by hydrolytic bacteria. Hydrolytic bacteria produce enzymes (such as amylases, lipases, proteases, celluloses,
among others) that transform the complex organic molecules into final products such as soluble sugar, amino acids, glycerol and long-chain carboxylic acids [4]. Thus, cellulose hydrolysis involves at least three groups of enzymes: endoglucanase, exoglucanase and glucosidase [5]. While hemicellulose degradation requires more specific enzymes for its degradation due to its complex structure compared with cellulose. On other hand, the main group of anaerobic bacteria that degrade the cellulose are *Clostridium celobioporus, Clostridium lochhadii, Clostridium stercorarium, Clostridium acetobutylicum, Clostridium thermoculum, Ruminococcus flavfaciens, Ruminococcus albus, Micromonospora bispora*, among others [4], [6].

Hydrolysis has been considered as a relatively slow process and generally the rate-limiting step of AD. For example, when lignocellulosic substrates are used, hydrolysis is limited by complex structure in the form of cellulose, hemicellulose and lignin. Thus, pretreatments are considered as a tool to make hydrolysis more efficient. These can been divided in physical, biological, chemical and physicochemical pretreatments. In the next chapter the effect of pretreatment on AD and biogas production will be discussed. So, the hydrolysis step can
also be affected by other factors such as pH (optimum range from 5.5-6-5), particle size of substrate and source of microbial inoculum [7].

1.1.2. Acidogenesis.
In the second step, acidogenesis, the product of hydrolysis sugars, amino acids and fatty acids are converted into a mixture of different volatile fatty acids (VFAs) (mainly acetate, propionate, and n-butyrate), alcohols, ammonium, carbon dioxide (CO2) and hydrogen (H2). Byproducts such as ammonia (NH3/NH4+) and dihydrogen sulfide (H2S) are also generated, which can prove toxic to the methanogenesis step later [8]. In general, the most efficient metabolic pathway for primary fermentative bacteria is the production of acetate and hydrogen from pyruvate [9]. However, at high partial pressure of hydrogen forms different VFAs and alcohols. The phylogenetic diversity of the bacteria in AD are very dependent on inoculum is used. Therefore, different bacteria are presented on the medium such as Clostridia, Bacteroida, Gammaproteobacteria, Actinobacteria and Bacilli [10]. Finally, it should be mentioned that the acidogenesis the quicker step in AD compared to hydrolysis.

1.1.3. Acetogenesis.
Acetogenesis is the third step during AD where products of the acidogenesis are further transformed to acetate, CO2, H2, and other C1 compounds by acetogenic bacteria. The hydrogen partial pressure is an important factor for these reactions, where a high hydrogen partial pressure causes CO2 reduction and involves the inhibition of acetate production resulting in accumulation of fatty acids [11]. The first steps are together known as acid fermentation, where acetogenic microorganisms of the genus Syntrophobacter and acetogenic microorganism denoted homoacetogens convert the acid-phase products into substrates for methanogenic bacteria [12]. The main types of bacteria that perform the acetogenesis are Synergistales, Syntrophobacterales, Clostridiales, and Thermoanaerobacterales [10]. For example, Methanobacterium suboxydans degrade the pentanoic acid to propionic acid, whereas Methanobacterium propionicum convert the propionic acid to acetic acid [4].

1.1.4. Methanogenesis.
In the final step of AD, methanogenesis, methane (CH4) is formed from the products of the acetogenesis step by strict anaerobic bacteria and archaea. Two routes are known for
methane formation: the acetoclastic methanogens transform acetate into CO₂ and CH₄, and the reduction of CO₂ into CH₄ through H₂ as an electron donor [13]. However, around 70% of CH₄ is produced from acetate because H₂ is limited in AD [13]. Methanogens are considered to be the most sensible microorganisms, inhibited by ammonia concentration, temperature and pH. For example, a majority of methanogens usually develop under inert conditions, with an optimal growth of a pH between 6.8 and 7.2 [4]. Additionally, these have a low growth rate compared to other bacteria in the above steps [15]. In this sense, when easily hydrolysable feedstocks are used in AD, methane production is the rate-limiting step.

Seven different methanogenic archaea are known, *Methanobacteriales*, *Methanococcales*, *Methanocellales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanomassiliicoccales*, and *Methanopyrales*, where the majority catalyze hydrogenotrophic and methylotrophic methanogenesis [10]. While, only few bacteria can solely use acetate for CH₄ production.

### 1.2. Important parameters for anaerobic digestion.

#### 1.2.1. Temperature.

The temperature is an important parameter for survival, optimum growth rate and metabolism of the microbial consortia, and performance of AD. AD can be occur under the three temperature ranges defined as psychrophilic (up to 25 °C), mesophilic (25-40 °C) and thermophilic (50-65 °C). In general, the range between mesophilic and thermophilic conditions is commonly used in different applications. However, currently, new researches are been carried out on the AD application under psychrophilic conditions.

On the other hand, an increasing temperature has several benefits, including a direct impact on conversion kinetics. The higher the temperature allows higher degradation rates, yield, substrate digestion and methane production. However, the application of high temperatures will cause an increase of the fraction of free ammonia, which has an important role in the kinetic inhibition process. Additionally, the control of the temperature is more difficult under thermophilic conditions compared to mesophilic conditions, affecting the activity of the archaea methanogenic. Mesophilic operation is robust and works with consortia that tolerate environmental changes, is easier to operate and maintain, and has a lower investment cost. However, it generally produces less biogas.
Finally, it should be emphasized the importance of maintaining stable temperature due to the affect that fluctuations can have on the bacteria, especially the methanogens. So, the temperature change of 1°C/day causes a process failure; and changes in temperature of more than 0.6 °C/day should be avoided.

1.2.2. pH and alkalinity.
The pH is directly related to the acidity or alkalinity degree in the biodigester. This parameter has optimal values depending on the microorganism. In the AD, a wide range of pH can be observed due to the different microbes implied in the process. Methanogenic bacteria are the most sensitive to pH with an optimum between 6.5 and 7.2 [16]. While the other types of microorganisms are more robust and can work in a pH range between 4.0 and 8.5 [17]. The pH in the process can be affected by different substances such as carbon dioxide (CO₂), organic acids, ammonia, hydrogen sulfide and salts as organic acids and bases. In this sense, the optimal performance of the AD has been established by different researchers of a neutral pH level between 7-7.5 [11]. On other hand, it is important that the pH can be controlled by CO₂ concentration in the gas-phase and the alkalinity in the liquid-phase. For example, when CO₂ remains constant, the pH value is increased with the addition of HCO₃. A buffer capacity of 70 meq CaCO₃/L is favorable to maintain the biochemical degradation and a stable transformation process [11]. Consequently, in the industrial biogas production the pH is monitored in order to control the buffer capacity of the system.

1.2.3. Ratio C/N.
The ratio of carbon (C) and nitrogen (N) plays an important role in AD. This parameter can give an idea of ammonia inhibition and nutrient deficiency, where the carbon acts as energy source and nitrogen serves to enhance the microbial growth. In general, an optimal ratio of C and N is between 20 and 30 [18]. A high relationship of C/N lead to a fast nitrogen consumption by methanogenic bacteria, which leads to a low biogas production. On the other hand, a low C/N ratio causes ammonia accumulation and the pH value can exceed 8.5, a level that is toxic for methanogenesis [4]. Optimum C/N ratio can be achieved by the combination of two or more organic materials in a processes called co-digestion. For example, lignocellulosic substrate that have a high carbon content can be combined to a material with a high nitrogen content (such as manure). In addition, nitrogen is considered the limiting factor in AD, therefore, supplements such as urea and biosolids are necessary
1.2.4. **Nutrients.**

Besides the carbon and energy source, microorganisms need a wide variety of essential nutrients for synthesis and growth. In this sense, methanogenic bacteria require mineral nutrients to resolve environmental changes and develop a robust metabolism [19]. The macronutrients and micronutrients must have the correct proportions in the biodigester due to their deficit inhibiting the overall process. Elements like phosphorus, sodium, potassium, calcium and other trace elements such as iron, zinc, cobalt, nickel and selenium are essential for microorganisms. Interesting information about the trace elements in the AD can be found in the literature [20]. However, a specific concentration of each element is difficult to establish because this depends on the specific substrate and inoculum of the AD.

1.2.5. **Total solids.**

AD is usually carried out in an aqueous environment, where the water is transported and distributed into the reaction medium. Substrates with a high moisture and low content of non-degradable compounds are preferred. However, there are also processes that operate at high solids content. Therefore, moisture content is a fundamental factor in the design and startup of AD plants.

In general, the biogas production technology is adjusted to the substrate’s requirements. Substrate requirements are considered a main characteristic for AD design including total solids (TS) content. The organic raw material most used in the industry can be divided into different categories considering their content of TS. Liquid substrates like wastewater (6% <TS), sludge like liquid manure (6%> TS <15%) and solids substrates like lignocellulosic material (TS > 15%) [10]. In addition, the amount of TS defines the technologies that are necessary in the handling of materials, affecting the rheological characteristics of the medium and the separation systems of by-products. For example, mixing, transport and separation of by-products are affected [10]. Consequently, systems containing more than 15% of TS usually operate with plug flow digesters in continuous operation. Meanwhile, continuous stirred tank reactors (CSTR) are the most used in systems containing less than
Chapter 1. Principles of anaerobic digestion.

15% in TS. Finally, for systems with low TS content and particle size, the most common systems used are anaerobic upflow sludge blankets.

1.2.6. Inhibitory compounds.
The performance of the AD is adversely affected by the formation of inhibitory compounds during the substrates degradation. These compounds affect the balance between acid concentration and methane formation, which inhibits the AD process and decreases methane production. Among the main inhibitory compounds are ammonia, hydrogen sulfide and organic acids. Additionally, inhibitory substances may sometimes include heavy metals, salts, surfactants, among others.

The impact of the inhibitory compounds depends on their concentration, interaction with other substances and adaptation of the microorganisms. The effect of an individual compound is specific for each microorganism and metabolic process. For example, the bacteria most susceptible to ammonia inhibition are methanogenic bacteria, which are inhibited at a concentration over 200 mg/L [21]. In this way, ammonia affects the microorganism and leading to proton imbalance and/or potassium deficiency [21].

On the other hand, volatile fatty acids (VFAs) are intermediary compounds in AD, and their accumulation causes an inhibitory effect. This effect is caused by the penetration of VFAs through the membrane of microorganisms, causing cytoplasm acidification and decreasing the pH level [22]. Table 1-1 shows the inhibitory concentration of certain inhibitory compounds.

<table>
<thead>
<tr>
<th>Component</th>
<th>Inhibitory concentration [g/L]</th>
<th>Component</th>
<th>Inhibitory concentration [g/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>0.2</td>
<td>Calcium</td>
<td>4.8</td>
</tr>
<tr>
<td>Sodium</td>
<td>5-15</td>
<td>Sulfide</td>
<td>0.03</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.8</td>
<td>Short-chain fatty acids</td>
<td>pH dependent</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.9</td>
<td>Heavy metals*</td>
<td>0.01-0.34</td>
</tr>
</tbody>
</table>

* Dependent of the type of heavy metal (Ni, Cu, Cr, Zn, …)
1.2.7. Mixing.
Mixing is an operative parameter that is a necessary application in AD. This is vital to maintain full homogeneity in the biodigester and to reduce the scum formation [4]. In addition, this affects the mass and energy balance in the digester. There are a number of different types of mixing equipments used in the AD industry. These include mechanical mixing, hydraulic mixing and pneumatic mixing. The most commonly used is mechanical mixing, however, gas recirculation too is utilized depending the TS concentration. It should be noted that excessive mixing can disrupt and/or stress of the microbes, so a slow mixing is established. More details about this parameter can be found in the literature [23].

1.2.8. Organic loading rate.
The organic loading rate (OLR) is defined as the amount of organic raw material added per day (F in kg volatile solids/day) and the reactor volume (V in m³).

\[
OLR = \frac{F}{V}
\]

OLR is the biological conversion capability of a reactor and depends on the substrate concentration in the influent and the retention time of the process. This parameter is used to measure the development of AD and its treatment effectiveness due to the relation between the input and output OLR values. Thus, a high concentration in the biodigester can cause VFAs to accumulate [24]. According to literature, typical values of OLR are between 1.0 kg VS.m⁻³.day⁻¹ and 9.9 VS.m⁻³.day⁻¹ [10]. However, depending on the biogas plant, these values are optimized considering techno-economic aspects.

1.2.9. Hydraulic retention time.
The hydraulic retention time (HRT) is the mean time in which the substrate remains inside the biodigester, that is, is the ratio between the volume of the biodigester and the flow input. Where, these definition only applies for a constant reactor volume. HRT is a crucial parameter for AD design in CSTR system.

\[
HTR = \frac{V}{Q}
\]
Chapter 1. Principles of anaerobic digestion.

There is optimal retention time for complete degradation, 12-14 days for thermophilic and 15-30 days for mesophilic digesters [4]. However, this depend of the type of substrate, environmental conditions and process characteristics. This other parameter that is monitored to reduce instability in the digester.

1.3. Final remarks.

Anaerobic digestion is a complex process, where diverse types of microorganisms interact and act in synergy in four sequential stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The fourth step is the most important, given the sensitivity of methanogenic bacteria taking account the operating parameters that control the transformation process of organic matter to biogas. Among the main parameters are temperature, pH, nutrients, total organic load and hydraulic retention time.

References.


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Chapter 1. Principles of anaerobic digestion.


2. Biogas production from agroindustrial and industrial waste.

Fossil fuels substitution through the search for clean energies with fewer effects on the environment is an issue of interest. The use of organic raw material for transformation to biofuels arises as an alternative [1]. Different raw materials are used for biogas production depending on the region where the technology is applied. Table 2-1 summarizes the main features for AD as a function of the different geographical areas. In less developed countries where there are large rural areas, low-level technology is being implemented, such as South America and Africa [2]. However, each biogas producing country seeks the best technology according to an application. United States, Germany, Italy, France, and the United Kingdom use biogas for electric power production and direct injection to the home gas network [2], [3]. In contrast, biogas production in China and India is focused on decreasing energy deficits in rural areas [4], [5]. Taking into account the above, it is possible to optimize these technologies by joining together an industrial network that optimizes the profits within the biorefinery framework [6], [7].

In this chapter, a detailed description of potential raw materials in biogas production is presented, and a comparison of these in terms of viability is made. Also, it describes the main technologies in the process of anaerobic digestion, the differences in the scale of production, the potential market of biogas and its potential in a biorefinery.
### Table 2-1. Main characteristics of anaerobic digestion according to geographical areas.

<table>
<thead>
<tr>
<th></th>
<th>Europe</th>
<th>North - American</th>
<th>Africa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biodigesters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Farm scale:</td>
<td>There are many types. They are currently used the vertical tanks with flexible membranes [8].</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Agroindustrial and industrial residues:</td>
<td>Organic waste (vegetable and fruit residues), sludge, and others [8].</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Industrial scale:</td>
<td>Principally in wastewater treatment are UASB, CSTR and other modifications.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Domestic digesters:</td>
<td>Plastic bag, brick and motor fixed dome, floating dome digesters, DIY biobag digesters [10].</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Agricultural digester:</td>
<td>Covered lagoon, plug flow, CSTR and UASB.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Substrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Animal manure:</td>
<td>non-specific materials (e.g. cow, pig, and others).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Energy crops:</td>
<td>grass and different types of biomass (approx. half of biogas production).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Agroindustrial and industrial residues:</td>
<td>Organic waste (vegetable and fruit residues), sludge, and others.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Animal manure:</td>
<td>Manure from dairies (cow and pig) [11].</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Low development in energy crops for biogas production [11].</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Agroindustrial and industrial residues:</td>
<td>Sludge, crop residues, food waste [11].</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Industrial waste:</td>
<td>Slaughterhouses waste, food waste, municipal solid waste [12].</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biogas upgrading</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Absorption:</td>
<td>Water scrubbing (35.51%), organic physical scrubbing (4.76%), and chemical scrubbing (20.56%).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Low development in upgrading for biogas production. However, biomethane is now included for vehicular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• There is no relevant development in the field of biogas upgrading. Biogas production carried</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Chapter 2. Biogas production from agroindustrial and industrial waste.

<table>
<thead>
<tr>
<th>Biogas Use</th>
<th>New Development</th>
</tr>
</thead>
</table>
| **Adsorption:** Pressure swing absorption (16.82%).  
**Others:** Membrane upgrading (20.56%), cryogenic upgrading (0.23%), unknown (1.64%).  
Biogas is used for electricity and heat production (>90%) [8].  
Biogas is used for vehicle fuel (<4%); Sweden is the most important producer.  
Gas grid injection: 480 plants (Netherlands, Germany, Switzerland, Austria, France, Italy, UK, Sweden) [14].  
Biogas is used for heat and electricity production. Treatment plants use biogas to support their productive process energetically. Additionally, biogas grid injection and new strategies about vehicular fuel become important [15].  
Biogas upgrading: Developments in new process for purification. Wagabox and cryogenic distillation [16].  
**Strong policy** around biogas (advanced legislation on biogas in France)  
New premises about subsidies (Austria, fuels from European technology [13].  
out on digesters of domestic and medium scale. Few digesters operate on an industrial scale [10].  
**Domestic digesters:** The mainly use of biogas is for cooking and lighting [10], [12].  
**Agricultural digester:** The use of biogas is heating, lighting and electricity generation.  
New applications of upgrading technologies in biogas production.  
Research in technology and projects for biogas grid injection.  
Membrane-based gas separation technologies.  
Development of policies to encourage anaerobic digestion technology.  
Advances in large-scale commercial digesters in principals cities.  
|
Chapter 2. Biogas production from agroindustrial and industrial waste.

- Biogas plants with less than 60% corn as raw material to applied for subsidies) [14].
- Expansion of projects about landfill biogas (UK mainly) [14].
- **Biogas market** around construction and service loans.

<table>
<thead>
<tr>
<th>Asia</th>
<th>Latin - American</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biodigesters</strong></td>
<td><strong>Biodigesters</strong>: Fixed dome digester, floating drum and tubular digester [19].</td>
</tr>
<tr>
<td>- Domestic digesters: Fixed Dome Biodigester (Chinese), floating dome digester, fixed-dome [17].</td>
<td>- Agricultural scale: covered lagoons, UASB reactor in PTAR. Low development of technologies in industrial scale [19].</td>
</tr>
<tr>
<td>- Agricultural and industrial scale: Upflow solid reactor (USR), covered in ground anaerobic reactor (CIGAR), CSTR and UASB [18].</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrates</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Low use of energy crops.</td>
<td>- In this moment, there are <strong>not use</strong> of energy crops.</td>
</tr>
<tr>
<td>- Industrial waste: waste of palm oil, wastewater, food and vegetable waste.</td>
<td>- Industrial waste: organic waste for food industry, urban residues.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biogas upgrading</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Mainly, biodigesters are on a domestic scale due to government subsidies, the upgrading developments are minor. However, the technology is developed within the framework of the waste use in large cities; where the central</td>
<td>- There are not developments in this sense, because European technology is hardly being implemented on a large scale. The countries that address this technology are Brazil and Argentina.</td>
</tr>
</tbody>
</table>
Chapter 2. Biogas production from agroindustrial and industrial waste.

Focused is the biogas upgrading [18].

**Biogas Use**

- **Domestic digesters**: The mainly use of biogas is for cooking and lighting.
- Biogas is used for **heat and electricity production** from combined heat and power (CHP) configuration.
- **Domestic biodigesters**: Mainly for cooking and heating.
- **Industrial digester**: Biogas is used to generate electricity.

**New Development**

- Models of **self-sustained farms** based on biogas [20].
- Programs to **reduce chemical fertilizers**. The **digestate** product of anaerobic digestion to will **replaced their demand** [20].
- **Expansion of the industrial biogas** in mainly countries.
- Implementation of **biogas as a strategic point of development**, taking into account the other technologies (biodiesel and ethanol).
- **Biogas technology to spread** with support networks at a Latin American level, it is a case of RedBioLAC and technology centers in strategic countries such as Brazil.

2.1. Introduction

2.1.1. Raw materials

Organic raw materials are used for biogas production. These include animal manure (pigs, cows, chickens), lignocellulosic materials (agroindustrial residues, plant materials, grass), crops (maize, wheat, barley), industrial waste and microalgae [21]. However, the accessibility of resources depends on their availability. The majority of biogas production in Germany is based on energy crops [22]. In the United States, wastewater and agriculture residues are used [23]. In India, the potential of raw materials is crop residues and animal manure [24].
In recent years, studies in biogas production have included various raw materials, dairy manure, microalgae, wastewater, livestock manure and other materials. Nevertheless, the traction rate is different for each substrate; undissolved compounds such as cellulose take several days, while soluble carbohydrates take a few hours [25]. Differences in biogas yield can be observed depending on the morphological characteristics of the raw materials (Figure 2-1).

**Figure 2-1.** Mean biogas yield from different feedstocks. Adapted from Weiland [25].

2.1.2. Production technologies

Technologies of biogas production are well developed nowadays, depending on the feedstock used in the transformation. For example, anaerobic digestion of energy crops in Germany is already established at the industrial level [3]. However, the operating conditions in anaerobic digestion are limited by pH, temperature, retention time, solid charge, C/N ratio, particle size and other factors. This causes multiple states during the action of the microorganisms in the decomposition stages (hydrolysis, acidogenesis, acetogenesis and methanogenesis).
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Taking into account the temperature at which AD is carried out, the process can be classified into psychrophilic, mesophilic and thermophilic. Consequently, optimum mesophilic temperatures between 35-42 °C are employed in most biogas plants and their operating times are between 15-30 days [26], [27]. Thermophilic digesters work at a temperature close to 55 °C with a residence time of 10-12 days. Operating under these conditions methane production is higher, but the application technology is more expensive [27].

Although mesophilic digestion is the most widely used in the industry, studies to develop improvement strategies in thermophilic digestion are being investigated. Thus, Madsen et al. [28] concludes that monitoring techniques cause additional costs to the process, making the process unaffordable when biogas production is on a small scale. Additionally, for wastes with specific composition as excess of the nitrogen, high temperatures can be favor the formation of ammonia, which inhibits the process.

Based on the amount of total solids (TS) in the fermenter, wet digestion (TS <10%) and dry digestion (TS up to 40%) are considered. Biogas plants are dominated by the wet route and 90% of them use mechanical agitation equipment to homogenize the mixture [25]. Also, piston flow digesters are used on a small scale to process animal manure. Montes et al. [29] were reported that conventional digesters can work at a mesophilic temperature, with a solids content of 11-13%, while sophisticated full-mix digesters work with manure slurries with a solids content of 3-10%. One technology often applied to municipal organic waste, industrial waste and solid manure is the two-stage reactor configuration. Here, hydrolysis and methanization occur in both reactors [30]. In section 6, a deeper understanding of the types of reactors used in biogas production today is presented.

2.1.3. Byproducts

After the AD, an effluent called digestate remains as a byproduct. This byproduct is commonly applied directly to crops since it consists of mineral compounds such as phosphorus, potassium and nitrogen [31]. The other byproduct is a sludge that can be used as fertilizer in field application. For example, Möller and Müller [32] established that animal manure sludge is a source of effective nutrients in the production of vegetables.

In 2015, 95% of the digestate produced in biogas plants in Europe was used for agricultural crops mostly with direct application, replacing chemical fertilizers [33]. This is the case in Germany, where 95.1% of the liquid digestate is used for the conventional agricultural
sector. Eighty three percent (83%) of the solid digestate is for agricultural use and the other 17% is for private gardens, soil manufacturers and others [34].

The use of digestates has strict legal impediments in the European Union which restrict its use. Biogas plants for example using domestic waste as a raw material have to comply with the German bio-waste regulation. This prohibits the use of unprocessed digestates in grasslands. Evaluations of quality criteria of pathogens and diseases can be performed with the sanitization. Therefore, an alternative for this byproduct has been proposed in works related to synthetic turf, encapsulation media, pellets as solid fuel and construction material [35]. Pellets and pearls can be priced at 0-20 €/t, and pearl fertilizers and pellets reach prices between 0-100 €/t [33]. Using these approaches, Abubaker et al. [36] demonstrated that the digestate from biogas production improves soil for wheat cultivation.

Otherwise, it is emphasized that knowledge about the influence of individual raw materials on the digestate composition is an important factor for raw material and byproducts optimization [32].

2.1.4. Trends

The biogas production is in a stage of balanced development, where different raw materials are studied for the industrial level application. With regard to European, American and Asian countries, the availability of resources varies depending on the action area. Energy crops with subsidized government support for electric power production are the best option in European countries [30]. European countries have established targets for 2050 for renewable energy use such as biogas, which has an influence on investments in the economic feasibility study for potential raw materials.

The powers of industrialized countries in the Asian continent depends on the technological development of biogas technology to treat a large amount of waste generated. So far, domestic biogas plants in rural areas, municipal, industrial and agricultural waste treatment plants are in continued growth in China [37].

Feedstocks for biogas production is dominated by industrial waste, animal manure and municipal organic waste. However, current trends in biogas production are focused on the use of different resources depending on their availability and logistics. Thus, in rural areas, their development is promoted through the use of native resources, such as waste
generated from animal farms. In contrast, urban areas focus on the research of technologies for biogas production at the industrial level. In general, the biogas applications are focused on the generation of electric power, heating and upgrading (mainly fuel and injection).

2.2. Biogas from municipal solid waste.

Urban solid waste comprises waste from everyday use, food waste, construction waste, paper and plastic. These wastes cause environmental problems when they degrade in landfills. Therefore, alternative studies to treat these residues focused on biogas production are described in the literature [38]–[40].

2.2.1. Food waste

Food waste comprises 25-60% of urban solid waste. In Italy, the plant “Rovereto” transforms 10 ton/day of food waste using activated sludge as a substrate and it generates 400 kW of electrical energy [41]. The above is closely related to yields for every kg of food waste, up to 100 L of CH₄/ kg food waste [42].

The biogas production is directly affected by the type of substrates. Food residues don’t have a homogeneous composition due to the structural characteristics acquired over time and the medium interaction (Table 2-2).

<table>
<thead>
<tr>
<th>Total solids (TS)</th>
<th>Volatile solids (VS)</th>
<th>Carbohydrates (% w.b)</th>
<th>Proteins (% w.b)</th>
<th>Lipids (% w.b)</th>
<th>C/N ratio</th>
<th>CH₄ yield (L CH₄/kg VS)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.10</td>
<td>17.10</td>
<td>11.20</td>
<td>3.30</td>
<td>2.30</td>
<td>13.20</td>
<td>480</td>
<td>[42]</td>
</tr>
<tr>
<td>23.20</td>
<td>21.70</td>
<td>13.70</td>
<td>2.90</td>
<td>6.50</td>
<td>N.R</td>
<td>481</td>
<td>[42]</td>
</tr>
<tr>
<td>20.05</td>
<td>19.21</td>
<td>33.22</td>
<td>14.03</td>
<td>25.25</td>
<td>28.40</td>
<td>260</td>
<td>[43]</td>
</tr>
<tr>
<td>18.90</td>
<td>17.50</td>
<td>N.R</td>
<td>N.R</td>
<td>N.R</td>
<td>21.30</td>
<td>430</td>
<td>[44]</td>
</tr>
<tr>
<td>30.90</td>
<td>26.35</td>
<td>N.R</td>
<td>N.R</td>
<td>N.R</td>
<td>14.80</td>
<td>435</td>
<td>[45]</td>
</tr>
</tbody>
</table>

C/N: Carbon ratio Nitrogen. NR: No Reports. w.b: wet basis.
Fruits and food residues are transformed over time. Mature fruits with a higher carbohydrate content in their structure show a faster conversion to biogas compared to residues containing lipids [42]. However, the latter contributes to a higher biogas yield due to the length of the carbon chains, which slows down their degradation.

Other factors involved in the efficient development of biogas production are related to the C/N ratio. This relationship is very important to obtain maximum potential because it mitigates the inhibition processes in the AD. In turn, studies [2], [46] on this parameter report that the optimal relationship between these two variables is 20-30. However, the co-digestion studies reported in the literature that there are optimal relationships different from the established range taking into account the amount of food waste [47].

Studies of anaerobic co-digestion show that biogas yield increases when different materials are combined [42], [48]. Food residues can reduce undesirable substances by improving the C/N ratio and mitigating possible inhibitors such as volatile fatty acids (VFA) and ammonia.

The maximum biogas production was achieved by adding a percentage of the solid residues in the range 40-60% in the total mixture as a function of volatile solids (VS) [42]. However, Rongping et al [49] reported an optimum percentage in relation to food residues and dairy manure of approximately 85%. These authors claim that this ratio increased from 0.8 to 5.5 biogas production compared to dairy manure based on VS.

Co-digestion of lignocellulosic waste and food waste improves CH\textsubscript{4} production when used in low proportion. For example, Chiu and Lo [42] reported that CH\textsubscript{4} production increases 39.5% compared to AD of individual residues using a 5:1 ratio to food residues and wheat straw. While Pavi et al. [31] reported that using a 1:3 ratio to food residues and plant residues, have a biogas and CH\textsubscript{4} yield of 493.8 L/kg VS and 396.6 L/kg VS, with increases of the yield of 141% and 43.8%, respectively.

In contrast, other studies of co-digestion with another variety of substrates have a negative influence on methane yield. It is the case of excess in lignocellulosics with high C/N ratio, which results in a considerable drop in nitrogen. Therefore, the specific nutrient rate can be depleted and decrease the CH\textsubscript{4} yield. Cuetos et al. [50] found that the CH\textsubscript{4} production decreased by 53 to 61.9% when using co-digestion of food waste and slaughterhouse waste compared to control (without co-substrate).
Chapter 2. Biogas production from agroindustrial and industrial waste.

2.2.2. Municipal wastewater

Wastewater (WW) is a source of pollution constituents of human and/or animal metabolic wastes, which can be affects society, the environment and the planet in general. However, there are considerable gaps in treatment of WW. According to Tauseef et al. [51], of 1,500 km³ of WW, more than 80% is not collected or treated. WW treatment is an essential process for the global community. Current studies are looking for a reduction in the physico-chemical characteristics of the WW, in order to reduce the environmental impact. Additionally, the unification of a treatment strategy and a search for added value to this waste can increase the urban development.

Conventional AD technology for the treatment of WW is the anaerobic upflow sludge blanket (UASB). This reactor is used commercially for the treatment of industrial and domestic water. UASB reactor efficiency for removing organic components ranges from 60 to 70% [52]. This reactor solves the problems of the conventional completely mixed anaerobic digesters (CAD) treating municipal WW sludge, where CAD is characterized by relatively low organic solids and fewer efficiencies (<50%) [40].

According to Shen et al. [15] there are 1315 biogas plants from WW in the United States; of these 1054 plants use biogas beneficially for displacement and energy production. While 720 plants only produce electricity, 74 of them export this energy to the grid. Additionally, Deng et al. [37] reports that China had 2842 sewage treatment plants in 2010. These plants had an average production of 7.5 m³ of biogas/m³ of WW (humidity 96%). In general, industrial WW provide 71% of production.

The WW are different in origin and composition. Thus, the content of ST, SV, nitrogen, total carbon, among others, differs depending on the source. Studies about biogas production for a specific water is commonly found in the literature [27], [40], [53]. Then, domestic residual water or a specific type of WW has different characteristics and composition. As an example, the WW effluents have different total nitrogen concentrations ranging from 0.9-4.3 mg N/L [54].

Recent research conducted on WW slaughterhouse found a maximum biogas yield of 116.56 ml/min [53]. This had a CH₄ production of 2.21 L/kg total organic carbon (TOC) when the TOC was between 85.03% and 72.10%. As for biogas productivity in the treatment of lemon industry WW, Navarro et al. [55] reported a loading speed of 5 g/L.day and a biogas productivity of 0.87 L/L.day.
2.3. Biogas from lignocellulosic biomass

Lignocellulosic biomass has a complex polymer structure. It is composed of a heterogeneous complex of polymers cellulose, hemicellulose, and lignin, small amounts of pectin, protein, ashes, salt and minerals. Lignin is a hydrophobic compound and has aromatic characteristics. It is composed of phenylpropanol units and is very resistant to degradation [56]. Hemicellulose is a branched polymer which consists of different sugars, mainly arabinose, xylose, galactose, fructose, mannose, glucose, or glucuronic acid. The most common form of hemicellulose is xylan, which forms an important part of plant walls with 30-35% dry weight [57]. Cellulose is the most abundant constituent of the cell wall of the plant. This is a polymer of glucose by the binding of β-1,4-O-glucosidic bonds. The cellulose has a linear and fibrous structure that allows intermolecular bonds of hydrogen which makes it insoluble in water [57].

2.3.1. Pretreatments

The purpose of pretreatment is to reduce the crystallinity in the cellulose, increase the porosity of the material, and remove of lignin and hemicellulose, in order to increase the surface area and to have more active sites in the AD [58]. Pre-treatment of raw material is important in the AD for to improve methane yields. This increases the biodegradable components used as substrates for the microorganisms [59]. However, pretreatment depends on the physico-chemical characteristics of the lignocellulosic material. These treatments have advantages and disadvantages in their application causing various effects when evaluating different substrates. In this context, mechanical, thermal, biological and chemical treatments have a positive effect on the production of CH₄/biogas. These treatments increase production by 25-60%, 4-46%, 37% and 30-50% respectively [60].

Factors to be taken into account in the choice of pretreatment include the yields and the advantages in its economic efficiency. A comparison of the pretreatment influence on the biogas production is reported by Li et al. [2]. This study showed a 48.5% increase in yield when maize stubble was treated with 6% NaOH compared to untreated maize stubble. The same author indicates that CH₄ yield increased by 88% when the rice straw was previously treated with H₂O₂. However, Rodriguez et al. [60] informed that pretreatments with microwaves decrease the biogas production by 13%. Further studies referring to pretreatment are available in the literature [61]–[63].

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Chapter 2. Biogas production from agroindustrial and industrial waste.

2.3.2. Agricultural crops

Crops for biogas production are common in Europe where subsidies by governments favor the use of renewable energy. In the European Union they are subsidized at values about $69/ha [64]. In Germany, more than 80% of the raw materials come from agriculture and animal manure [25]. However, generalized concerns have arisen over the use of crops for biogas production as it can affect food security. Current researchers Pacetti et al. [65] and Senghor et al. [66] evaluate the environmental quality of related transformation processes, the water footprint, life cycle analysis and CO₂ emissions.

The search for a sustainable energy using agriculture wastes is a main issue for the present century. The characteristics of each crop play an elementary role. For example, the net energy yield per hectare of a crop is an important parameter in the biogas production. Energy potential for different crops is illustrated in Table 2-3.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Crop yield (t FM/ha)</th>
<th>Biogas yield (Nm³/(t VS))</th>
<th>CH₄ content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet</td>
<td>40–70</td>
<td>730–770</td>
<td>53</td>
</tr>
<tr>
<td>Fodder beet</td>
<td>80–120</td>
<td>750–800</td>
<td>53</td>
</tr>
<tr>
<td>Maize</td>
<td>40–60</td>
<td>560–650</td>
<td>52</td>
</tr>
<tr>
<td>Corn cob mix</td>
<td>10–15</td>
<td>660–680</td>
<td>53</td>
</tr>
<tr>
<td>Wheat</td>
<td>30–50</td>
<td>650–700</td>
<td>54</td>
</tr>
<tr>
<td>Triticale</td>
<td>28–33</td>
<td>590–620</td>
<td>54</td>
</tr>
<tr>
<td>Sorghum</td>
<td>40–80</td>
<td>520–580</td>
<td>55</td>
</tr>
<tr>
<td>Grass</td>
<td>22–31</td>
<td>530–600</td>
<td>54</td>
</tr>
<tr>
<td>Red clover</td>
<td>17–25</td>
<td>530–620</td>
<td>56</td>
</tr>
<tr>
<td>Sunflower</td>
<td>31–42</td>
<td>420–540</td>
<td>55</td>
</tr>
<tr>
<td>Wheat grain</td>
<td>6–10</td>
<td>700–750</td>
<td>53</td>
</tr>
<tr>
<td>Rye grain</td>
<td>4–7</td>
<td>560–780</td>
<td>53</td>
</tr>
</tbody>
</table>
Nutrients in the crop, harvesting time and planting technology directly affect the yield. Weiland (2010b) reported that maize harvested after 97 days of vegetation has a higher yield compared to the crop harvested at full maturity (37% higher). Likewise, Amon et al. [67] confirmed that CH$_4$ production decreases with maturity of maize and that at later maturity a maximum biogas yield per hectare of 7100-9000 Nm$^3$/ha was obtained. Additionally, the authors reported the corn cob alone and the corn mixture decrease the yield by 43-70% compared to the corn grain alone.

From a techno-economic point of view, Markou et al. [68] evaluated the biogas production in Greece from 7 raw materials (triticale, maize, alfalfa, sunflower, clover, barley and wheat). According to the authors, these studies affirm that triticale was the most competitive energy crop under selected climatic conditions for northern Greece. However, maize shows a higher yield of biomass and biogas potential than triticale in technical assessment. Therefore, the use of cereal-based farmland such as maize and triticale present potential raw materials to achieve positive environmental and energy balances. Another study Luca et al. [69] compared the giant cane and maize for biogas production. The investigation concluded that the productivity of giant cane is lower than maize due to CH$_4$ production per hectare cultivated. The giant cane has better results (12,292 Nm$^3$/ha).

The acceptability of these raw materials for biogas production changes continuously due to renewable energy policies and constant research. However, there are barriers to the utilization of these raw materials due to mainly to that can have noble proposes than biogas (e.g. animal food) and the low costs of other raw materials. Economic incentives then perform an important role in making this option more viable. The selection of crop taking into account collection and transport logistics, energy viability, environmental impact, production costs and high biomass yield are an important focus for the biogas plants with a good economic margin [70].

2.3.3. Crop residues

Agricultural residues such as corn stover, grass, rice straw, wheat straw and waste sunflower are promising raw materials for AD. Comparative advantages over other raw materials are known, such as potential biogas yields and low costs. However, waste generation depends on the crop, the sowing intensity and productivity.
In this context, millions of waste are wasted worldwide without consideration of their added value. A mathematical model to predict the total crop residues and manure available realized by Einarsson and Persson [71] found that there are nearly 200 million dry metric tons per year in Europe. Residues such as straw represent approximately 800 million dollars (ton/year) of crop residues in China; where rice straw represents 32.2% of total production [72]. In India, the main contributors to agricultural waste are cereal crops, rice and wheat [73]. However, the pretreatment of crops perform an important role and affects the final yields of methane. A comparison based on methane production from promising wastes is presented in Table 2-4.

Table 2-4. Methane production from different crop residues.

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Pretreatment</th>
<th>HTR (days)</th>
<th>Temp. (°C)</th>
<th>CH₄ Yield (L CH₄/kg SV)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice straw</td>
<td>Cut (3-5 mm).</td>
<td>120</td>
<td>22</td>
<td>280.0</td>
<td>[76]</td>
</tr>
<tr>
<td></td>
<td>Cut/pre-digested with biogas sludge for 46 h</td>
<td>146</td>
<td>26-28</td>
<td>273.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2% NH₃</td>
<td>24</td>
<td>35</td>
<td>190.0</td>
<td></td>
</tr>
<tr>
<td>Wheat straw</td>
<td>4% NaOH (120 h)</td>
<td>60</td>
<td>37</td>
<td>165.9</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>Hydrothermal (200 °C, 10 min) + 5% NaOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Without pretreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley straw</td>
<td>Mechanical (particle size 0.5 cm)</td>
<td>NA</td>
<td>40</td>
<td>370.0</td>
<td>[78]</td>
</tr>
<tr>
<td>Maize stalk</td>
<td>Thermal (120 °C, 30 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mechanical (particle size 0.2 cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cassava</td>
<td>Biological</td>
<td>NA</td>
<td>55</td>
<td>260.0</td>
<td></td>
</tr>
<tr>
<td>Corn cob</td>
<td>Fast extrusion (FE)</td>
<td>32</td>
<td>35</td>
<td>270.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaOH + Fast extrusion (A+FE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ultraflo L hydrolysis (H)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A+FE+H</td>
<td></td>
<td></td>
<td></td>
<td>[79]</td>
</tr>
</tbody>
</table>
Chapter 2. Biogas production from agroindustrial and industrial waste.

Another type of waste are contaminated crops that are a source of environmental problems. Tian and Zhang [74] found that the production of biogas from cadmium contaminated corn (27-63 L/kg VS) is higher compared to sweet sorghum (19-58 L/kg VS) contaminated with the same compound. In the same way, Jekayinfa and Scholz [75] investigated the waste as tuber cassava, cassava peels, palm kernel shell and palm kernel cake. The results of the study claim that biogas yields were 660, 660, 580, and 80 (L/kg VS), respectively, after 30 days of digestion time.

2.4. Biogas from animals manure

2.4.1. Livestock manure

World production of biogas from livestock manure is approximate 28% of obtaining renewable energy [80]. In countries such as Denmark, 80% of the potential to produce biogas is based in manure [81]. For example, a kilogram of manure can produce from 0.26 to 0.28 m³ of biogas, containing 50-60% CH₄. However, biogas production from manure has some disadvantages such as mismanagement causes odors, insect attraction, and water pollution. Additionally, since the amount of manure produced by an animal digestion differs in characteristics, it can affect the biogas produced.

From the standpoint of performance, dairy manure in AD suffers from low biogas production per mass unit, therefore it is expensive per volume unit. This causes countries like the United States to not accept the AD of this raw material because the costs of producing 1 kWh of energy (0.05-0.5 $/kWh) is less economically competitive than traditional energy (0.09 $/kWh) [82]. However, this is an approximate cost because that in biogas production lead to lower cost of treatment and disposal of manure.

The economic limitations have stimulated the search to increase the biogas productivity. Methods for increasing biogas productivity by co-digestion and improving methane quality by eliminating compounds as H₂S have been studied. When dealing with animal waste the production of intermediate compounds (H₂S) affects the quality of the methane produced. However, researchers are currently investigating the elimination of this compound in biogas generation from livestock manure are carried out Liang and Liang [83]; Sun et al. [84]). According to Macias-Corral et al. [85], CH₄ content increases in the co-digestion of two substrates, approaching about 70%. Similarly, Atandi and Rahman [82] reported that the co-
digestion of cattle manure cake with mustard increases 63% in the biogas production compared to a single substrate. Another study Takeuchi et al. [86] investigated a centralized biogas plant from three types of co-substrates (WW, crop residues and cow manure) found that the CH$_4$ yield was 354 L/kg VS, representing 67% more than a plant operating with higher content of manure feedstock.

2.4.2. Pig manure

Pig waste is produced in large quantity around the world. It causes serious environment problems due its high chemical oxygen, suspended solids and phosphorus compounds [87]. The unified treatment of this residue is complex and there are shortcomings in disposal methods [88].

AD has potential for the treatment of pig manure reducing emissions of organic compounds and odor control. In general, pig manure has better production of biogas compared to other animals waste due to its moisture content (96%) and easy biodegradability [88], [89]. Approximately 500 to 600 L/kg of waste can be produced from solid pig manure [30].

The AD from pig manure is widely used on small farms for waste treatment. A study by Thu et al. [90] in Vietnam reported that farmers know the benefits of biogas, but the lack of money impede from installation. However, benefit studies focusing on electricity generation from this fuel were investigated by Chang [91]. Benefits estimated at pig farms with more than 1,000 pigs in Taiwan generate 2.67 Kwh of electricity per year (Electricity charge saved: US $26.7 million per year).

To increase the yield on biogas production, different technologies have been studied Hidalgo et al. [92] different reactor configurations, types of co-substrates, temperature, among others. However, researches have focused on studying the mesophylls conditions both for conventional digestion and AD with co-digestion.

2.4.3. Chicken manure

The possibility to use chicken waste for energy in biogas form exists since the chicken manure can be fresh and used almost without energy losses. These residues are characterized by VS 20-25%, and a higher fraction of biodegradable material with high
content of organic nitrogen and low carbon [89]. Historically, the conversion of these residues to biogas is challenging since this has certain obstacles: substrate heterogeneity, high solids substrate (> 25% TS) and inhibition on the methanogenic stage due to nitrogen concentrations (3 -5% dry weight) [93].

The major barrier in the AD of chicken manure is the excessive nitrogen amount. Based on this, this parameter is critical in the conversion to biogas. Implementation of co-digestion at low organic loads of manure is a possible solution to this problem. Recent studies (Dalkılıç and Uğurlu [94]) suggest that organic load with less than 3% in TS could give high biogas yields (459-551 L/kg VS). While studies of Bayrakdar et al.[95] about the co-digestion of chicken manure and crop residues have a yield of 360 L/kg VS when the concentrations of ammoniacal nitrogen are below 4000 and 400 ppm.

Others studies addressed the importance of the organic loading rate for CH₄ yield. Thus, Mei et al. [96] evaluated an organic load in the co-digestion of rice straw and chicken manure in the range 3-8 kg/m³ VS.day. The study established an optimal load 4.8 kg VS/m³.day. In this condition, the biogas yield was 380 L/kg VS and volumetric biogas production rate (VBPR) of 1.8 m³/m³.day. Another study of chicken manure co-digestion and corn silage without dilution with water was carried out by Sun et al. [97]. The research results showed a specific production of 309 L CH₄ /kg VS. This research showed that adding manure concentrations above 30% is critical for the digester in nitrogen content.

Improvements in AD of chicken manure are frequently investigated (Sun et al. [97]; Weerayutsil et al. [98]; Braeutigam et al. [99]) in order to implement these systems in farms. Complementary technology assessments have been launched by recent research. Here, Braeutigam et al. [99] have apply ultrasound to improve the production of biogas. This study concluded that the biogas production and CH₄ content could be increased up to 41%. Finally, a review of biogas production from different manure sources is presented in the Table 2-5.

From the table it can be concluded that the individual yield is much higher for pig manure than cow manure. For chicken manure, there aren’t many studies as a point of comparison. However, it can be concluded that the highest yields occur when carrying the AD with a co-substrate. It is predicted that the structural features of the substrate are more suitable for the microorganisms. This statement is strengthened by other co-digestion studies Macias-Corrал et al. [85]; Takeuchi et al. [86]; Yamashiro et al. [100]; Li et al. [101]. Considering the results of these studies co-digestion improves the yield of biogas production.
Chapter 2. Biogas production from agroindustrial and industrial waste.

Table 2-5. Biogas production from waste manure: livestock, pig and chicken. Adapted from Ismail et al. [89].

<table>
<thead>
<tr>
<th>Reactor type and volume</th>
<th>Feed</th>
<th>Temp. (°C)</th>
<th>HRT (days)</th>
<th>CH₄ yield (L CH₄/kg VS)</th>
<th>Biogas yield (L/kg VS)</th>
<th>% CH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch (10 L)</td>
<td>CM</td>
<td>53</td>
<td>17</td>
<td>184</td>
<td>259</td>
<td>65</td>
</tr>
<tr>
<td>Batch</td>
<td>CM slurry</td>
<td>21–23</td>
<td>14</td>
<td>NR</td>
<td>103</td>
<td>55</td>
</tr>
<tr>
<td>Batch (1 L)</td>
<td>Dairy manure</td>
<td>37</td>
<td>NR</td>
<td>166</td>
<td>245</td>
<td>NR</td>
</tr>
<tr>
<td>Batch</td>
<td>CM + MM</td>
<td>37</td>
<td>NR</td>
<td>168</td>
<td>303</td>
<td>56</td>
</tr>
<tr>
<td>Batch (128 L)</td>
<td>CM + whey mix</td>
<td>35</td>
<td>56</td>
<td>211</td>
<td>423</td>
<td>51.4</td>
</tr>
<tr>
<td>Batch (1 L)</td>
<td>CM + KW</td>
<td>35</td>
<td>12</td>
<td>297</td>
<td>NR</td>
<td>50.9</td>
</tr>
<tr>
<td>Stirred batch (565 L)</td>
<td>PM</td>
<td>35</td>
<td>60</td>
<td>176</td>
<td>NR</td>
<td>50</td>
</tr>
<tr>
<td>Non-stirred batch (245 L)</td>
<td>PM</td>
<td>35</td>
<td>60</td>
<td>165</td>
<td>NR</td>
<td>45</td>
</tr>
<tr>
<td>PFR (250 L)</td>
<td>SM</td>
<td>26</td>
<td>40</td>
<td>290</td>
<td>NR</td>
<td>70</td>
</tr>
<tr>
<td>PFR (250 L)</td>
<td>SM + CG</td>
<td>26</td>
<td>40</td>
<td>310</td>
<td>NR</td>
<td>54</td>
</tr>
<tr>
<td>Batch (225 L)</td>
<td>SM + water</td>
<td>22.6</td>
<td>80</td>
<td>NR</td>
<td>207</td>
<td>22</td>
</tr>
<tr>
<td>Batch (225 L)</td>
<td>SM + urine</td>
<td>32.5</td>
<td>80</td>
<td>NR</td>
<td>262</td>
<td>49</td>
</tr>
<tr>
<td>CSTR (9 L)</td>
<td>SM + CM</td>
<td>55</td>
<td>15</td>
<td>307</td>
<td>NR</td>
<td>70</td>
</tr>
<tr>
<td>Batch (0.5 L)</td>
<td>Treated CHM</td>
<td>55</td>
<td>10</td>
<td>195</td>
<td>NR</td>
<td>67</td>
</tr>
<tr>
<td>Batch (0.125 L)</td>
<td>CHM + SM</td>
<td>35</td>
<td>113</td>
<td>130</td>
<td>200</td>
<td>NR</td>
</tr>
<tr>
<td>Batch (0.125 L)</td>
<td>CHM +</td>
<td>55</td>
<td>55</td>
<td>103</td>
<td>NR</td>
<td>55 (55°C)</td>
</tr>
<tr>
<td>Batch (0.125 L)</td>
<td>Seed sludge</td>
<td>37</td>
<td>55</td>
<td>31</td>
<td>NR</td>
<td>30 (37°C)</td>
</tr>
</tbody>
</table>

CM, cattle manure; KW, kitchen waste; SM, swine manure; PM, pig manure; CHM, chicken manure; MM, mink manure; CG, cooking grease; PFR, plug flow reactor; CSTR, continuous stirred tank reactor; HRT, hydraulic retention time; NR, not reported; Temp., temperature; VS, volatile solids.

2.4.4. Other animal manure residues

There are several possibilities for the use of animal waste such as sheep, horses, buffaloes, elephants, among others. African countries can assess the performance and production of methane with respect to elephants’ fecal matter. Specific places like horse farms can implement energy harvesting systems for small-scale AD on their own.
In this context, research in the production of biogas from various animal fecal waste are presented Liu and Liu [102]; Phetyim et al. [103]. Therefore, the yields estimated in biogas production are presented in Table 2-6.

Table 2-6. Biogas production from manure. Adapted from Department of Alternative Energy Department and Efficiency [104].

<table>
<thead>
<tr>
<th>Type</th>
<th>Biogas production (L/kg VS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>307</td>
</tr>
<tr>
<td>Buffalo</td>
<td>286</td>
</tr>
<tr>
<td>Pig</td>
<td>217</td>
</tr>
<tr>
<td>Chicken</td>
<td>242</td>
</tr>
<tr>
<td>Duck</td>
<td>310</td>
</tr>
<tr>
<td>Elephant</td>
<td>241</td>
</tr>
</tbody>
</table>

2.5. Biogas from other raw materials

2.5.1. Algae

Algae are cataloged as third-generation biomass. It can be divided into two large groups: microalgae and macroalgae. The first are composed mostly of cellulose and hemicellulose, the rest are proteins (50-60%) and lipids (20-30%) [59]. The second group has a structure similar to that of terrestrial plants.

Microalgae can reach 2-20 times the production potential of energy crops and algae can double their biomass within 24 h [78]. Furthermore, compared to terrestrial crops these are low in polymeric complex responsible for incomplete hydrolysis and have a lesser pretreatment intensity [59]. The pretreatment methods for algae can be reviewed in the investigation by Jankowska et al. [105]. Table 2-7 shows a comparison of CH₄ yield from different species of algae.
Chapter 2. Biogas production from agroindustrial and industrial waste.

Table 2-7. Methane production at different algal species.

<table>
<thead>
<tr>
<th>Algae</th>
<th>Temp. (°C)</th>
<th>HTR (days)</th>
<th>CH₄ yield (L CH₄/kg SV)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uvla</td>
<td>35</td>
<td>15</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Laminaria hyperborea</td>
<td>35</td>
<td>24</td>
<td>280</td>
<td>[78]</td>
</tr>
<tr>
<td>Laminaria sp.</td>
<td>35</td>
<td>22</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>35</td>
<td>35</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Chlorella sp. + CM</td>
<td>35</td>
<td>35</td>
<td>170</td>
<td>[106]</td>
</tr>
<tr>
<td>L. Japonica</td>
<td>35</td>
<td>6-20</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>L. Japonica</td>
<td>35</td>
<td>9-30</td>
<td>240-300</td>
<td>[107]</td>
</tr>
<tr>
<td>L. Saccharina</td>
<td>35</td>
<td>24</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>L. hyperborea</td>
<td>35</td>
<td>24</td>
<td>280</td>
<td></td>
</tr>
</tbody>
</table>

* CM: Cattle manure.

The AD from algae is restricted because of certain disadvantages compared to other raw materials. These disadvantages include the limited load of organic fraction, variation in nutrient content, low C/N and costs growing algal biomass and harvesting. For this reason, microalgae biomass is restricted and require sophisticated technologies for conversion into biogas, resulting in a low economic benefits of the process [30], [78]. However, economic incentives for CH₄ production from microalgae it's necessary for a competitive process [105].

On other hand, biorefinery concept together with AD is a promising option to improve the economic feasibility of the microalgae processes. The above translates into a profit, due to that the different fractions of microalgae that come out, as byproducts of the processes can be generation greater energy recovery and reduction of costs growing algal [108].

2.5.2. Industrial waste

Industrial waste is a source of substrates for biogas production. In general liquid and solid waste with favorable organic nitrogen content loads may have potential in biogas production. In section 2.2 some potential production of biogas is illustrated from industrial WW. However, this topic is expanded in the present section.
AD studies with dairy WW have typical operating conditions. In the case, technologies such as upflow anaerobic sludge bed reactors (UASBR), hybrid UASBRs, expanded granular sludge bed reactors (EGSBR), and anaerobic filters are used [109]. Lobato et al. [110] evaluated the biogas production from pig manure and residual glycerin. Researchers noted an increase in the CH₄ production from 2.1 to 5.4 L/day when applied glycerin of 2.5% (v/v) and 8% (v/v) respectively. Similarly, Paixão et al. [111] evaluated residues from the manufacture of cassava flour where the biogas production was 14.5 L/day with 80% CH₄.

The biogas production from the palm oil industry for long-term energy planning show that its power generation is competitive with other forms of electricity generation [112]. Future research focuses on the use of industrial waste to obtain added value product in different production lines. Among the products that can be obtained from the mucilage, the biogas and bioethanol are the most promising. In this sense, Zambrano et al. [113] reported that from the anaerobic decomposition of the mucilage, an equivalent of 336 L of CH₄ per kg of COD can be produced.

### 2.6. Production technologies

Biodigesters are generally cylindrical insulated containers made of reinforced concrete, steel or fiberglass, and a hermetic cover which traps gases. The mixture can implement a mechanical system, with a recirculation pump liquid as completely mixed CSTR [27].

Varol & Ugurlu [114] show the livestock manure in HR degrades more efficiently than in a CSTR methane single stage. Moreover, Romero-Cedillo et al. [115] showed that efficiency in a two stage process is higher. The researchers compared the conventional process versus the two-step process (hydrolysis-stage and methanogenesis-step). They confirmed 65% more potential energy, which can be attributed to metabolites produced in the hydrolysis-stage that can be assimilated in the methanogenesis-phase.

The wet fermentation requires specific conditions in the reactor, where one of the most utilized is a vertical CSTR (more than 90% of current biogas plants in Germany). Other reactors are also used, including UASB, EGSB, FBR and AnMBR. However, these reactors are inadequate to handle solids, even if used in a multistage system that can be operated with high solids content. Mustafa et al. [40] developed an anaerobic fluidized bed reactor (AnFBR) with zeolite for stillage resulting in yields with TS of 60 g/L and 3.5-day hydraulic retention time (HRT).
The KSAMBR reactors are utilized on a large scale in the food industry, the advantage of this reactor is its ensure stability and good CH₄ performance. It’s suitable for handling food waste with high nitrogen content. Moreover, membrane reactors are useful for separating AD inhibitors, reducing the washed biomass. They have disadvantages such as same membrane fouling, maintenance and operating costs.

Plug flow digesters are long and narrow vessels (5:1 ratio respectively), that have the advantage of operating at mesophilic and thermophilic temperature. Additionally, they don’t have agitation because operating load is 11-14% in TS, and they have a retention time of 15-20 days. PBR are another type of anaerobic reactors, which are most commonly used to treat WW. They can also handle short holding times (3-5 days) and the load has a low TS concentration [27].

New reactor technologies have been implemented on the market, such as the SS-AD DRANCO, system developed and marketed by two companies in Europe (Valorga and Kompogas). It is the most widely used reactor technology to treat solid urban waste, kitchen waste and yard waste. More than 75 enterprises have implemented this technology and 24 of these technologies have had a life-time of 10 years. Such reactors are based on a variability between 20-40% of the TS content, operated in a mesophilic stage with thermophilic conditions. Thermophilic conditions yields are much lower when operating reactors in continuous batches.

In Europe, 90% of the plants at large-scale operate with a single stage in anaerobic digesters. ABR reactors enable treatment of waste in a short time; also tubular reactors are significant because they can separate into two steps: acidogenic and methanogenic [116]. The organic loading rate is important in this type of reactors because influence the performance and design of AD plants on a large scale. These reactors have high yields of biogas and methane content (0.78 Nm³/kg VS, methane yield of 0.43 Nm³/kg VS from a load of 35 kg/day with a retention time of 27 days [35]).
2.7. Comparison of the main types of raw materials

2.7.1. Productivity

The origin of feedstocks is relevant to evaluate the biogas production. The global benchmarks constitute a way to predict the potential for resource exploitation in specific regions. Comparing regions, biogas production is governed by energy crops in European countries. However, the possibility of using energy crops without government incentives leads to a bottleneck in less developed countries. In contrast, countries with large rural extension seek to resolve the energy deficit using raw materials of animal origin.

From another point of view, the co-digestion of raw materials can represent an output in specific regions that don’t have a stable sector of material to produce biogas. According to researchers Pavi et al. [31]; Zhang et al. [47]; Rongping et al. [49], the combination of substrates for the biogas production is a key option to increase productivity. The increase in productivity is evident when lignocellulose biomass and animal manure are combined. This improves the C/N, a critical factor for suitable performance in the AD. Recent research on the biogas production from different raw materials is presented in Table 2-8.

To achieve high productivity in biogas plants, the pretreatment of the raw material is an important factor. The biogas yield is influenced directly by this stage, where the technology and substrate types for pretreatment affect the process economy.

In this sense, the waste of animal manure, WW and some industrial wastes do not require rigorous treatment for higher productivity. However, the lignocellulosic biomass requires a pretreatment due to its complex structure in terms of cellulose and hemicellulose.

Technologies of AD applied an industrial WW are the UASB and CSTR reactors. However, the technology to be applied to depend on amount raw material. For example, the German technology in biogas production from energy crops differs of the Denmark technology used in biogas production from animal manure. Therefore, a poor choice in technology affects biogas productivity.
### Chapter 2. Biogas production from agroindustrial and industrial waste.

#### Table 2-8. Biogas production from different feedstocks.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Raw material</th>
<th>AD Conditions</th>
<th>Yield (L biogas/ kg SV)</th>
<th>% CH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>[117]</td>
<td>Livestock Manure and waste kitchen: VS (61.96 %w)</td>
<td>Volume: 128 L. Temperature: 35°C. Time: 12</td>
<td>297</td>
<td>50.9</td>
</tr>
<tr>
<td>[118]</td>
<td>Mink manure and livestock manure: TS (1.8 % w)</td>
<td>Volume: 5L. Inoculate 25%. Temperature: 37 °C.</td>
<td>303</td>
<td>56</td>
</tr>
<tr>
<td>[119]</td>
<td>Pig manure: TS (26.38±0.77 % w); VS (20.82±0.85 %w)</td>
<td>Volume: 4L. Inoculate 30%. Temperature: 35 °C. Time: 22</td>
<td>207.1</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Corn cob: TS (89.01±1.29 %w); VS (87.41±0.95 %w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge: TS (8.72 ± 0.92 %w); VS (5.24 ± 0.18 %w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[120]</td>
<td>Livestock waste: VS (78 %w)</td>
<td>Volume: 10L. Temperature: 53 °C. Time: 17</td>
<td>159</td>
<td>65</td>
</tr>
<tr>
<td>[121]</td>
<td>Chicken manure: TS (25 %)</td>
<td>Volume: 0.5 L. Temperature: 55 °C. Time: 10</td>
<td>NR</td>
<td>67</td>
</tr>
<tr>
<td>[123]</td>
<td>Pig manure + Used cooking grease: VS (95.4 %w)</td>
<td>Volume: 250L. Temperature: 23 °C. Time: 40</td>
<td>67.3*</td>
<td>70</td>
</tr>
<tr>
<td>[124]</td>
<td>Pig manure: TS (27.16 % w); VS (20.12 %w)</td>
<td>Volume: 2L. Temperature: 37 °C. Ratio of raw material: 0.4: 1.6: 1</td>
<td>674.4</td>
<td>50-70</td>
</tr>
<tr>
<td></td>
<td>Rice straw: TS (93.72 %w); VS (83.18 %w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kitchen waste: TS (20.23 % w); VS (18.16 %w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[125]</td>
<td>Livestock manure: TS (16.2 %w); VS (12.5 %w)</td>
<td></td>
<td>81**</td>
<td>47.8</td>
</tr>
<tr>
<td></td>
<td>Llama manure: TS (58.3 %w); VS (34.4 %w)</td>
<td></td>
<td>101**</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>Sheep manure: TS (67.6 %w); VS (50.3 %w)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>Sheep manure + Livestock manure (50:50)</td>
<td></td>
<td>152**</td>
<td>44.8</td>
</tr>
<tr>
<td></td>
<td>Llama Manure+ Sheep manure (50:50)</td>
<td></td>
<td>51**</td>
<td>45.6</td>
</tr>
<tr>
<td>[126]</td>
<td>Effluents from the palm oil plant (12-68 g/L)</td>
<td>Volume: 40 L. Temperature: 30-35 °C. Time: 67</td>
<td>NR</td>
<td>41</td>
</tr>
</tbody>
</table>

VS: Volatile solids. TS: Total Solids. Time: (days). NR: Not reported. * L/day ** L CH₄/kg VS.day
2.7.2. Quality

Energy crop growing techniques and harvest time influence the quality of the substrate. For example, lignocellulosic waste can change the physicochemical properties with agro-ecological differences. Animal manure is unique to each animal due to the differences in the metabolism. Similarly with the algae, where each species has unique physicochemical characteristics.

Consequently, raw materials of the same type have differences. Animal manure, food waste, waste crops are clear examples. Thus, chicken manure compared to other types of manure contains an excessive amount of nitrogen, which causes a decrease in pH and affects AD. The characteristics of grass differ from each other. Wheat straw has greater potential in biogas production that the maize stalk with a mechanical pre-treatment (Table 2-3). Food waste like fruits contains a high amount of simple sugars, which facilitates degradation by microorganisms. However, this feature increases the production of CO₂ and hence decreases the methane content.

The quality of the raw material can also be assessed by the biogas quality. A high percentage of CH₄ indicates good quality. Accordingly, when analyzing the recent studies (Table 2-8), the highest percentage of CH₄ was obtained for rice straw and co-digestion (manure, anaerobic sludge and corn cob). These have a yield of CH₄ of 92% and 80% respectively. However, accessing the quality of raw materials is essential to individual studies as there are many factors that affect the production.

2.7.3. Availability

The differences between the various evaluated raw materials lead to the search of opportunity in the biogas production. The AD from crops is viable as there are energy subsidies, as in the case of corn grown in Germany. In contrast, the biogas cost from this feedstock is non-competitive respect the conventional energy.

Opportunities for the biogas production from manure in big cities focus on the possibility of centralizing the production facilities of the waste. A good example is the economy of Denmark that focuses on centralized biogas plant manure. However, its viability decreases if it does not have an integrated co-digestion system to compensate for the deficit in the C/N ratio.
Likewise, the exploitation of manure from cows and pigs by rural populations are focused on the African and Asian continent. This idea involves small-scale production. It is a possibility for family clusters to take advantage of the energy it produces for heating and power generation. However, restrictions on initial investment for technology prevent free exploitation of this resource.

The transformation of municipal solid waste, food waste and WW exist several centuries ago. However, in some case the production systems is affected by the difference in the waste characteristics. The optimization of these systems is carried out with advances in technology and energy efficiency.

Lignocellulosic residues are influenced by different logistic systems and feedstock disposition. This can cause high investment costs and supply problems. The viability of these wastes can be improved with the co-digestion of raw materials already mentioned. Finally, the AD by algae is a promising possibility for the future. However, there is not enough technological advancement to compete with other types of energy. The feasibility of biogas production from this raw material is very low at present.

In conclusion, to determine the viability of a certain type of raw material, specific logistic, techno-economic and environmental studies are required in each region. In turn, the integration of this product as part of a biorefinery with energy supply chains and value-added products has potential. In general, these aspects should take into account to determine the feasibility of the AD of a feedstock in particular.

2.8. Market, production scale and natural gas

2.8.1. Market

The largest producer of biogas in the world is Europe with 60%, followed by North America (22.0%), Asia-Pacific (11%), Latin America (6%), Middle East and Africa (1.0 %). In 2016, Europe produced 16,093.6 ktoe/year in terms of primary energy through biogas. Germany is the largest producer in Europe representative with a production up to 7,956.3 ktoe/year, followed by the UK with 2406.9 ktoe/year, Italy (2,028.9 ktoe/year), Zzech Republic (601 ktoe/year) and France (579.6 ktoe/year) [14]. In Europe they operate more than 17,560 biogas plants with a biogas electricity production of 62.5 TWh [14]. Where 63% of the plants
installed are from German (plants responsible for AD of organic raw materials and mainly of CH4 and CO2). Per the UNU, Europe will have built more than 100,000 plants by 2025. As in the rest of the world, in the EU biogas production is intended for power generation, cell cogeneration or fuel, in several generations of heat, steam, electricity and refrigeration industry, injection into the gas networks, transport fuel, and the production of chemicals [30]. The use of this alternative energy source can save natural gas, oil consumption and reduce ambient air pollution [80].

The biogas market is influenced by growth in the transport sector and the energy requirements of today's society. Worldwide, 25 million households get their energy for lighting and cooking from biogas, including 20 million households in China and 3.9 million in India. According to EurObserv’ER [14], in Europe about 16 million tonnes oil equivalent (Mtoe) of biogas primary energy were produced in 2016. A description of biogas production between 2015 and 2016 in Europe is presented in Figure 2-2.

**Figure 2-2.** Primary production of renewable energy by biogas (in ktoe). Data from EurObserv’ER [127].
Biofuels represent 3.3% of the total fuel used in road transport. In the European Union, the use of CH$_4$ in the transport sector is distributed mainly in Sweden and Switzerland [25]. Asia and the Pacific have low proportions of biofuels (below 1%), Argentina 5.3% and the largest consumer is Brazil, with a consumption of 18.13% [128].

The technology for power generation in gas engines (45% efficiency) are dual fuel engines. Additionally, fuel cells are a new class of technologies with high efficiency in generating electricity and in reducing harmful emissions [30]. In this sense, biogas production will have a striking value chain for the following years. The above oriented on the new developments of use in technological sectors such as biomethanization for a transport and injection grilles. In Europe it is expected that by 2030, the CH$_4$ production will increase from 2.7-3.7% respect to 2016 [14]. Furthermore, the diversification of policies and new biogas applications make this prediction can be carried out. France with its new advanced legislation regarding the biogas applications. Sweden, born leader in the biogas use in vehicles. The market opening of UK respect to the export of biomethane to Nederland and finally, the German power regarding its technological diversification and the commitment to upgrading.

### 2.8.2. Production scale

The scale is important when starting a project. This is directly related to investment and maintenance costs. The higher the scale of a plant investment, the higher the costs. However, Net Present Value (NPV) and production costs are sensitive to the scale of the process due to energy consumption and the depreciation of equipment [129], [130].

The biogas production scale is limited by the availability of raw materials. Energy crops have a limited extension for planting. Animal manure and sewage are available in specific places and sufficient amounts of food waste are only found in big cities. Pig manure from rural farms has higher productivity if it operates on a small scale compared to energy crops, which can be used on a large scale [37]. Therefore, to find a viable scale for AD of organic materials, it is necessary to study individual cases separately, taking into account the logistics and resources available.
2.8.3. Biogas and natural gas

Countries with the highest production of natural gas in the period 2016 are the United States, Russia, Iran, Saudi Arabia, China and Japan with an average of 2,000 billion cubic meters per year (56.37% of world production) [131]. The same year, the world consumption of natural gas was 3542.9 billion of meters cubic per year with an increases of 1.5% respect to 2015 year [131]. Countries with the highest consumption of natural gas in the period 2016 are the United States, Russia, Iran, Qatar, Canada and China with an average of 1,800 billion cubic meters per year (50.8% of world consumption). The natural gas has significant advantages over other kinds of fuel as it is found freely in nature and it does not need technologies for purification. Unlike natural gas, AD needs organic matter transformation and biogas purification.

A comparison between the two energy sources is established by the calorific power. The heating value of natural gas is higher compared to biogas, 9800 kcal/m$^3$ and 5300 kcal/m$^3$ respectively [80]. This difference is due to CH$_4$ content. Natural gas has a CH$_4$ content of more than 80%, while biogas has a content of 50-70%. Additionally, natural gas has not contaminants, while biogas contains hydrogen sulphide and carbon dioxide.

Despite the disadvantages of biogas compared to natural gas, it can achieve almost the same energy efficiency. The biogas can have a high CH$_4$ content (>95%) when removing contaminant compounds such as CO$_2$ and H$_2$S. This percentage is the ideal for various gas appliances to function properly [25]. Other contaminant compounds such as siloxanes, halogenated carbon hydrates and NH$_3$ depend on the biogas source. These compounds are generally produced when the biogas comes from landfills or municipal waste [30].

Due to these problems, the use of biogas is restricted to injection into the natural gas grid or as fuel for vehicles. For the utilization of gas it is necessary to perform a purification process to remove contaminants. Technologies such as water scrubbing, activated carbon, and molecular sieves are common in removing CO$_2$ [25]. Membrane separation, biological filtration and activated carbon adsorption are applied to remove H$_2$S. Finally, adsorption on silica gel and activated carbon is commonly applied to meet the maximum permitted levels of siloxanes (0.03 to 28 mg/m$^3$).

The biogas upgrading technology has not expanded worldwide. Germany and Sweden top the list of approx. 400 biogas upgrading plants in the world [30]. In conclusion, the biogas can maximizer its potential when used in injection grids, domestic gas and a fuel for vehicles.
However, technologies to remove contaminants compounds are essential on energy efficiency.

2.9. Biogas in biorefineries

2.9.1. Biogas for Self-supply energy
The biorefinery concept is an alternative potential to transform different types of biomass into value-added products. The biorefinery concept is related to the current oil refineries, working in the generation of multiple products. However, it differs in the operational characteristics both in raw material and their technologies [132]. The first can work with various types of biomass, while the second works only with a specific oil field.

Consequently, different authors Quintero et al. [132]; Martínez-Ruano et al. [133]; batista et al. [134] assessed technical and economic conditions of different biomass emphasize in a biorefinery concept. Therefore, economic yield and energy integration have a relationship with the integration of different platforms. Accordingly, the integration of AD in a biorefinery concept can be carried out with the energy integration. So, the production cost decreases with the integration of the fuels in the productive process.

Biogas in a biorefinery concept may be related to self-sufficiency as energy inputs. This can be used in heating equipment lines, electric energy production and as fuel for boilers. Fuess et al. [135] presented a specific production of biogas in the framework of a biorefinery. In the study, the authors stand out the economic feasibility of scaling-up two-phase of AD plants in sugarcane-based biorefineries for the vinasse treatment. They conclude that biogas and electricity production cost in biorefinery concept have a similar or lower compared with stand-alone process. Similarly, Martinez-Ruano et al. [133] concluded than biorefinery increase the economic profit compared to stand-alone biogas production.

2.9.2. Biogas as platform
An integrated biorefinery uses the combination of raw materials and bioenergy generation platforms routes through transformation, processes integration, and equipment [136]. The
biorefinery can combine different chemical platforms, such as sugar and biogas, syngas and sugar, or combinations of the different platforms. Therefore, the biogas can be used as a platform for value-added products such as electric power and natural gas concentration.

AD in a biorefinery concept has significant advantages in biogas production respect to the stand-alone process. It can be produced by multiple organic raw materials. Thus, waste generated by individual industries can adopt the concept of integrating biorefinery. Waste integrated into sustainable energy production causes a decrease in environmental impact and social problems.

In this sense, Hagman et al. [137] studied the roll of biogas in biorefineries respect to three sectors (agricultural, marine and forest). These research indicated that biogas contributes with value-added of the biorefinery, including through making the biorefinery more sustainable and competitive. However, the design and implementation of biorefineries is ruled by analysis from technical, economic and environmental terms. Factors such as the transformation route, the process scale and energy integration represent a decisive role in the feasibility of fuel within a biorefinery. To solve this problem, techno-economic evaluation [133], [138], scale [139] and energy integration [140] have been studied in the biorefineries. Additionally, Moncada et al. [130] approaches the biorefinery design from the conceptual design.

2.10. Final remarks

Many organic materials to produce biogas are available today, such as lignocellulosic biomass, municipal solid waste, animal manure, industrial waste, among others. Each of these possibilities is limited by the physicochemical characteristics, which determine the quality of the substrate in the anaerobic digestion. For example, an optimal C/N ratio is fundamental to carry out the transformation. Lignocellulosic residues have a high C/N ratio. This feature favors AD but the need for pretreatment may limit its use.

Each raw material behaves differently depending on the conditions and the technologies used. Availability, productivity, and quality determine the viability for its industrial use. Individual experimental studies can determine physicochemical characteristics and possible yields. From another point of view, a techno-economic and environmental analysis can determine the best way of producing one raw material as compared to another. This takes
globalized costs into account depending on the technology used in the process line. In general, it is necessary to carry out this type of analysis to determine the feasibility of each feedstock, while also considering the experimental studies reported in the literature.

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Chapter 2. Biogas production from agroindustrial and industrial waste.


Chapter 2. Biogas production from agroindustrial and industrial waste.


Chapter 2. Biogas production from agroindustrial and industrial waste.


3. Milk whey as a residue from milk industry: Colombian and Chilean Case.

Colombia is a tropical country localized in the northwestern region of South America. The country is recognized as the most mega-biodiverse of the planet, for this reason you can find many species, crops and industries. The main agricultural products of the country are coffee, sugarcane, bananas, rice, potatoes, palm oil and among. Different industries are located throughout the country, including the dairy, oil and food industry. On other hand, Chile is a country located in the extreme southwestern region of South America. The country has one of the successful economies in the continent due to the exploitation and export of raw materials such as copper, fruit, fishery products, wine, among others. However, the country is energetically dependent given the import of petroleum, carbon and natural gas [1]. In consequence, the price of fuel is governed by the international market. Also, should be noted that livestock farming is one of the main agricultural activities in the central and southern regions of the country. These coupled with the great progress of the dairy industry due to the growth of cheese consumption in the population.

3.1. The milk industry.

The dairy industry is one of the most important in the food sector, from milk you can produce a diversity of products for human consumption. Among the most representative products with high demand for worldwide marketing are cheese, yogurt, butter, whole milk, milk powder, and milk caramel. According to the latest data from the Food and Agriculture Organization (FAO), world milk production was 827 million tons in 2017. Milk originates from different sources such as cows, camels, sheep, goats, and buffalo. However, cow milk is the most widely produced worldwide, representing 81.6 percent of the total, while sheep milk represents just 1.5 percent [2]. In addition, according to the International Trade Center [3], the value of world exports of milk and milk products were US$ 80.5 million in 2018.
Chapter 3. Milk whey as a residue from milk industry: Colombian and Chilean Case.

The American continent produces more than 27 percent of the world's milk, where the United States is the largest producer, followed by Brazil, Mexico, and Argentina. In Latin America, Colombia and Chile are the fourth and sixth in the world rankings of the milk production nations. However, Colombia is a larger milk producer than Chile, with an annual average of 6,803,316 tons (23.3% more than Chile) [2]. The milk of both countries comes from only cows, however, Chile also produces goat milk (10,000 tons/year) [2]. A more detailed perspective of milk and cheese production in both countries can be seen in the Figure 3-1 and Figure 3-2. On the other hand, in Colombia, the raw milk differs in chemical composition (although all excellent quality) depending on the different cattle-raising areas. A key factor of Colombian milk is its high protein content (3.56%), which is higher compared to other producing countries such as Germany (3.41%) and New Zealand (3.52%) [4]. The above allows increased performance when used in the generation of finished products such as cheese. Similar to Colombia, in Chile milk varies in chemical composition and has of good quality. In Chile, milk production is mainly centered in the south of the country with 31 dairy plants, and its characterized by a strong seasonality (it is higher in the spring-summer period) [5]. According to the FAO data, all milk in both countries is used for internal consumption in its different forms such as butter, cheese, powdered milk, condensed milk, and others.

![Figure 3-1. Production of milk and cheese in Colombia.](image)
3.2. Cheese production.

The cheese is one of the main products that can be obtained from milk at an industrial level. According to the International Trade Center [3], the value of world exports of fresh and aged cheeses were US$ 32 million in 2018. This corresponds to 39.8 percent of the import and export of the total dairy products.

The cheese is obtained from the extraction, cutting and pressing of the curd, which may or may not be subjected to maturation by specific microorganisms. There are about 2,000 varieties of cheese in the world, which depends on the production process. Only in Colombia, there are about 12 varieties of cheese, apart from Paipa cheese (semi-aged cheese) and Costeño cheese (salty cheese). In Chile, there are also different varieties like the Gauda and Chanco, where the per capita consumption of cheese reached 8.4 kg, while in Colombia 1 kg of cheese is consumed a year per person [6]. Thus, the annual production of cheese is greater in Chile than Colombia, where Chile produces around 81,000 tons of cheese each year. While in Colombia, it is 58,500 tons. However, these figures were very similar for both countries between 2009 and 2010 (57,000 tons/year). Furthermore, it is to be noted that the amount of cheese produced in Chile cannot supply the Chilean population.
Thus, cheese and curd are the main import products respect to dairy products (in 2016, 31.326 tons were imported) in Chile, which from countries like Germany, Argentina and the United States. In contrast, Colombia imported only 10 percent of the amount imported by Chile. According to FAO data [7], the import and export rate of cheese of both countries can be observed in Figure 3-3 and Figure 3-4.

![Figure 3-3. Import and export of cheese in Colombia.](image1)

![Figure 3-4. Import and export of cheese in Chile.](image2)
Taking account that the cheese is one of the main products of the dairy industry, its represents the lowest yield considering the milk used for the process. In this sense, cheese production has a yield between 10 and 13 percent of the milk used as a raw material in the production process. In other words, for each 10 kg of milk only produced between 1-1.3 kg of cheese [8]. The remnant is the byproduct of cheese manufacture, which is called cheese whey/milk-whey (MW). This byproduct is main composed by water, proteins, lipids, vitamins and minerals. Therefore, MW is attractive as a substrate in biotechnological applications. However, MW is the most difficult problem to solve in the dairy industry due to its high organic, corresponding between 60 and 70% of the total industrial effluents [9].

3.3. Milk-whey as a residue from milk industry.

The waste generated from the transform of milk into cheese is mainly MW, which is an important problem due to its high organic load and difficult to manage at the industrial level. MW is a light-colored liquid substance (green-yellow) resulting from cheese production. The cheese production consists in five or six steps depending on the final product (fresh cheese or aged cheese). These consist of coagulation, cutting and draining, molding and pressing, salting, drying and maturation. However, before initiating the cheese manufacturing process, the collected and stored milk should be treated and prepared by filtration, clarification and normalization processes [10]. The above with the purpose of removing protein-bound, particulate matter and conditioning their physical, chemical and biological characteristics.

The first step is the coagulation, which consists in the precipitation of milk proteins (mainly casein), giving rise to a gelatinous mass denominated curd. This can be done in three different ways: acid coagulation, enzymatic coagulation and mixed coagulation (comprises the mixture of acidic and lactic coagulation). The enzymatic coagulation is the most used system in cheese production, which is produced by the action of protease-like enzymes. Unlike enzymatic coagulation that uses enzymes, acid coagulation uses lactic bacteria.

The next steps are cutting and draining operations, these operations are necessary to separate the gel formed from the liquid phase (milk-whey). Where the curd is cut into cubes, and the liquid phase is separated by heat (raising temperature) and/or mechanical treatments (stirring, molding, pressing, etc.) [11].

The MW can be classified depending on the type precipitation/coagulation of casein into two types: sweet whey and acid whey [12]. The sweet whey (produced during the enzymatic coagulation) originates from manufacture of pressed cheeses from cow and/or sheep´s milk,
Chapter 3. Milk whey as a residue from milk industry: Colombian and Chilean Case.

which has a poor lactic acid content and has a pH value above 6 [13]. In contrast, the acid whey (produced during the acid coagulation) originates from manufacture of fresh cheeses from cow and/or goat’s milk, which has a lower proportion of fat and a higher lactic acid content compared to sweet whey [10]. The typical composition of the two types of MW is presented in Table 3-1. However, despite this classification, the physicochemical composition of the cheese varies depending on the pH for coagulation, the milk origin and the moisture content of the final product. In particular it is important to emphasize that regardless of the type of MW, it is estimated that the elaboration of 1 kg of cheese produces 9 kg of MW as waste [13].

Table 3-1. Composition of sweet and acid milk-whey. Taking from Panesar et al., [14].

<table>
<thead>
<tr>
<th></th>
<th>Sweet milk whey (g/kg)</th>
<th>Acid milk-whey (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry mass</td>
<td>55 - 75</td>
<td>55 - 65</td>
</tr>
<tr>
<td>Lactose</td>
<td>40 - 50</td>
<td>40 - 50</td>
</tr>
<tr>
<td>Lipids</td>
<td>0 - 5</td>
<td>0 - 5</td>
</tr>
<tr>
<td>Protein</td>
<td>9 - 14</td>
<td>7 - 12</td>
</tr>
<tr>
<td>Ash</td>
<td>4- 6</td>
<td>6 - 8</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.4 - 0.7</td>
<td>0.5 - 0.8</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0 - 0.3</td>
<td>7 - 8</td>
</tr>
<tr>
<td>pH</td>
<td>&gt;6.0</td>
<td>&lt;4.5</td>
</tr>
</tbody>
</table>

** The values are reported on a wet basis (kg of crude product).

The next stage (after cutting and draining) are molding and pressing. This step consists in pressing the curd pieces into molds in order to drain the intergranular MW. MW is also generated during this stage, however, it is lower compared to the previous stage [10]. In addition, MW generation varies depending on the pressing intensity according to the type of cheese.

The cheese production is complete with the stages of salting, drying and maturation. The salting stage is an important factor due to because it influences on the cheese flavor and stimulates its maturation. Generally, the brines (ranged to 16-22 percent) used to salt cheese produce wastewater with high conductivity [10]. Finally, the drying and maturation
stages (if required) of the final product are carried out in chambers under specific humidity and temperature conditions. A general overview of the MW generation and cheese production is presented in **Figure 3-5**.

**Figure 3-5.** Overview of the process flow for MW generation and cheese production. Modified from [10].
3.4. Colombian and Chilean context.

According to FAO data, cheese production worldwide was 21'166,000 tons in 2014, which represents an approximate of 190'500,000 metric tons of MW per year. Or those, 45 percent is disposed of in the environment (mainly in rivers, lakes and wastewater treatment plants), which causes serious pollution problems [15]. In the case of Colombia, the annual MW production considering a cheese production of 58.500 is approximately 526.00 tons. While in Chile, the annual MW production is around of 780.966 tons, taking account 86.774 tons of cheese produced. These figures are substantial considering the pollution problems that this byproduct can generate throughout these territories.

In Colombia, the MW is a raw material used in the elaboration of different products such as butter, some types of cheese, milk caramels, desserts, and others. According to the Ministerio de Agricultura de Colombia y desarrollo rural [16], imports of milk products reached 31,000 tons in 2015, where powdered milk constitutes 55%, MW 13% and 11% cheese. However, despite the various applications of the MW, this not fully used in the national dairy industry. In general, most small and large industries, this by-product is eliminated directly to water sources, sold as animal feed or treated with coagulation-flocculation processes. The situation in Chile is not very different, although large dairy companies can transform this by-product into whey powder (which is used for the production of different drinks and other products), the small companies in the southern regions of Chile have the same final disposal problem as in Colombia.

On the other hand, the characteristics of the MW are promising alternative in the transformation into value-added products such as proteins [17], bioactive compounds [18], biopolymers [19] and biofuels (eg biogas, ethanol, hydrogen) [20]–[22]. However, there are still barriers to take advantage of this byproduct due to high capital costs. In this sense, milk processing companies and small producers in developing countries such as Colombia and Chile do not fully use this resource. Although its use as a raw material to make other foods is an alternative, the supply of these foods is higher compared to the demand. Therefore, the remaining MW is poured directly into the environment causing serious environmental problems due to its high organic load in terms of $\text{BOD}_5$ (40.000 – 60.000 mg/L), COD (50.000 – 80.000 mg/L) [23] and low alkalinity (2500 mg/l as CaCO$_3$) [24]. In consequence, alternative research for new transformation paths is necessary, which must be affordable to technologies of both countries. An alternative is the production of renewable energy in the form of liquid or gaseous fuels such as ethanol, biogas and hydrogen. For example, anaerobic digestion is a process that reduced the pollution discharge and produced an
energy source as biogas. Here, it is a one of the main point of this work. **To give value-added to milk-whey in developing countries (such as Colombia and Chile) through biogas production**, which can bring benefits in economic and environmental terms for both countries.

### 3.5. Final remarks.

The milk-whey is one of the main by-products of the dairy industry in the cheese manufacture. This can be used to obtain value-added products through biotechnological processes due to their physicochemical characteristics. However, milk-whey is not used effectively, due to high cost of technologies. Additionally, an inefficient final disposal of this waste leads to serious environmental pollution problems. Such is the case of Colombia and Chile, where there are still technological gaps to fully use this by-product. In this sense, the production of renewable energy (such as biogas) is a promising alternative for the valorization of milk-whey, which can bring benefits in economic and environmental terms for both countries.

### References


Chapter 3. Milk whey as a residue from milk industry: Colombian and Chilean Case.


4.1. Potato.

Potatoes are stem tubers that are native to the Andean mountain region of South America. There are about 5,000 varieties from around the world. Three thousand of them are in Peru, Bolivia, Ecuador, Chile and Colombia [1]. These varieties are categorized into few groups with common characteristics, such as russet potatoes (rough Brown skin), red potatoes, White potatoes, yellow potatoes and purple potatoes. However, the largest species cultivated around the world is *Solanum tuberosum*.

Potato is used as raw material for various types of foods and preparations due to their excellent nutritional values. The main contribution in human nutrition are carbohydrates in the form of starch (88% of the carbohydrates is starch). In general, this tuber is composed of water (79%), carbohydrates (17%), proteins (2%), contains negligible amounts of lipids and others such as fiber, vitamins and minerals [2]. For these and other reasons, potato is the fourth food crop in the world after wheat, rice and corn [2]. The world production of potatoes in 2017 was 388 million metric tons with an average productivity of 20 tons per hectare. According to FAO [3] data, the main potato producing countries in the world are China, India, Russia, Ukraine and the United States. Latin America produces 16’979.165, being the main producers Peru, Brazil, Argentina, Colombia and Chile. The distribution of potatoes production in Latin America is presented in Figure 4-1.
Chapter 4. Potato stem – A potential waste for biogas production.

Figure 4-1. Production of potatoes in Latin America. Adapted from FAO data (2017) [3].

4.2. Potato plant description.

Potato plants are herbaceous that grow about 60 cm (24 in) high, depending on variety. The potato is made up of two sections: an underground section consists of root, stolons, tubers and mother tuber; and an aerial section composed by stems (main and secondary), leaves, flowers and fruits. A description of the main parts of the plant is showed in Figure 4-2. The tubercle is constituted by approximately 2% of peel, 75% to 85% of potato and from 14% to 20% of stem [4]. In general, potatoes are mostly cross-pollinated by insects such as bumblebees, which carry pollen from other potato plants, though a substantial amount of self-fertilization occurs as well.

Potato cultivation needs specific conditions for an ideal tuber development. In general, the potato is grown with a soil pH between 5.5 to 7.0 and temperatures ranging between 12 °C and 14 °C [5]. In addition, the potato requires an effective depth of 40 cm due to its limited root system (in relation to other plant species). In this way, the plant makes sure to extract the major amount of the nutritive elements from the soil. The potato has a very short vegetative cycle, around 80 days after sprouting [6].
4.3. Potato in Colombia and Chile.

Potato is currently the second most produced food product in Colombia with 2.8 million tons per year [7], [8]. Potato cultivation occupies the first place in production and third in planted area with around 130 thousand hectares. The main producers are concentrated in the departments of Boyacá, Antioquia, Nariño and Cundinamarca (around 90%) [9]. It should be noted that the potato production is focused on small producers, which sow less than one hectare and correspond to 80 percent of total producers. In Colombia, there are 60 different varieties of potatoes, which participate with 3.5% of agricultural GDP [10]. On the other hand, the average potato productivity is 22.7 tons per hectare at national level.

In Chile, around 50 thousand hectares of potatoes are under cultivation and 1’162.568 ton produced every year [11]. Potato is the four most important crop in terms of land occupied and work the largest number of farmers. According to regional harvest results (2018/2019), Los Lagos is the leading production region of potato with 379.285 tons by year, followed by
La Araucania region with 259.522 tons. On the other hand, the highest potato productivity per hectare is found in the Los Lagos and Los Rios regions, with a yield of 43.7 and 42.5 tons per hectare [12]. While the national yield is around 27.8 tons per hectare. However, this amount does not supply the country’s demand since the potato imports reached a level of 64,720 tons in 2018/2019 [12].

On the other hand, potato cultivation is carried out under a certain number of stages to reach the final product. Initially the crop requires special soil conditioning, where the soil is decompressed or a good root development. Subsequently, the soil is crossed with a separation between them of 0.9 and 1.1 meters before the planting. Sowing is done with one seed per plant, establishing a distance of 0.20 and 0.30 meters between them [5]. After the planting stage, the farmer must follow up of weeds, pests, water and nutritional requirements. For Colombia and Chile cases, phosphorus is a nutritional requirement and is applied to most soils where potatoes are produced [5], [6]. In addition, it should be noted that the water requirement of the potato crop are high (5,000 to 6,500 m³/ha). The above is a most important agronomic factor in the central north and central south regions of Chile [6].

Another important economic factor in potato production is the final stage of the crop: the harvest. The harvest requires more people and organization than any other stage. This is carried out when the crop reaches its physiological maturity, characterized by plants yellowish and flaccid, stems open on the ground and tubers easily detach from their stolons. In Figure 4-3 is showed a typical harvest of potato in the southeast region of Colombia.
The harvest corresponds to the separation of the tubers from the mother plant and contemplates: removing the soil; collect the tubers; separate soil tubers, lumps and plant debris; transport to the place of classification and storage. In this operation should remove all tubers in poor condition. Thus, this stage is where a large quantities of residues are generated (mainly stems and tubers). Without considering the residues that can be generated an industrial level such as peel, which have been researched in order to obtain value-added products [13], [14].

Despite the large amount of waste generated at the harvest stage, these have not been thoroughly investigated. Thus, similar to the industrially discarded potato peel, the potato stem can be a valuable organic resource to obtain molecules of interest.

On the other hand, it should be noted that the potato plant consists of a set of stems, each of whom forms roots, stolons and tubers. In general, crops are adjusted to produce between 200,000 and 300,000 main stems per hectare, depending on the variety. However, it should be considered that each plant has approximately 5.59 stems [6].

Taking into account productivity per hectare and annual potato production in Colombia and Chile, the amount of stems generated in both countries can be estimated. Thus, considering that Colombia annually produces 2.8 million tons of potatoes with a crop productivity of 22 tons per hectare, the number of hectares planted per year is 127,000 hectares. Therefore, the amount of waste would be 25.4 billion main stems considering 200,000 main stems per hectare. Similarly, potato stem waste would be 8,363 million stems per year in Chile.

The potato stem generated in the harvest stage is disposed in landfills, without exploiting its potential to produce value-added products despite its lignocellulosic composition. In the literature, practically no information was found on the production of biotechnological products from this residue. Additionally, it can be observed that this crop and its residues is emerging with an interesting potential to be used for different purposes than food. For example, the milk whey (described in the above chapter) has been investigated for biogas production but potato stem only in mixtures with peel and other residues.

Chapter two demonstrate that the strategic integration of raw materials in anaerobic co-digestion processes for biogas production is an interesting alternative to reduce availability and characteristics problems of single substrate anaerobic digestions. However, there is no report where the potato stem alone was a substrate in anaerobic digestion or co-digestion processes. Therefore, it could be interesting the integration of potato stem and milk whey as an innovative alternative to recover energy with economic benefits as a result of the increase of the overall raw material availability for the anaerobic digestion. The real possibilities of
using any biomass or residue through different energy conversion strategies (including the use of more than one residue simultaneously for the same energy purpose) should be analyzed seriously based on techno-economic analysis [15]. Last, it allows developing non-expensive calculations and simulations based on the composition of the raw material and experimental experiences for following steps in developing this type of anaerobic digestion technologies. In this context, the aim of this thesis is established and evaluated different biotechnological routes for biogas production from potato stem and milk whey.

4.4. Final remarks.

Potato is one of the most important foods around the world. In the case of Latin America, the largest producers are Peru, Brazil, Colombia, Argentina and Chile. In the cultivation stage, specifically in the tuber harvest, the potato stem is generated as a residue. This waste is generated in large quantities in Chile and Colombia, however, it is not used. On the other hand, it can be used as raw material to obtain products of interest such as biogas. However, this has not been explored by the scientific community, which makes it attractive and promising for its transformation to biogas.

References


Chapter 4. Potato stem – A potential waste for biogas production.


The present chapter outlines the raw materials characterization including reagents, methods and equipment that are used in this thesis. In this context, milk whey (MW) was obtained from an industrial milk company named Colum in the Fifth Region of Chile, specifically in the industrial zone in Santiago de Chile localized at latitude 33° 22’ 45.854” south and longitude 70° 40’ 57.165” west. The Colum Company commercializes different milk products such as cheese, whole milk, yogurt, butter, and others. MW is a subproduct of the Colum company, it is generated in the interline steps of the milk products production as cheese elaboration (e.g. cream cheese, cheddar cheese, mantecoso cheese, etc.).

After obtention of MW, this was transported to the school of biochemical engineering from Pontificia Universidad Católica de Valparaíso in the Valparaíso Region for carry out the physicochemical characterization analysis. Before the sample’s analysis, these were homogenized and deepfreeze in a cooler at -20 °C. This was done in order to preserved the physicochemical characteristics until further usage.

Potato stem (PS) samples were collected from a farm located in the south west region of Colombia, municipality of Guaitarilla, Nariño at latitude 1° 07’ 37” north and longitude 77° 32’ 59.8” west. In this region are cultivated diverse agricultura’s products as potato, corn and wheat, there is great livestock influence. Families live from the milk production, which sell to medium-sized milk processing industries for the cheese production. Accordingly, PS samples were immediately transported to the Instituto de Biotecnología y agroindustria (IBA), Block T, Campus la Nubia, Universidad Nacional de Colombia – Manizales.

PS samples were conditioned, these were dried during two days at room temperature and placed a convective oven (Thermo Precision model 6545) at 40 °C for 48 hours until to obtain a moisture content of 10%. The moisture content of the raw material was measured in a Shimadzu moisture balance MOC - 120H. In addition, PS samples were crushed with a gyratory mill (Retsch GmbH SR200 Gusseisen) until a particle diameter of 04 mm using
Chapter 5. Milk whey and potato stem characterization.

Milk whey and potato stem characterization.

Wiley® mill. Also, these were homogenized and stored at room temperature before use and characterization in Valparaíso city.

5.1. Milk whey.

Physicochemical characterization of milk whey (MW) involves the determination of the chemical composition in terms of the protein, carbohydrates, lipids and ash content. On other hand, additional characterization was necessary for quantified the standard biogas and methane production explained in subsequent sections. These additional experiments consisted in the determination of moisture, total solids (TS), volatile solids (VS), pH and the chemical oxygen demand (COD). All experimental procedures described in the following sections were carried out in triplicate, in order to maintain data reproducibility. Finally, the characterization of MW was carried out at Escuela de Ingeniería Bioquímica (EIB), Pontificia Universidad Católica de Valparaíso in Chile.

5.1.1. Carbohydrates content.

Carbohydrates are long-chain neutral compounds formed by carbon, hydrogen and oxygen. These are found in living tissues in the form of sugars, starch and cellulose. MW is composed mainly of sugars, in the form of lactose (4.5-6.0%) [1], [2]. Carbohydrates content is important because of the main biodegradable source in the MW, which can be metabolized almost completely in AD and generate methane (374 mL CH₄/g VS) [3].

Carbohydrates determination was done thought colorimeter method following the protocol proposed by Dubois et. al, “Phenol Sulphuric Acid Method for Total Carbohydrate” [4]. This protocol was described and claimed with most details in the fourteenth book chapter of the book entitled “Food Science Text Series Food Analysis Laboratory Manual” [5]. The necessary materials to carry out the method were hydrochloric acid (2.5 N), glucose solution (100 mg/L), phenol (80% w/w) and concentrated sulfuric acid (approx. 96% w/v). Additionally, a group of materials as analytic balance, volumetric flasks, water bath thermostated, boiling tube, volumetric pipettes and spectrophotometer were used.

The method consists in the total conversion of carbohydrates to hydroxymethyl furfural (HMF) in acid medium, where the quantification of this reaction with phenol producing a green colored solution with a maximum absorbance at 490 nm. First, 100 mg of the sample was passed into a boiling tube. These were led to hydrolysis with 5mL of 2.5 N HCl for three hours in boiling water. The contents were cooled at room temperature and neutralized with
sodium carbonate to complete a volume of 100 mL. Thus, the standard curve was realized for duplicate with a standard glucose solution (100 mg/L), taking 0.2, 0.4, 0.6, 0.8 and 1 mL in a series of standard tubes. Simultaneously, 0.1 and 0.2 mL of sample are added to two individual test tubes. Each tube was fully until 1 mL of work volume with distilled water and was added 1 mL of the phenol solution. For each tube, concentrated sulfuric acid (5 mL) was added to established 10 reaction minutes. For each tube, concentrated sulfuric acid (5 mL) was added with a reaction time of 10 minutes. The final mixture must be shaken vigorously before it hating in a water bath thermostated at 30 °C for 20 min. In the end, the samples for standard curve for total carbohydrates determination were measured using a UV/Visible Spectrophotometer 6405 (Jenway) operating at 490 nm of absorbance. The equation of the line for the standard curve was calculated and was determined the total carbohydrates expressed in terms of glucose ($A_{490}$ versus µg of glucose/ 2 mL).

5.1.2. Protein content.
The protein content makes reference to biopolymeric compounds former from linked-amino acids by peptide bonds. These proteins are an organic material easy to degrade, thereby you are very valuable in AD processes. However, the activity in the biogas reactors operated with milk residues decreases when the substrates have a high protein content [6].

The quantification of total protein was carried out with the Biuret method, which is based on the complexation of Cu2+ to functional groups in the protein’s peptide bonds. In this section, a method summary showed, however, it can be consult with greater detail in previous research [7], [8]. Biuret reagent composed by sodium hydroxide (3 g), potassium sodium tartrate (0.6 g) and copper sulfite (0.15 g) diluted in 100 mL of distilled water was used to develop the methodology. In addition, bovine serum album was used as protein standard. Alternatively, the employed materials are volumetric pipettes, volumetric flasks, test tubes, analytical balance and spectrophotometer.

Before characterization of protein content, a calibration curve was performed with protein concentrations of 0, 0.4, 0.8, 1.2, 1.6 and 2 g/L in successive test tubes from an albumin stock-solution of 2 g/L. Thus, 300 µL of each solution was added to 1 mL of Biuret reagent (this was previously prepared and stored in the absence of light) in separate tubes. Each tube was vigorously homogenized for 30 seconds and allowed to stand during 10 minutes. Later, for each tube, the absorbance was measured using a UV/Visible Spectrophotometer 6405 (Jenway) at 540 nm. A linear regression of the data was made and the equation of the line with the correlation between $A_{540}$ versus g of protein/L. Meanwhile, the same process
was considered for MW samples so as to quantify the total protein content with the equation line of the standard curve.

5.1.3. Lipids content.

Lipids contain the same elements as carbohydrates, however, there is a higher ratio of hydrogen and oxygen (> 2: 1). This characteristic makes it interesting in biological systems because of these are not soluble in water. The formation of lipids in the MW differs mainly in the transformation route, where the obtaining of fat and non-fat dairy products is important. Moreover, lipids are attractive for AD and co-digestion due to causes a positive effect in biogas and methane production compared with proteins and carbohydrates. The potential of biogas production from lipids is 1.72 and 1.53 twice higher that the biogas potential that carbohydrates and protein, respectively [9]. However, high lipid content can cause inhibition in biogas production, low mass-transfer, blockage and loss of active biomass [9], [10].

The total lipid content was carried out with the soxhlet method, which is based on a semi-continuous extraction, with an organic solvent at a temperature of approximately 70 °C. The solvent used was a mixture of ethyl ether and petroleum ether according to the twelve book chapter entitled “Food Science Text Series Food Analysis Laboratory Manual” [5]. The materials used in this experimental method were analytic balance, desiccator, glass soxhlet extraction tubes (soxhlet syphon), condensers (NS 45/40), beakers (250 ml), hoses and porcelain boiling chips.

To perform the lipid content determination 3 g of dehydrated MW were added to pre-weighted and pre-dried extraction thimble. Continually, 250 mL of mix composed by petroleum ether and ethyl ether in relation 1:1 was added in 500-mL flat-bottom flasks with several boiling chips to prevent bumping. The flask was placed on the turn off heating plates. After, the thimble was placed on the Soxhlet syphon tube considering that the thimble must not exceed the Soxhlet syphon height and was assembled with the flat-bottom flasks, hoses and external condensers. The heating mantles were adjusted to provide a minimum of 4-5 siphon cycles per hour. Carry out the extraction in a well-ventilated chemical fume hood for 24 h.

After 24 hours of water extraction, the heating plates were turned off and the cooling water flow was stopped. The samples of milk whey were placed in an oven during 24 hours at temperature of 45°C. The dried thimble was transferred to a desiccator for two hour and weighted. Then, the lipids content was calculated as the loss of weight per weight of dry sample used in the analysis. Thus, it is necessary use the following equation:
5.1.4. Ash content.

Ash refers to the inorganic residue that remains after ignition or complete combustion of organic matter. The ashes are original oxidized forms of oxides or carbonates, and the organic salts are lost by oxidation during the intense thermal treatment. It is possible to identify some minerals such as Aluminum (Al), silicon (Si), potassium (K), magnesium (Mg), manganese (Mn) and iron (Fe).

The method for measured ash content is based on the combustion of dehydrated organic sample at a temperature of 523 °C during three hours this was established in the book titled “Standard Methods for the Examination of Water and Wastewater” [11]. The necessary materials for the ash characterization were desiccator, muffle, crucibles and analytic balance. Initially, 5 g of sample were incorporated to a crucible previously dried and weighed. Thus, the sample was placed in a furnace with a temperature ramp program as indicated below: Ramp from room temperature to 105°C during 24 h (dehydration), ramp to 523°C at 10°C per minute and hold at 523 °C for 180 min. Finally, the temperature is decreased until reach a temperature of 105°C. In this moment, the crucibles were removed from the muffle and placed directly into a desiccator. When cooled to room temperature, the weight was recorded on the analytical balance.

The ash content was calculated according to following equation:

\[
\text{Ash (\%, db)} = \frac{W_{\text{crucible + ash}} - W_{\text{crucible}}}{W_{\text{sample before extraction}} - W_{\text{sample after extraction}}} \cdot 100
\]

5.1.5. Total and volatile solids determination.

Total solids (TS) make inference to the organic sample free of water after a previous drying at a temperature of approximately 105 °C. This is a parameter of interest in AD processes to specify the digestion type: conventional wet (≤ 10% TS), semi-dry (10-20% TS) and modern dry (≥ 20% TS). Furthermore, this has a direct influence on the efficiency of biogas production.
and methane production [12], [13]. On other hand, volatile solids (VS) can give an approximation of the available organic matter to be biodegraded by biological treatments. This is used as a control parameter in AD plants [14].

The followed methodology to find the TS and VS was based on the book “Standard Methods for the Examination of Water and Wastewater” [11]. The method used for TS and VS determination were 2540 B and 2540 E, respectively. The materials used in this step were analytic balance, crucibles, agitator, graduated pipette, magnetic stirrer, desiccator and muffle furnace for operation at 105 °C and 550 °C.

To determine TS in the MW, MW samples was homogenized with a glass rod. After, 10 ml of sample was added inside the porcelain crucible previously dried and weighed in order to obtain between 25 mg to 200 mg of solids. These crucibles were placed to a muffle furnace at a temperature of 105 °C. Finally, the crucibles were removed of the muffle and was placed into desiccator (1 hour approx.). Similarly, dried VS samples were carried out a controlled calcination process for a period of 2 hours at temperature of 550 °C in the muffle furnace. The samples were retired from muffle and were passed to a desiccator for 1 hour. Also, the samples were weighed for to calculate TS and VS content.

The following equations were used for total and volatile solids determination:

\[
TS \left(\% \frac{w}{w}\right) = \frac{W_{\text{crucible}} + \text{solid (Dried to 105 °C)}}{W_{\text{sample}}} \cdot 100 \quad \text{Eq. 5-3}
\]

\[
VS \left(\% \frac{w}{w}\right) = \frac{W_{\text{crucible}} + \text{solid (Ignition to 105 °C)}}{W_{\text{sample}}} \cdot 100 \quad \text{Eq. 5-4}
\]

### 5.1.6. Oxygen chemical demand determination.

Chemical oxygen demand (COD) is defined as the oxygen equivalent (in milligrams per liter of product) needed to oxidize a certain amount of organic matter with strong chemical oxidants (potassium permanganate \([\text{KMnO}_4]\), or potassium dichromate \([\text{K}_2\text{Cr}_2\text{O}_7]\)). This parameter is used to measure water quality or the degree of organic pollution of wastewater [15]. In the case of anaerobic digestion, COD allows determining the quality of a substrate due to the organic matter is known indirectly for the use of the oxygen in the cells [16].
COD was determined by the colorimeter method, which is based on the color change of the oxidation reaction among potassium dichromate ($K_2Cr_2O_7$) and the test sample. A brief description of this method is presented following, however, it is advisable to consult the complete method in the book titled "Standard Methods for the Examination of Water and Wastewater" [11], section 5220 D. The reagents used in this analysis were potassium dichromate ($K_2Cr_2O_7$), mercury sulfate ($HgSO_4$), concentrated sulfuric acid ($H_2SO_4$), silver sulfate ($Ag_2SO_4$), potassium phthalate ($HOOCC_6H_4COOK$) and distillated water. As regards materials, test tubes with lid, equipment of digestion at 150 °C, spectrophotometer, analytic balance, pipettes and flask of 1 L.

Before start the determination of COD, three standard solutions were prepared. A liter of digester solution prepared by addition of 10.216 g of potassium dichromate, 33.3 g of mercury sulphate, 167 mL concentrated sulfuric acid and water. A liter of catalytic solution prepared by addition of 10.2 g of silver sulfate in 1000 mL of distillated water. A liter of standard solution of potassium phthalate prepared dissolved 0.425 g of potassium phthalate in 1000 mL of water distillated, where the Cod in this solution was of 800 mg $O_2$/L. The process initiated by the elaboration of calibration curve with standard solution where the COD concentration will be between 200 to 800 mg/L. For this, 8 tubes were prepared taking account a concentration of 100, 200, 300, 400, 500, 600, 700 y 800 mg $O_2$/L in an effective volume of 2.5 mL. Subsequently, for each tube was added carefully 1.5 mL of digester solution and 3.5 mL of catalytic solution (exothermic reaction). The tubes were closed and put on the vigorous agitation before start the digestion at 150 °C for 2 hours. At the end of two hours, the tubes were removed from the equipment and cooled left out a room temperature.

On other hand, 2.5 mL of diluted MW sample (1:100) was added catalytic and digester solutions following the same procedure. Finally, the standard samples together MW samples were measured in the spectrophotometer at 600 nm. Thus, a line equation (R=0.9958) was calculated with the $A_{600}$ nm and COD values of all samples were obtained.

The COD in initial sample can be measured by Equ. 5-5:

$$\text{COD as mg } O_2 \cdot L^{-1} = \frac{mg \ O_2 \ \text{in} \ \frac{\text{final volume}}{\text{mL sample}}} \cdot 100 \ \text{Equ. 5-6}$$
5.1.7. Moisture content and pH

Moisture is defined as the amount of water present in a body and its of vital importance in biological systems to survive. In the case of AD the moisture affects the degradation of organic matter and the biogas production [17]. Furthermore, pH indicates the concentration of hydrogen ions in solutions, therefore, it is a direct measure of acidity or alkalinity in the medium. This is important due to only under certain conditions can survive the biological systems. In the case, a change in pH can inhibit the action of methanogenic bacteria in AD (see Chapter 2).

Moisture content determination was based on previous characterization of TS due to it an indirect measure. In consequence, the total sample weight minus TS content is the moisture (see numeral 5.1.5). The formula for moisture content is presented in the next equation. Furthermore, pH was measured with in-situ sample using a pH Tester also features manual or automatic temperature compensation.

\[
\text{Moisture (\%)} = 100 - W_{\text{sample}}\cdot\%TS
\]

A summary of the main methods for milk whey characterization can be consulted in Table 5-1.

<table>
<thead>
<tr>
<th>Analysis type</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Phenol Sulfuric Acid Method</td>
<td>[4], [5]</td>
</tr>
<tr>
<td>Protein</td>
<td>Biuret Method</td>
<td>[7], [8]</td>
</tr>
<tr>
<td>Lipids</td>
<td>Soxhlet extraction</td>
<td>[5]</td>
</tr>
<tr>
<td>COD</td>
<td>ASTM 5220 D</td>
<td>[11]</td>
</tr>
</tbody>
</table>

5.2. Potato stem.

Physicochemical characterization of potato stem (PS) involves the determination of the chemical composition in terms of the extractives, cellulose, hemicellulose, lignin and ash.
content. At the same to MW, an additional characterization was carried out in terms of TS and VS. The characterization of PS was realized in the Instituto de Biotecnología y Agroindustria (IBA), bloque T, campus la Nubia, Universidad Nacional de Colombia sede Manizales. Finally, it is noted that all procedures will be described in the next sections were implemented for triplicate.

5.2.1. Moisture content.

Moisture content in biomass have an inverse relation with the calorific value. In this sense, the increased of moisture (0 to 40 %) for some specific types of biomass can be decrease the calorific value around of 66% [18].

The moisture content determination was carried out with PS previously dried and milled. The PS samples were analyzed using Shimadzu moisture balance MOC - 120H equipment. Thus, approx. 0.5 g of sample was put on the plate-equipment and the read of moisture percent was realized after 10 minutes.

5.2.2. Extractives content.

Extractive are a group of compounds present in lignocellulosic biomass that are not part of the cell wall structure and can be extracted through different organic solvents and water [19]. These are mainly composed by a complex mixture of inorganic compounds, such as sugars, amino acids, lipids, salts, waxes, terpenes, phenolic compounds and chlorophyll [19], [20]. It is necessary to remove these compounds before next characterization steps because of they cause interference in analytical methods such as lignin determination. The methodology used to perform the characterization is based on the National Renewable Energy Laboratories (NREL/TP-510-42619) [20], in which ethanol and water are used as extraction solvents.

The materials used in this method were extraction tubes (soxhlet syphon), condensers (NS 45/40), heating plates, flat-bottom flasks (500 ml), hoses, analytic balance and desiccator. While that reagents were ethanol and water. In first instance, 10 g of material was aggregated into a tared extraction thimble. Then, the thimble was placed on the Soxhlet syphon tube considering that the thimble must not exceed the Soxhlet syphon height. Then, 250 mL of distillated water was added to the tared receiving flask with several boiling chips to prevent bumping. In this moment, the mounting was start and the Soxhlet syphon with the condensers assembled and it’s was placed over the flat-bottom flask. Once, the cooling
water flow was started and stabilized, the heating plates were adjusted to ensure a minimum of 4-5 syphon cycles per hour.

After 24 hours of extraction, the thimble with the raw material was dismantled and placed in a convective oven overnight at temperature not exceeding 45°C. The dried thimble was removed from the oven and was transferred to a desiccator for one hour and weighted. The same procedure was repeated with ethanol as a solvent. The formula for determining the content of extractives with ethanol or water is presented in Eq. 5-8:

\[
\text{Extractives (\%, db) = } \frac{W_{\text{sample before extraction}} - W_{\text{sample after extraction}}}{\text{Oven dry weight}} \cdot 100
\]

5.2.3. Holocellulose determination.

Lignocellulosic biomass such as PS, there are composed by a mixture of different carbohydrates’ polymers which are denominated cellulose, hemicellulose and lignin. Holocellulose (~65 to 85%) comprises of the polysaccharides total fraction of extractive free biomass in the form of cellulose (~ 30%), hemicellulose (~ 20 to 30%) and pectin (~ 5 to 30%) [21]. On the other hand, it is noted that this calculation is an indirect form to determine the cellulose and hemicellulose.

The method used in the present thesis for holocellulose determination was chlorination method described by ASTM standard method entitled “Method of Test for Holocellulose in Wood”, which could be found as ASTM D1104-56(1978) [22]. The reagents used in this experimental method are sodium chlorite industrial grade, distilled water and acetic acid. Additionally, the materials used for development this method were analytic balance, thermostatic bath, Erlenmeyer flasks (100 and 250 mL), 1 mL pipettes and qualitative filter paper.

Free-extractives potato stem sample (2.5 g) was added to 250 mL Erlenmeyer flask with water (80 mL), acetic acid (0.5 mL) and sodium chlorite (1g). Subsequently, an inverted Erlenmeyer flask (100 mL) was put in the neck of the reaction flask because of these reaction produces toxic gases after a short time. In addition, the entire process must be done with the extraction cabin on. Simultaneously, the thermostatic bath must be set to 70°C, the mix reaction was homogenized and heated. After 60 min, 0.5 mL of acetic acid and 1 g of sodium chlorite were added. After each succeeding hour, fresh portions of 0.5 mL acetic acid and 1
g sodium chlorite are added with shaking during six reaction hours. Afterward, the samples were left 24 hours without further addition of sodium chlorite and acetic acid. In the end of 24 hours, the holocellulose samples were filtered on filter paper using a Buchner funnel with acetone (20 mL for each sample) and distilled water until yellow color and the odor of chlorine dioxide are removed. Afterwards, the filter was placed in a porcelain tray in the oven at 50°C for 24 hours until constant weight. The dried sample was transferred to a desiccator for 1 hour and weighed.

The holocellulose content was calculated according to following equation:

\[
\text{Holocellulose (\%, db) = } \frac{W_{\text{holocellulose}}}{W_{\text{dry sample}}} \cdot 100
\]

Equ. 5-10

5.2.4. Cellulose determination.

Cellulose is the main component of the plant cell wall. These is a linear polymer of glucose attached through β-1,4-O-glycosidic bonds. The cellulose structure is lineal and fibrous and have a hydrogen intermolecular bounding force, hence are insoluble in water [23]. In addition, this natural polymer is considered as renewable resource and widely used in biochemical processes for obtain value-added products.

The process for determination of cellulose content is after the holocellulose determination. This process is based on the test method T222 os-74 described in the book entitled “Paper and Composites from Agro-Based Resources” [24]. For develop the methodology was necessary the use of materials as watch glass, qualitative filter paper, water bath, 5 mL pipettes, 250mL beakers, glass rod, glass cover, dissector and analytical balance. Also, distilled water, acetic acid and sodium hydroxide were used in this method.

In first place, 2 g of vacuum-oven dried holocellulose were weighed and placed into a 250 mL glass beaker provided with a glass cover. Simultaneously, two NaOH solutions (one at 17.5% w/v and the other one 8.3% w/v) and a solution of acetic acid at 10% v/v were prepared. Afterwards, 10 mL of NaOH solution (17.5% w/v) was added to the holocellulose in the 250 mL beaker, cover with a watch glass, and maintain at 20°C in the water bath. Then, at five minutes intervals, it is necessary to add 5mL of NaOH solution (17.5% w/v) and thoroughly stir the mixture with the glass rod, until the NaOH is gone. Allow the mixture to stand at 20°C for 30 minutes, making the total time for NaOH treatment 45 minutes. After
the finish this treatment, 33 mL of distilled water at 20 °C was added to the reactive mixture and this was let repose for one hour before filtering.

In the second place, the reactive mixture was filtered with 100 mL of the second NaOH solution and distilled water at 20 °C. In addition, 10 mL of acetic acid solution was added with contact time cycles of three minutes. The remaining solids was washing with 250 mL of distilled water at 20 °C until all acetic acid was completely drained. Finally, the sample was dried at 60°C and weigh every hour until constant weight. Then, determine the hemicellulose mass through the difference between holocellulose and hemicellulose with the next formula:

\[
\text{Cellulose (\%, db)} = \frac{W \text{ Cellulose}}{W \text{ Holocellulose}} \times \frac{W \text{ Holocellulose}}{\text{Dry sample}} \times 100
\]

**Equ. 5-11**

### 5.2.5. Hemicellulose content determination.

Hemicellulose is a branched polymer composed by different types of sugars such as arabinose, xylose, galactose, mannose, fructose, glucose and others. The most common form of hemicellulose is a xylan, which corresponds to approximately 35 to 35 %db. of the plant cell wall [25]. Additionally, it is also remarkable that this percentage change depending of the biomass source, climate, soil conditions, and others. Taking account that, xylan content is higher in comparison with other compounds, the hemicellulose content was calculated from the subtraction between the holocellulose and cellulose content.

The hemicellulose content was calculated using the following equation:

\[
\text{Hemicellulose (\%, db)} = W \text{ Holocellulose} - W \text{ Cellulose}
\]

**Equ. 5-12**

### 5.2.6. Lignin content determination.

Lignin is a hydrophobic amorphous polymer composed by phenylpropanoids (i.e. p-cumarilic alcohol, coniferyl alcohol and sinapyl alcohol) resistant to hydrolysis and with aromatic characteristics [26]. In general, the lignin content is less in grass plants compared to wood plant due to the lignin is as an encrusting agent in the cellulose/hemicellulose matrix. The
method for determining the lignin content is "acid insoluble lignin" which is used extensively for the characterization of lignocellulosic biomass [27].

The procedure for the lignin determination is a modified version of TAPPI T222 acid-insoluble lignin in wood and pulp which is described in the book entitled "Paper and Composites from Agro-Based Resources" [24]. The reagents used in this experimental method are distillated water and sulfuric acid. Otherwise, the employed materials are test tubes (100 mL), test tube rack, thermostatic bath, analytical balance, 100 mL Erlenmeyer flasks, qualitative filter paper, vacuum filtration system and an autoclave.

This procedure can be realized at the same time the holocellulose determination. Approximately 200 mg of potato stem sample previously dried and milled was added into the test tubes. Then, 2 mL of sulfuric acid solution (72 % w/w) was added to each tube. These tubes were mixed with glass rod twice and were placed in a water bath at 30 °C for one hour. Afterward, 56 mL of distillated water was added to reduce the acid concentration. However, this process must be performed carefully. The diluted solution was transferred into a 100 mL Erlenmeyer flask and covered with aluminum foil. The mixture was autoclaved at 121 °C and 15 psia during one hour. The mixture was removed of the autoclave and its washed through the filtering process with hot water (100 °C). Before weighing, the remnant solid lignin sample was placed in an oven at 105 °C for a period of 24 hours. Then, the sample was moved to a desiccator for one hour and it was weighted.

The acid insoluble lignin content was calculated according to Equ. 5-13:

$$\text{Acid insoluble lignin (\%, db)} = \frac{W_{\text{acid insoluble lignin}}}{\text{Dried sample free extractives}} \cdot 100$$  

Equ. 5-14

**5.2.7. Ash determination.**

The same ash description in the item 5.1.4 for milk whey is applied in the potato stem context. In sense, for the ash determination were used similar materials and conditions. However, for lignocellulosic biomass is advisable to use the methodology reported by Renewable Energy Laboratories (NREL/TP-510-42622) [28].

First, 500 mg of extractives-free sample was added into crucible and the weight was recorded. After this, the crucible was put inside of the muffle and a series of heating ramps are programmed. Ramp from room temperature to 105°C. Hold at 105°C for 12 minutes.
Ramp to 250°C at 10°C per minute. Hold at 250°C for 30 minutes. Ramp to 575°C at 20°C per minute. Hold at 575°C for 180 minutes. Finally, the temperature is decreased until 105°C and the samples are removed. These were placed directly into a desiccator for two hours and weighted.

The ash content was calculated according to following equation:

\[
\text{Ash (\%, db)} = \frac{W_{\text{crucible + ash}}}{W_{\text{oven dry weight}}} \cdot 100
\]

Equ. 5-15

5.2.8. Total and volatile solids determination.

As indicated in the past description (Chapters 1 and 2) and methods (Item 5.1.5). TS and VS are a parameter of high important in biochemical processes. In this sense, the methodology for its characterization is a little different that TS and VS in milk whey.

The methodology for the characterization of potato stem was based on the norm ASTM E1756-08 standard method [29], which is described detail in the article of Sahito et al. entitled “Estimating Calorific Values of Lignocellulosic Biomass from Volatile and Fixed Solids”[30]. The materials used for this method were analytic balance, crucibles, desiccator and muffle furnace for operation at 105 °C and 550 °C.

TS content was measured using 2 g of potato stem sample. A sample of 2 g of potato stem was taken into the pre-weighted silica crucible, which was pre-dried at 105 °C for one hour. The crucible with the sample was put on the oven at 105 °C for six hours. Afterwards, the crucible was placed into desiccator during 2 hours and its was weighted. Following VS content, this was determinates by ignition of dried samples at 550 °C. After the TS determination, the crucible was placed into a muffle at 550 °C for two hours. Similarly, TS content, the crucibles was removed of the oven and this was weighted.

The TS and VS content was determined using the following equations:

\[
\text{TS (\%, w/w)} = \frac{W_{\text{crucible + solid (Dryed to 105 °C)}} - W_{\text{crucible}}}{W_{\text{sample}}} \cdot 100
\]

TS (\%, w/w) = \[
\text{Equ. 5-16}
\]

\[
\text{VS (\%, w/w)} = \frac{W_{\text{crucible + solid (Ignition to 105 °C)}} - W_{\text{crucible}}}{W_{\text{sample}}} \cdot 100
\]

VS (\%, w/w) = \[
\text{Equ. 5-17}
\]
A summary of the main methods for potato stem characterization can be consulted in Table 5-2.

**Table 5-2.** Standard methods used for potato stem characterization.

<table>
<thead>
<tr>
<th>Analysis type</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractives</td>
<td>NREL/TP-510-42619</td>
<td>[20]</td>
</tr>
<tr>
<td>Holocellulose</td>
<td>ASTM D1104-56</td>
<td>[22]</td>
</tr>
<tr>
<td>Cellulose</td>
<td>T222 os-74</td>
<td>[24]</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>Subtraction Holocellulose and cellulose</td>
<td>[22]</td>
</tr>
<tr>
<td>Lignin</td>
<td>TAPPI T222</td>
<td>[24]</td>
</tr>
<tr>
<td>Ash</td>
<td>NREL/TP-510-42622</td>
<td>[28]</td>
</tr>
<tr>
<td>TS and VS</td>
<td>ASTM E1756-08</td>
<td>[30]</td>
</tr>
<tr>
<td>Moisture</td>
<td>Shimadzu moisture balance</td>
<td>- -</td>
</tr>
</tbody>
</table>

5.3. Final remarks.

The physicochemical characterization methods described above comply with international standards. This translates into truthful data which will be easier to analyze considering the researches of scientific literature. In consequence, these methods will allow to know in more detail the feedstocks taking account its origin, crop age, climate and intermediate processes. Also, the data obtained can be compared with other resources such as book chapters, scientific articles, theses and others.

References


Chapter 5. Milk whey and potato stem characterization.


Chapter 5. Milk whey and potato stem characterization.


Biochemical Methane Potential (BMP) test was performed in the School of Biochemical Engineering from Pontificia Universidad Católica de Valparaiso, Chile. The present chapter gives a detailed description of the methodology used to evaluate the biogas and methane potential from milk whey (MW) and potato stem (PS). A detailed description of the equipments, materials, reagents and external procedures is given with deeply knowledge about procedure like inoculum acquisition, gas measurement, modeling and determination of kinetic parameters. In addition, a series of recommendations for the right implementation of the BMP test are described.

6.1. Experimental design.

For the BMP establishment of the MW and PS, a statistical analysis was considered with two extremes temperature. These extremes temperature were 37 °C and 55 °C. However, after previous experiments, the analyses were not considered due to similarly in the BMP results (data not showed). Therefore, the better temperature (37 °C) for biogas production was used for the following experimental procedures.

On other hand, two biotechnological routes were evaluated in the experimental biogas production from MW. The first route was the stand-alone production of biogas from MW and the second route is the production of biogas from co-digestion of MW and PS. So, co-digestion process was evaluated in three scenarios where different relation of MW and PS were evaluated. Consequently, the experimental design consisted of 5 experiments: stand-alone PS, stand-alone MW, and three co-digestion relations of both materials. Also, an extra BMP test was realized for the inoculum as the only substrate as blank of the others experiments. Finally, it should be noted that all the experiences were implemented in triplicate for a total of 18 experiments (Figure 6-1).
6.2. Inoculum preparation.

The inoculum is considered as a consortium of interdependent microorganisms that act in synergy to degrade organic matter. It comes from functioning anaerobic digesters; however, the origin of the inoculum is not limited to special types of digesters. In practice, the inoculum can be categorized in one of the following sources: (i) sewage sludge digesters, (ii) agricultural biogas plants (ABPs), (iii) biowaste treatment plants (BWTPs) and (iv) granular sludge [1].

Before performing the BPM test, it is necessary to condition the substrate and inoculum for the experiments. In the case of the inoculum, it is required to homogenize, remove non-homogeneous material and degassed. The latter in order to deplete the residual biodegradable organic material present in it. This process lasts typically between 2 and 5 days of incubation at working temperature when inoculum is non-taken from a reactor fed with relatively high fat/oil concentration [2], [3].
In this context, inoculum was obtained from a biodigesters of the residual water treatment plant named La Farfana in the Fifth Region of Chile, specifically in the industrial zone in Santiago de Chile localized at latitude 33° 27' 45.5'' south and longitude 70° 46' 59.4'' west. The La Farfana plant is the main water treatment center in Santiago, purifying about 60% of the region's wastewater and generating an average of 50,000 and 60,000 m³ of biogas.

After acquisition of Inoculum, it was transported to the School of Biochemical Engineering and afterward transferred to 1000 mL recipient. It was degassed for a period of 7 days in a hot chamber at 37 °C. Subsequently, this was homogenized and completely withdraw non-homogeneous material. Additionally, the inoculum was characterized with the same protocols of MW in terms of TS, VS and pH (see chapter 5).

6.3. Batch digestion test.

To evaluate the application of anaerobic treatment systems in the degradation of a solid organic substrate it is necessary to previously determine its methanogenic potential [2].

The BMP test was developed using the German standard VDI_4630 [3] entitled: "Fermentation of organic materials – Characterization of the substrate, sampling, collection of material data and fermentation test" and the protocol proposed by Angelidaki et al., [2] entitled: "Defining the biomethane potential (BMP) of solid organic wastes and energy crops: protocol for batch assays ". These gives all the know how of the main characteristics and procedures to carry out the assay. Within the information provided a detailed of the substrate and inoculum conditions, materials, and reagents, among others.

The Biochemical Methane Potential procedures employed were developed taking account a series of considerations. These are listed below:

1. The particle size of the solid substrates (in this case PS) was the same used for characterization: a standard size of 40 mm. This in order to maintain the reproducibility of measurement data and thus prevent the effect of particle size on the biodegradability kinetics.

2. The preliminary characterization was developed according to the recommendations presented by Angelidaki et al., [2]. In this way, for the solid substrates as PS was necessary to calculate biodegradable and non-biodegradable compounds such as lignin.
3. The inoculum for BMP tests was always "fresh". It was obtained from an active anaerobic reactor and was prepared according to the recommendations in the section above.

4. A medium containing a great amount of minerals/vitamins/macro and micronutrients was prepared for the proliferation of microorganisms consortium. Therefore, could be assumed that there is no nutrients limitation in the BMP tests.

5. Blank and controls assays were carried out in triplicate for statistical significance. The first was used for measuring the methane production from the inoculum using medium and no substrate. The second was used for obtaining an idea of the inoculum response toward "standard" substrate as glucose.

The materials to carry out the procedure were glass vessels (120mL), septum and aluminum caps, beakers (250 and 500 mL), pipettes, micropipettes, magnetic stirrer, burettes, incubation chamber (37 °C), pH-metro, hoses, pressure gauge, among others. The reagents used to prepare macronutrient, micronutrient and pH adjustment solutions will be described below.

The solutions for the BMP test correspond to macro and micronutrient solutions, sulfur solution, yeast extract solution, alkaline solution of sodium hydroxide, and sodium hydroxide and chloride acid (2N) solutions. It must be noted that the sulfur solution have to be fresh and therefore it was prepare at same time when the rest was carried out follow 0.025% Na2S.9H2O. Yeast extract solution was prepared whit 0.2 g of yeast extract in a volume 1000 mL of distillated water. The alkaline solution of sodium hydroxide contain 25 g of NaOH in 1000 mL of distillated water. Finally, the preparation of macro and micronutrient solutions can be consulted in Table 6-1 and Table 6-2.

The anaerobic process in batch systems depends on several environmental factors that influence in methane production. Therefore, it is necessary to maintain optimal and constant experimental conditions to obtain a reproducible and reliable results. The methanogenic activity was carried out in glass bottles (120 mL) with a liquid effective volume of 100 mL.

Table 6-1. Macronutrients solution.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Concentration [g/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>100</td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
</tr>
<tr>
<td>MgCl₂.6H₂O</td>
<td>10</td>
</tr>
<tr>
<td>CaCl₂.2H₂O</td>
<td>5</td>
</tr>
<tr>
<td>K₂HPO₄.3H₂O</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 6-2. Macronutrients solution.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Concentration [g/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₂.4H₂O</td>
<td>2</td>
</tr>
<tr>
<td>CoCl₂.6H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>MnCl₂.4H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>CuCl₂.2H₂O</td>
<td>0.038</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>0.05</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.05</td>
</tr>
<tr>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>Na₂SeO₃.5H₂O</td>
<td>0.1</td>
</tr>
<tr>
<td>NiCl₂.6H₂O</td>
<td>0.092</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.5</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>0.05</td>
</tr>
<tr>
<td>HCl 36%</td>
<td>0.5</td>
</tr>
<tr>
<td>Reassuring</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The volume of inoculum was estimated considering an inoculum concentration of 4.0 g VS/L in the reaction medium [4]. Simultaneously, the macro and micronutrient solutions were mixed in 500 mL beakers, considering that 100 mL of reaction medium requires 1 and 0.4 mL of the macro and micronutrient solutions, respectively. Then, water and sodium bicarbonate were added to the previous mixture. In this way to neutralize the organic acids production and maintain the pH (approx. 7.0) during the test. The experimental ratio was 1 g bicarbonate/g VS of the inoculum. The inoculum/substrate ratio for BMP test was 2:1 in terms of grams of Inoculum’s volatile solids and substrate’s volatile solids [5]. After the substrate addition, the glass bottles were gassing with nitrogen for 3 min to eliminate the air, and hermetically sealed with gums and aluminum caps. Finally, all biodigesters were incubated for 40 days at a temperature of 37 °C, until the cease of gas production.
6.4. Gas measured and sampling.

6.4.1. Methane and biogas production.

Different methods are used to perform a measure of biogas production in digesters. Each method differs in terms of reactors, units, techniques, gas sampling, gas collection, among others. However, the volume of gas produced can be measured using manometric and volumetric methods. The measurement of the gas volume by the manometric method requires a gas pressure measurement instrument and a gas chromatography (GC) and/or an alkaline solution system for the determination of the composition of the gas.

The methane and biogas production were measurement daily in the biodigesters for a period of 10 days. From there, the measurement was between 2-5 days of gas production. The quantification of biogas volume production was carried out with a gas pressure measurement instrument where the individual pressure is registered in each bottle. The above was made with prior calibration of the equipment at 0 mbar of pressure.

On the other hand, the methane production was determined taking into account the NaOH displacement method [6]. This method consists in passing the gas through a concentrated solution of NaOH, where the CO₂ in the fermentation is absorbed and the methane concentration can be estimated. The above, takes into account that other possible gases such as sulfides are negligible and that they are in a minimum proportion [7]. Figure 6-2 showed the method implemented in this Thesis for gas measured and sampling.

Figure 6-2. Gas volume measurement with a gas pressure measurement instrument and displacement method.
6.4.2. Normalized methane and biogas production.

The environmental conditions of the BMP tests experiments must be corrected for the measurement of the gas volume. This is because of the drastically changes of gas volume due the environmental conditions as pressure and temperature, causing an underestimation or overestimation of the real value. In this sense, the scientific community has made the effort to corrected this error. However, the data analysis of the reported papers does not emphasizes on this subject and could potentially induce errors.

Accordingly, the BMP data can be calculated and report in a standardized manner. This means that the gas volume can be normalized to standard conditions of pressure (1 bar) and temperature (25 °C). Accordingly, the biogas and methane volume produced during the test is expressed in terms of NmL of biogas or NmL of CH4/g VS of substrate using the ideal gas law as presented in the next equation [8]. Therefore, standardization of the BMP test is required to ensure that the results from different research groups are reliable and comparable.

\[
\text{VSTP} = \left( \frac{P_{\text{gas}}}{P_{\text{stp}}} \right) \cdot \left( \frac{T_{\text{stp}}}{T_{\text{gas}}} \right) \cdot V_{\text{gas}}
\]

Equ. 6-1

VSTP is the volume adjusted to standard conditions, \( P_{\text{gas}} \) is the pressure of the measured gas, \( P_{\text{stp}} \) is the standard pressure, \( T_{\text{stp}} \) is the standard temperature in Kelvin (K), \( T_{\text{gas}} \) is the temperature of measured gas in K, and \( V_{\text{gas}} \) is the measured gas volume.

6.5. Digesters characterization.

The digesters characterization is important because gives relevant information about the biodegradable material not consumed, the acids not consumed by methanogenic bacteria and inorganic material. In this sense, the digester characterization consisted in the quantification of the main acids generated in the hydrolysis stage, the pH measurement, the ST and SV characterization in the reaction medium. Factors such as SV, ST and organic acids are important to know the complete or incomplete degradability of the substrate [9], [10]. In this way, higher amount of organic matter, lower the amount of solids, and lower the quantity of acids in the medium, greater biogas production. On the other hand, considering the pH, this is a key factor in the performance of the test. It is responsible for maintaining
stable the consortium of microorganisms without causing stress and/or death of them. Thus, a neutral pH is recommended to maintain this condition [9].

The analyzes of the reactors were performed for a period of 10, 20, 30 and 40 days after started the BMP test. The analytical methods used to characterize the biodigesters with respect to ST, SV and pH are described in the chapter above.

6.6. Theoretical maximum yield and biodegradability.

The maximum methane (TMY) and biogas yield can be calculated from the experimental characterization described in the previous section. In this sense, based on the physicochemical characterization can be calculated the maximum biogas and methane potential from MW and PS. The most common equation for this propose is using the Buswell’s equation [11]; which considers that all organic material is biodegraded in the BMP test. However, lignocellulosic substrates contain non-biodegradable organic material such as lignin. In this case, Chen’s formula [12] is applied due to be more rigorous. The Buswell’s formula and Chen’s formula are shown in equations Equ. 6-2 and Equ. 6-3, respectively.

$$C_aH_bO_cN_d + \left( a \cdot \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} \right) \cdot H_2O \Rightarrow \left( \frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} \right) \cdot CH_4 + \left( \frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} \right) \cdot CO_2 + dNH_3$$

Equ. 6-2

$$TMY \left( \frac{mL \cdot CH_4}{g \cdot VS} \right) = \frac{22.4 \cdot 1000 \cdot \left( \frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} \right) \cdot \left(1 - lignin \% - ash \%\right)}{12a + b + 16c + 14d}$$

Equ. 6-3

On the other hand, the theoretical yield value and the experimental yield are used to calculate the level of biodegradability (BD) under defined test conditions. Then, once the experimental assays had finished the biodegradability of the substrates and co-digestions were analyzed in order to evaluate the level of anaerobic biodegradability under the defined BMP test conditions. The equation Equ. 6-4 is used to calculate the BD of the substrates [13].

$$BD(\%) = \frac{B_{Exp}}{B_{oTh}} \cdot 100$$

Equ. 6-5
Where $B_o\_Exp$ is the experimental methane production, $B_o\_Th$ is the theoretical methane production and BD(%) is the percentage of biodegradability.

Finally, the co-digestion of certain substrates can produce synergistic effects, which refers to an additional biogas/methane production compared to the average of the individual substrates. In contrast to the synergistic effect, the antagonistic effect has the opposite influence because of its causes a lower of biogas/methane production. Such effects can emerge from the conditions in the AD considering variables such as alkalinity, enzymes, nutrients, volatile solids, trace elements, or any other improvement which a substrate by itself may lack [14]. The synergistic effect index (SEI) for the anaerobic co-digestion proposed by Li et al [13] was calculated as shown in Equ. 6-6.

$$SEI = \frac{B_o\_Exp\_\text{(co)}}{X_1\cdot B_o\_Exp\_\text{(1)} + X_2\cdot B_o\_Exp\_\text{(2)}} \cdot 100$$

Equ. 6-6

Where $B_o\_Exp\_\text{(co)}$ is the $B_o\_Exp$ of a co-digestion. $B_o\_Exp\_\text{(1)}$ and $B_o\_Exp\_\text{(2)}$ are the $B_o\_Exp$ of the mono-digestions of PS and MW, respectively. $X_1$ and $X_2$ are the VS fractions of PS and MW in the co-digestion, respectively.

### 6.7. Modeling and kinetic parameters determination.

The BMP test assays provide information on the degradation kinetic of the substrates. The parameters of degradation kinetics are of vital importance for the development and operation of anaerobic treatment systems. This is the key information for the selection of substrates, process analysis, optimization and process design. The modeling of the degradation kinetics also allows to know the biodigester performance, a more stable operation and a better control of the process.

The kinetic parameters of degradation are sensitive to experimental conditions such as temperature, inoculum, particle size, substrate concentration, substrate ratio, among others. Mathematical models are used for an increased understanding of the degradation and biogas production phenomenon. Thus, mathematical models provide information on the rates of the material degrades, selection of a potential substrate and/or the evaluation of synergistic effects in codigestion process. It has been generally assumed that the hydrolysis of the substrate follows the first order model or a modified first order model. A model that
has been widely applied to simulate and predict the performance of anaerobic digestion in the BMP test is the modified Gompertz model (Equ. 6-7) [13], [15], [16]. Which is the model applied in this thesis.

\[
B = B_o \cdot \exp \left( -\exp \left( \frac{\mu_m \cdot e}{B_o} \cdot (\lambda - t) + 1 \right) \right)
\]

Equ. 6-7

where, \( B \) represents the simulated cumulative methane yield (mL/g-VS); \( B_o \) refers to the simulated maximum cumulative methane yield (mL/g-VS); \( \mu_m \) means the maximum methane production rate (mL/g-VS/day); \( e \) is equal to 2.718; \( \lambda \) stands for the lag phase time (day); \( t \) is the digestion time (day).


The biochemical methane potential test described above is a relevant aspect for the study of anaerobic digestion. This chapter take into account important knowledge like the main international standards for the study of the organic matter degradation and biogas production, reporting experimental procedure like inoculum preparation, materials for batch digestion, gas measured and sampling, digester characterization and finally, the modeling and kinetic parameters. These data are very important for development of this thesis, which provide important information about the comparison between stand-alone and the effect of co-digestion process in biogas production from milk-whey and potato stem. Additionally, this is the first step for the selection of substrates, process analysis, optimization and process design executed in the followings chapters.

References.


materials – Characterization of the substrate, sampling, collection of material data and fermentation test,” 2016.


7. Characterization results and its potential for the production of biogas.

This chapter presents the results of the physicochemical characterization of the raw materials and inoculum used in this thesis. In this sense, MW and PS were analyzed in terms of organic matter, carbohydrates, lipids, extractives and protein. While that the Inoculum was analyzed in terms of the total and volatile solids. Additionally, the potential of biogas and methane production, from both raw materials, was analyzed. The production yield, gas composition, process efficiency and kinetic parameters were determined. The results of the raw materials characterization obtained in this work were compared with literature data. On other hand, the methanogenic potential was evaluated using each raw material independently or in co-digestion scenarios, comparing the kinetic variables of the digestion process and the biogas yield. These parameters are important in the design, operation and start-up of biogas treatment plants (it was observed in the previous chapters). Then, the experimental results allow to establish the quality of the waste and its potential converting to energy vector such as biogas.

7.1. Chemical characterization.

MW and PS were characterized in terms of its physicochemical composition and the potential of these residues for biogas production were evaluated. These results have a great importance biochemical process, being necessary to understand the mechanisms of conversion, and determining the specific organic matter that can be used. Also, knowing the composition - characterization process – of the raw materials used is very interesting, because of the differences that may exist with the same substrates of different origin (other
7.1.1. Milk whey characterization.

The composition of the MW is showed in Table 7-1. In the table can be observed that the most representative component of MW are water and carbohydrates, followed by proteins and lipids. The high moisture content (96.04%) of this raw material is agree than the reported by Saddoud et al., [1]. The content of lipids, carbohydrates, and proteins would allow to establish the potential use of the MW in biotechnological applications such as biogas production.

<table>
<thead>
<tr>
<th>Feature</th>
<th>This work</th>
<th>Saddoud et al. [1]</th>
<th>Beszédes et al. [2]</th>
<th>Orive M. [3]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>96.04 ± 0.19</td>
<td>94.2</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Protein</td>
<td>0.13 ± 0.13</td>
<td>0.27± 0.05</td>
<td>0.93</td>
<td>1.21</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>2.45 ± 0.64</td>
<td>4.59± 0.88</td>
<td>4.20</td>
<td>5.31</td>
</tr>
<tr>
<td>Fat</td>
<td>0.18 ± 0.20</td>
<td>0.94± 1.14</td>
<td>0.26</td>
<td>0.42</td>
</tr>
<tr>
<td>Total Solids</td>
<td>2.55 ± 0.15</td>
<td>5.93± 0.38</td>
<td>- - -</td>
<td>5.43</td>
</tr>
<tr>
<td>Volatile Solids</td>
<td>2.26 ± 0.15</td>
<td>5.61± 0.36</td>
<td>- - -</td>
<td>5.38</td>
</tr>
<tr>
<td>COD (g/L)</td>
<td>40.89 ± 1.16</td>
<td>68.6 ± 3.3</td>
<td>36</td>
<td>53.15</td>
</tr>
<tr>
<td>pH</td>
<td>4.8 ± 0.21</td>
<td>4.9 ± 0.27</td>
<td>2.0</td>
<td>6.56</td>
</tr>
</tbody>
</table>

On the other side, chemical composition values are low compared to scientific literature. The protein, carbohydrate and lipid content of our residue are 0.13%, 2.45% and 0.18%, respectively, and the COD (organic matter) is 40.89 g/L, which is lower than those reported for the whey obtained from the Tunisian Cheese Factory in Tunisia with a COD of 68.6 g/L. In addition, it can be observed that the content of total and volatile solids in MW is approximately 50% lower than reported by Beszédes et al., [2] and Orive M. [3]. In this way,
considering these characteristics, this residue (MW) is more like a cheese permeate than MW. This sentence is justified by the research of Barile et al., [4] when the content of lipid and protein in whey permeate are 0.1% and 0.17%, respectively. Finally, the MW can be classified depending by the type of precipitation/coagulation of casein into two types: sweet whey and acid whey (see chapter 3). Our results showed that the pH of the MW is 4.0, hence, which means that our whey is acidic. In addition, MW vary according to the environmental conditions, such as the milk origin and the production process in the cheese elaboration [5]. For example, the differences in the operation of the filtration where solid particles are suspended.

7.1.2. Potato stem characterization.

PS was characterized following the parameters presented in the chapter 5. The results from the physicochemical characterization are presented in Table 7-2. As can be seen in this table, the most significant component is the lignocellulose, which is an interesting resource for biotechnological applications such as to obtain fermentable sugars, syngas production thought a thermochemical process and biogas production from AD. Additionally, PS has a significant amount of extractives, which may be rich in antioxidants and/or phenolic compounds of industrial interest. These results indicate the potential of this emerging and new raw material for obtaining value-added products through different biotechnological routes.

Table 7-2. Physicochemical characterization of potato stem.

<table>
<thead>
<tr>
<th>Feature</th>
<th>This work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>36.03 ± 0.32</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>27.18 ± 0.40</td>
</tr>
<tr>
<td>Lignin</td>
<td>11.45 ± 0.56</td>
</tr>
<tr>
<td>Extractives</td>
<td>12.57 ± 0.11</td>
</tr>
<tr>
<td>Ash</td>
<td>2.10 ± 0.21</td>
</tr>
<tr>
<td>Moisture</td>
<td>9.67 ± 0.02</td>
</tr>
<tr>
<td>Total Solids (TS)</td>
<td>92.63 ± 0.05</td>
</tr>
<tr>
<td>Volatile Solids (VS)</td>
<td>90.06 ± 0.03</td>
</tr>
</tbody>
</table>
As for the lignin content in PS is 11.45%. This value is not as high as the other components of the cell wall. However, the lignin of PS can generate interference because of its non-degradable characteristics. For example, in the biogas production, the lignin has a direct influence on the yield because of causing problems of the accessibility and crystallinity [6].

On the other hand, the low ash content makes it suitable for application in thermochemical processes. However, the energy consumption associated with the removal of moisture increases their costs significantly. Finally, the high content of VS is ideal in biotechnological applications such as methane production. Nevertheless, the PS biodegradability is low because of the high recalcitrance and it difficult the access based on the action of microorganisms to break down organic matter. Despite not having information to compare in relation to PS in open access literature, this topic has been analyzed with respect to similar raw materials such as wheat straw, cotton stem and sorghum stem.

According to the information showed in Table 7-3, the chemical composition is similar in terms of cellulose compared to other stem residues such as stem of cotton, wheat and sorghum. Thus, the cellulose content in the three residues are between 35-39%. However, the hemicellulose can vary greatly among all stem waste analyzed, ranged of 19 to 40%.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Potato stem</th>
<th>Wheat straw</th>
<th>Cotton stem</th>
<th>Sorghum stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This work</td>
<td>Chandra et al., [6]</td>
<td>Patel V. R., [9]</td>
<td>Zhang et al., [10]</td>
</tr>
<tr>
<td>Cellulose</td>
<td>39.88 ± 0.32</td>
<td>38.09</td>
<td>39.56 ± 0.79</td>
<td>35.45 ± 1.53</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>30.09 ± 0.40</td>
<td>25.78</td>
<td>19.85 ± 0.56</td>
<td>40.21 ± 1.16</td>
</tr>
<tr>
<td>Lignin</td>
<td>13.79 ± 0.56</td>
<td>8.14</td>
<td>22.35 ± 0.58</td>
<td>12.32 ± 2.15</td>
</tr>
<tr>
<td>Extractives</td>
<td>13.92 ± 0.11</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Ash</td>
<td>2.32 ± 0.21</td>
<td>- -</td>
<td>5.02 ± 0.14</td>
<td>- -</td>
</tr>
<tr>
<td>Total Solids (TS)</td>
<td>92.63 ± 0.05</td>
<td>94.2</td>
<td>96.35 ± 1.25</td>
<td>94.14 ± 0.06</td>
</tr>
<tr>
<td>Volatile Solids (VS)</td>
<td>90.06 ± 0.03</td>
<td>88.9</td>
<td>82.12 ± 1.35</td>
<td>92.06 ± 0.18</td>
</tr>
</tbody>
</table>
On the other hand, the lignin percentage of the PS may be compared to the sorghum stem reported by Zhang et al., [7] with a value of 12.32%. The content of ash in PS is 2.1%, which is low compared to the 5.02% informed by Patel V. R. [8]. Finally, the amount of TS is equivalent in all the waste evaluated, with values greater than 90%.

Thus, it is possible to conclude, from the chemical characterization that the PS can be used in similar processes studied for other stem types. However, it is necessary to analyze other mineralogical aspects and the obtaining of value-added compound due to the high extractives content.

### 7.2. Biochemical methane potential (BMP).

The results of BMP test are described in this item, which contains relevant information about of the anaerobic digestion process. In this sense, the 6 scenarios outlined in the methodology are thoroughly analyzed in terms of gas production, yield, process efficiency, kinetic parameters and intrinsic variables such as pH.

#### 7.2.1. Daily and accumulated gas production in the BMP test.

**Figure 7-1 and Figure 7-2** shows the kinetic behavior of the biogas and methane production for the scenarios: stand-alone PS, stand-alone MW, and three co-digestion relations of both materials. The above in order to obtain key information concerning the effect of co-digestion on the stand-alone biogas production from MW and PS.

The accumulated biogas production is showed in **Figure 7-1**. In this figure, can be observed that the greater biogas production is for MW, followed by co-digestion 3 where the proportion of MW and PS is 2:1 in terms of VS. On the other hand, the production of biogas is lower for the PS compared to other scenarios. This last result can be explained for the physicochemical characteristics of the substrate, where the cellulose and hemicellulose are hard to degrade [11]. In addition, these compounds are characterized by a high cellulose crystallinity, which decrease the organic matter availability for the microorganism’s consortium [12].
Chapter 7. Characterization results and potential of raw materials for biogas production.

Figure 7-1. Accumulative biogas production in BMP assay.

Figure 7-2. Accumulative methane production in BMP assay.

On the other hand, the co-digestion process of both materials increase the yield of biogas production respect to the stand-alone biogas production from PS. However, this phenomenon does not occur using MW, which can be explained for the nature of the two substrates. Thus, the MW is composed by biodegradable matter (approx. 99%) in the form of carbohydrates, protein and lipids [13]. While, in the co-digestion there is also the presence
Chapter 7. Characterization results and potential of raw materials for biogas production.

of lignocellulosic material, which is difficult to degrade. For this reason, it can be inferred that the biogas generation in co-digestion scenarios decrease with the amount of PS used in the BMP test. In this sense, the biogas generation from co-digestion processes vary according to the raw material composition between 200 mL of biogas/g SV and 100 mL of biogas/g SV. Where the higher production is for the ratio 2:1, followed by 1:1 and 1:2 of MW:PS in terms of g SV.

The results of methane production have a similar profile that the biogas production described in the Figure 7-1. The maximum production was reached with MW, followed by the co-digestion scenarios and finally the digestion of PS. Thus, for the MW was reached a net production of 277 mL CH₄/g VS. In contrast, the lowest production occurred with PS with a yield of 92.18 mL CH₄/g VS. While the co-digestions were in the range of 100 - 200 mL CH₄/g VS.

The specific yield of biogas and methane in the proposed scenarios is shown in Figure 7-3. Figure 7-3 show that the highest specific biogas yield of BMP assays is the mono-digestion of MW with a value of 277.44 mL biogas/g VS. The mono-digestion of PS obtained the lowest specific yield with a value of 92.18 mL biogas/g VS, which is 66.77% lower than that of the MW experiment. The highest BMP yield of co-digestion experiments was 198.89 mL biogas/g VS, which is 28.31% lower than the digestion of MW. In contrast to the above comparison, the mono-digestion assay using PS showed to BMP value 53.65% lower than the highest value (C3) of the co-digestion using MW and PS. It is worth to mention that respect to the co-digestions of MW and PS, the best relationship for biogas production is 2:1 of whey:stem in terms of VS. This ratio has a yield 20.61% higher than the ratio 1:1 and 13.03% higher than the ratio 1:2.
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Figure 7-3. Specific biogas and methane production in BMP assay.

The findings of this study are also compared to the literature, where a range of values is establishing for biogas yield. Thus, a recent BMP study of cheese whey with granular sludge elaborated by Peña-Vargas and Durán-Moreno [14] reports a specific biogas yield of 60 mL biogas/g VS. This value is lower than the obtained in this study (277.44 mL biogas/g VS). On the other hand, these values are much lower than those reported by Bolen et al., [15] and Escalante et al., [16]. These last authors applied the BMP test for 5 dairy companies in Colombia. The BMP values were between 510 and 600 mL CH$_4$/g VS added. However, this study doesn't consider the synergistic effect of the inoculum, due to the its poor degassing. This could diminish the precision when measuring the net gas production and leads to an increase in the specific performance values of the substrates evaluated [17].

Co-digestion researches with stem and MW residues have not been widely explored (this is the first study in the case of PS). Recent results of co-digestion of MW with wastes also have been reported by Lovato et al., [18] and Carlini et al., [19]. In the latter, the results are comparable to those results obtained in this thesis with PS. The researchers Carlini et al. [19], obtained a specific biogas yield of 223 mL of biogas/g VS in the co-digestion of poultry manure and MW (ratio 1:1), which is a value a little higher than that obtained by C3 with a value of 198.89 mL of biogas/g VS with a 2:1 ratio of MW and PS. In the same way, when the ratio of poultry manure and MW increase of 1:1 to 3:1 reduced the biogas yield in 39.46%. This last phenomenon occurs when the ratio PS and MW (2:1) increased, where the biogas production decreased in 20.61%.
A very important variable for the biogas application is its percentage of methane, which affects the costs in the purification process when biogas is used as a substitute of natural gas [20]. In this sense, the results for methane percentage in BMP test are between 48% and 65% reported by Carlini et al.,[19], Rasi et al.,[21], and Pilarska et al.,[22]. Also, it should be noted that there is a high methane percentage in co-digestion 1 and 3, where its values are 70.51% and 74.46%, respectively. The above means, that the co-digestion of these materials is promising for the biogas production. These processes can be to reduce production costs in biogas applications due to high methane content.

The daily biogas and methane production can be seen in Figure 7-4 and Figure 7-5. These shows that for all scenarios the maximum daily production of biogas and methane is reached in the first 10 days, where there is an instantaneous generation of methane and biogas.
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Figure 7-5. Daily methane production in BMP assay.

The highest amount of biogas generated was approximate 31 mL biogas with the MW and C3 scenario on day 6. On the other hand, the gas production decreased considerably in all samples on day 12 and subsequently to increase until to 21 days. This effect can be explained due to the depletion of the easily biodegradable material (day 12) and the effort of the microorganisms to break the lignocellulosic matrix for the gas generation (until day 21). This is confirmed by Gurung et al., [23] where it explain the that usually the hydrolysis phase is slow for lignocellulosic compounds.

7.2.2. pH.

One of the most important parameters in biological systems is the amount of acid formed in the medium of reaction, which can be known through an indirect measurement by means of the pH. In this sense, the optimal pH varies depending on the author in systems of degradation by means of anaerobic digestion. However, the optimal pH according to Syaichurrozi et al., [24] could be in the range of 6.4-8.2. On the other hand, the output of the range can cause a low gas production, inhibition and death of the methanogenic bacteria, which are the most sensitive in these systems.
Figure 7-6 shows the behavior of the pH at different time intervals in the BMP test. It can be observed that initially there is an important decrease of pH value, which is reflected at day 5 with values ranged 6.74 to 7.1. This is correlated with the maximum gas peak generated in all the reactors (Figure 7-5). However, these values stabilize during the whole test around 7.1. So, taking into account the optimal pH values described in the Chapter 1, this pH (7.1) characterized by a good buffer property and adequate conditions for the microorganisms. Where the buffer property can been explained by the amount of calcium carbonate added to the medium in order to prevent acidification.

![Figure 7-6. pH measurement during BMP assay.](image)

7.2.3. Biodegradability and synergistic effects.

The results of the biodegradability of the substrates, considering the theoretical potential using the Buswell equation are presented in Table 7-4. In addition to the biodegradability, in this same table, the synergic effects in the co-digestion of MW and PS using equation Equ. 6-6 (see section 6.6) are presented.

Similar theoretical values of digestion using different MW and PS ratios can be observed, with biogas theoretical potential values in the BMP tests ranged from 402.76 to 418.82 mL/g
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VS. The highest and lowest value were for the mono-digestion of MW and the mono-digestion of PS, respectively. Unlike this similarity, biodegradability determined experimentally is very different in each scenario, where the highest value was for the mono-digestion of MW (68.88%) and the lowest biodegradability was obtained for PS (22.01%). Biodegradability values increased with the MW fraction in the co-digestion. The highest value was 48.89% with a ratio of MW:PS (3:1), followed by the ratio MW:PS (1:3) with 41.70% and the ratio MW:PS (2:2) with 38.44%. These values are lower compared to the mono-digestion of MW.

**Table 7-4.** Digestion performance in BMP test assay.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Theoretical yield (mL/g VS)</th>
<th>Experimental yield (mL/g VS)</th>
<th>BD (%)</th>
<th>SEI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS:MW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0:4</td>
<td>402.76</td>
<td>277.44</td>
<td>68.88</td>
<td>-</td>
</tr>
<tr>
<td>1:3</td>
<td>406.77</td>
<td>198.89</td>
<td>48.89</td>
<td>-13.95</td>
</tr>
<tr>
<td>2:2</td>
<td>410.79</td>
<td>157.90</td>
<td>38.44</td>
<td>-14.56</td>
</tr>
<tr>
<td>3:1</td>
<td>414.81</td>
<td>172.98</td>
<td>41.70</td>
<td>24.90</td>
</tr>
<tr>
<td>4:0</td>
<td>418.82</td>
<td>92.18</td>
<td>22.01</td>
<td>-</td>
</tr>
</tbody>
</table>


The results were consistent with literature review, where that co-digestion process can be decreasing the biodegradability with other substrates [27]. Comparatively, the results obtained differs from those reported by Li et al., [25] and Li et al., [26], where the co-digestions increased the biodegradability and methane yield compared to the mono-digestions. However, the substrates are different to our residues. Li et al., [25] evaluated the co-digestion of sheep dung and waste paper. While, Li et al., [26] evaluated the co-digestion of corn stover and chicken manure.

One explanation to the results obtained in this work can be associated with the substrates characterization. Thus, the high biodegradability of the MW is due to its composition, which contain mainly easily biodegradable material by the consortium of microorganisms (carbohydrates, lipids and proteins). Therefore, the PS is difficult to biodegrade due to the amount of cellulose and hemicellulose present. As a comparison, in previous researches
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with observed that 34-41% is biodegradable in corn stover and 6-21% in wheat straw [28]. The above causes a high resistance to degradation, considering also the non-digestible lignin [25].

On the other hand, the co-digestion of certain substrates can produce synergistic effects, which refers to an additional biogas/methane production compared to the average of the individual substrates. In contrast to the synergistic effect, the antagonistic effect has the opposite influence because of its causes a lower of biogas/methane production. Such effects can emerge from the conditions in the AD considering variables such as alkalinity, enzymes, nutrients, volatile solids, trace elements, or any other improvement which a substrate by itself may lack [29].

The SEI of the co-digestions of MW and PS were calculated. The highest SEI (24.9%) in the co-digestions of PS and MW was also attained at ratio of 3:1, which is the only one that had a synergic effect in the BMP test. Conversely, the results of BMP test of this research work indicate an antagonistic effect between the two substrates in the two remaining co-digestion experiments. Similar co-digestion researches were development for biological sludge, where the results indicated that antagonistic effects for mixtures between 5-25% weight of different sludge sources [30]. However, other results for co-digestion of organic fraction municipal solid and biological sludge [29] and co-digestion of sheep dung and waste paper [25] have a synergic effect in the methane production.

7.2.4. Modeling of BMP assay results.

The biogas production from MW and PS was evaluated using the Gompertz model, which is the closed mathematical model most used in this type of processes. In this sense, the results of the data adjustment to the Gompertz model equation (see Equ. 6-7) is observed in Figure 7-7.

The data adjustment shows values of $R^2$ between 0.97 and 0.99 for the 5 experiments. The main parameters in the modeling of biogas production can be observed in the Table 7-5. This model presents a lower difference in methane yield ($\beta_0$) for the evaluated substrates (1.85-10.47%). Where the prediction error is measured as the percentage difference between experimental data and estimated through simulation. Thus, the greatest difference in the prediction was for PS with an error of 10.47%, followed by C2 with 5% of error. The remaining tests have a predictive error below 2.88%. These results are higher compared to
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the error obtained Pengfei et al [31], which obtained values between 0.0-0.3 percent. However, our values are lower than obtained by Nielfa et al [29] with errors ranged from 5-8%.

![Graph](image-url)

**Figure 7-7.** Modeling of BMP assay using Gompertz equation.

**Table 7-5.** Parameters of Gompertz equation in BMP test assay.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MW</th>
<th>PS</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_o$</td>
<td>270.01</td>
<td>82.53</td>
<td>168.00</td>
<td>150.00</td>
<td>195.21</td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>29.13</td>
<td>8.16</td>
<td>24.50</td>
<td>20.66</td>
<td>26.43</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>1.47</td>
<td>0.10</td>
<td>1.13</td>
<td>0.86</td>
<td>1.23</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.99</td>
<td>0.97</td>
<td>0.99</td>
<td>0.98</td>
<td>0.97</td>
</tr>
</tbody>
</table>

$\beta_o$: simulated maximum cumulative methane yield (mL/g VS), $\mu_m$: maximum methane production rate (mL/g-VS/day), $\lambda$: lag phase time (day).
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On the other hand, the latency phase ($\lambda$) is between 0.10-1.47 days, where the short-range latency time is for PS with 0.10 days. These results are comparable with the BMP assays in the anaerobic digestion from crops residues and anaerobic digestion from wastewater screening with values between 0.00-1.11 days [31] and 1-3 days [32], respectively. This translates into a very low latency phase in all the experiments evaluated, which can be explained by the high availability of biodegradable compounds and a good activity of the inoculum.

An important concept is the biogas production rate which is governed by the $\mu_m$ parameter. In this experience, MW has the highest production rate due to the high content of biodegradable compounds (protein and carbohydrates) compared to PS (cellulose and hemicellulose). The difference is that the maximum production rate is 29.13 mL/g VS.day for the MW. While for PS, the maximum production rate is only 8.16 mL/g VS.day. However, despite the difference, the values in the co-digestion scenarios oscillate in a range of 20.66-26.43 mL/g VS.day using these substrates. So, based on kinetic analysis results it can be said that the Gompertz model predicts correctly the results obtained in the BMP tests of stand-alone and co-digestion scenarios with MW and PS.

7.3. Final Remarks.

The organic raw materials such as the potato stem and the milk-whey can be transformed to biogas through the anaerobic digestion process. Taking into account its chemical composition, both materials (milk-whey and potato stem) are qualified for transformation to value-added products. In this context, the potential for biogas production was determined using the Biochemical Potential Test (BMP). The results showed that the two feedstocks are capable of generating biogas through mono-digestion or co-digestion. Additionally, the results showed that although good performance in terms of pH, the co-digestions have different synergistic effects, which can be modeled employed the Gompertz equation.

Also, it is important to highlight the fact that this is the first research where the potential of the potato stem as a substrate in anaerobic digestion has been evaluated.
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References


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8. Biotechnological schemes for biogas production from milk whey and potato stem.

The main of this chapter is described methodological aspects of the biogas production from PS and MW by means of simulation process. In this sense, this chapter describes technical and economic details applied to the simulation of biogas production in the software Aspen Plus (Aspen Technology, Inc., Houston, USA). Seven scenarios using anaerobic digestion are described: the firsts are the combined production of heat and electricity from stand-alone process, and the seconds are the combined production of heat and electricity from co-digestion of milk whey and potato stem. Finally, the considerations of the environmental assessment using the Waste Reduction Algorithm (WAR) software are showed.


The integration of potato stem (PS) and milk whey (MW) as an innovative alternative to recover energy and potentially obtain economic benefits. The synthesis of the flowsheet of the process was carried out with process simulation tools. The main objective of this stage is to determine the mass and energy balances of the process. With the mass and energy balances, it is possible to calculate the requirements for raw material, utilities and energy. Therefore, a methodological simulation approach is presented in order to evaluate the production of heat and electricity using PS and MW as raw materials. Additionally, the main parameter for estimating the economic profitability and environmental impacts of the process to be considered. As a consequence, the capital costs (CAPEX) and operating costs (OPEX) were determined by an economic assessment using the tool Aspen Economic Analyzer (Aspen Technology, Inc., Houston, USA). The Waste Reduction Algorithm (WAR) developed
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by the Environmental Protection Agency of the United States (EPA) was used to environmental assessment considering eight (8) impact categories.

8.1.1. Simulation procedure.

The simulation of the AD and energy production was performed using the software Aspen Plus (Aspen Technology, Inc., Houston, USA). The simulation of energy production was carried out using the physicochemical characterization of the feedstocks and biogas generated in AD process. In this step, three different processes were simulated: stand-alone using PS as raw material, stand-alone using MW as raw material and the co-digestion of both feedstocks. So, co-digestion process was evaluated through five scenarios where different relation of MW and PS were evaluated. The fed ratio of both materials for biogas production was calculated considering the process implementation in high solids or dry-anaerobic digestion (>20% total solid) and low solids or wet-anaerobic digestion (<10% solid total). Finally, the economic assessment of all processes was evaluated in Aspen Plus Economic Analyzer (Aspen Technology, Inc., Houston, USA).

The flowsheet model in the Aspen Plus was assumed in continuous mode, based on 8000 hours of work per year. Additionally, important parameter in industrial process simulation are thermodynamic models, due to the fact that these can represent experimental data [1]. For this reason, the thermodynamic models for the modeling were established through the property method selection assistant of the software Aspen Plus. The non-random two-liquid (NRTL) was used to describe the liquid phase, Hayden O’Connel Equation of State for the vapor phase and Wong-Sandler mixing rules equations for high pressures (>10 bar). The thermodynamic properties reported by the National Research Energy Laboratory (NREL) were used to introduce components such as protein, cellulose, hemicellulose, lignin, and biomass that are not available in the software database [2]. The simulation did not consider energetic optimization for pinch analysis and total energy integration. However, the water recycling in some steps was considered (e.g., anaerobic digestion).

On the other hand, the simulation consists of two well-defined steps: the AD for biogas production and cogeneration for power and heat generation. The first step was subjected to the type of substrate that input as a raw material at the process, where the reactions involved in the biodigester are influenced by the physicochemical characterization. While the second step is subjected to the composition of methane in the biogas stream obtained. AD step was
simulated using kinetic model reported in the literature. While, the cogeneration system was designed using process engineering and previous researches. However, yields were used to complete the mass balances of processes where a kinetic model is not available. A schematic overview of the simulation steps for biogas and energy production from MW and PS are present below in the Figure 8-1.

![Figure 8-1. Flowsheet of energy production using biogas as a vector.](image)

8.1.2. Anaerobic digestion for biogas production

AD process for biogas production was simulated with a modification of the Novel Process Simulation Model (PSM) developed by Rajendran et. al [3]. This model was validated and established in order to predicts the biogas production from any substrate at any given process condition using the software Aspen Plus. The model considers the four stages of biogas production previously described in the chapter 1: hydrolysis, acidogenesis, acetogenesis and methanogenesis.

The model divides these phases into two separate groups of reaction-sets. The first set refers to the hydrolysis phase based on the fractional conversion (0.0 – 1.0) of the reactants into products. In this sense, the raw materials composed of complex structures such as carbohydrates, proteins and lipids are transformed into monomeric compounds. The reactants for this phase when used PS as raw material are cellulose, hemicellulose and extractives. While that for the MW, the reactants are the main protein and lipids. For the hydrolysis step, the stoichiometric reactor model was used for simulates the first set reactions. The second set involves the other phases of AD, which were simulated with first-order kinetic reactions, collecting the kinetic parameters of previous models (e.g. ADM 1)
and presented on the article developed by Rajendran et. al [3]. In this case different conversion routes are presented for PS and MW (Table 8-1). Finally, for the stand-alone process with PS a new conversion reaction for extractives was added to the model. Likewise, for simulation proposes, the protein and lipids from MW were considered as soluble protein and saturated triacylglyceride (when the composition is mainly triolein).

**Table 8-1.** Reactions for modeling biogas production from potato stem and milk-whey.

- Reactions used for PS.  
- Reactions used for MW.  
- Reactions used for both feedstocks.

<table>
<thead>
<tr>
<th>Group of the reactions</th>
<th>Parameter</th>
<th>Extent of reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydrolysis reactions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Cellulose</td>
<td>( (C_6H_{12}O_6)n + H_2O \rightarrow n (C_6H_{12}O_6) )</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>2 Cellulose</td>
<td>( C_6H_{12}O_6 + H_2O \rightarrow 2 C_2H_6O + 2 CO_2 )</td>
<td>0.6 ± 0.0</td>
</tr>
<tr>
<td>3 Hemicellulose</td>
<td>( C_5H_8O_4 + H_2O \rightarrow 2.5 C_2H_4O_2 )</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>4 Hemicellulose</td>
<td>( C_5H_8O_4 + H_2O \rightarrow C_5H_{10}O_5 )</td>
<td>0.6 ± 0.0</td>
</tr>
<tr>
<td>5 Xylose</td>
<td>( C_5H_{10}O_5 \rightarrow C_5H_4O_2 + 3 H_2O )</td>
<td>0.6 ± 0.0</td>
</tr>
<tr>
<td>6 Ethanol</td>
<td>( 2 C_2H_6O + CO_2 \rightarrow 2 C_2H_4O_2 + CH_4 )</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>7 Extractives</td>
<td>( CH_5N + 0.5 H_2O \rightarrow 0.5 CO_2 + 0.75 CH_4 + H_3N )</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>8 Protein</td>
<td>( C_{13}H_{25}O_7N_3S + 6 H_2O \rightarrow 6.5 CO_2 + 6.5 CH_4 + 3 H_3N + H_2S )</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>9 Lipids</td>
<td>( C_{57}H_{104}O_6 + 3 H_2O \rightarrow C_3H_8O_3 + 3 C_{18}H_{34}O_2 )</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acidogenic reactions</th>
<th>Kinetic constant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Dextrose</td>
<td>( C_6H_{12}O_6 + 0.1115 H_3N \rightarrow 0.1115 C_5H_7NO_2 + )</td>
<td>( 9.54 \times 10^{-3} )</td>
</tr>
<tr>
<td></td>
<td>( 0.744 C_2H_4O_2 + 0.5 C_3H_6O_2 + 0.4409 C_4H_8O_2 + )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( 0.6909 CO_2 + 1.0254 H_2O )</td>
<td></td>
</tr>
<tr>
<td>11 Glycerol</td>
<td>( C_3H_8O_3 + 0.04071 H_2N + 0.0291 CO_2 + 0.00005 H_2 \rightarrow )</td>
<td>( 1.01 \times 10^{-2} )</td>
</tr>
<tr>
<td></td>
<td>( 0.04071 C_5H_7NO_2 + 0.94185 C_3H_6O_2 + 1.09308 H_2O )</td>
<td></td>
</tr>
</tbody>
</table>
### Acetogenic reactions

![Image content]

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Chemical Equation</th>
<th>Kinetic Constant</th>
</tr>
</thead>
</table>
| 12 Propionic acid | \[
C_6H_8O_2 + 0.06198 \ H_2N + 0.314336 \ H_2O \rightarrow \\
0.06198 \ C_5H_7NO_2 + 0.9345 \ C_2H_4O_2 + 0.660412 \ CH_4 + \\
0.160688 \ CO_2 + 0.00055 \ H_2
\] | \[1.95 \times 10^{-7}\] |
| 13 Isobutyric acid | \[
C_4H_8O_2 + 0.0653 \ H_2N + 0.8038 \ H_2O + 0.0006 \ H_2 + \\
0.5543 \ CO_2 \rightarrow 0.0653 \ C_5H_7NO_2 + 0.8909 \ C_2H_4O_2 + \\
0.446 \ CH_4
\] | \[5.88 \times 10^{-6}\] |
| 14 Oleic acid | \[
C_{18}H_{34}O_2 + 15.2396 \ H_2O + 0.2501 \ CO_2 + 0.1701 \ H_3N \rightarrow \\
0.1701 \ C_5H_7NO_2 + 8.6998 \ C_2H_4O_2 + 14.4978 \ H_2
\] | \[3.64 \times 10^{-12}\] |

### Methanogenic Reactions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Chemical Equation</th>
<th>Kinetic Constant</th>
</tr>
</thead>
</table>
| 15 Acetic acid | \[
C_2H_4O_2 + 0.022 \ H_2N \rightarrow 0.022 \ C_5H_7NO_2 + 0.945 \ CH_4 + \\
0.066 \ H_2O + 0.945 \ CO_2
\] | \[2.39 \times 10^{-3}\] |
| 16 Hydrogen | \[
14.4976 \ H_2 + 3.8334 \ CO_2 + 0.0836 \ H_3N \rightarrow \\
0.0836 \ C_5H_7NO_2 + 3.4154 \ CH_4 + 7.4996 \ H_2O
\] | \[2.39 \times 10^{-3}\] |

For the stand-alone biogas production from PS was carried out a size reduction with a gyratory crushed system until a maximum particle diameter of 1 mm. The pretreatment stage was simulated with RSTOIC block considering the empirical and primary stoichiometric equations of each lignocellulosic component proposed by Demirbas et al. [4] (i.e. for the cellulose and hemicellulose hydrolysis during pretreatment). In order to simulate the mesophilic biodigester (37 °C) a stoichiometric reactor was used for the hydrolysis phase and a continuously stirred tank reactor (CSTR) for the other phases. The global residence time of the reactors was set at 30 days, which is typical for AD [5]. Sequentially, the separation of biogas stream and semiliquid effluent was performed through a flash separator at 37 °C. PS and MW are different in TS content (high TS for PS and low TS for MW), so these can be treated by two types of digestion: using continuous flow stirred tank reactor (CSTR) in High-Anaerobic Digestion (High-AD) or using an upflow anaerobic sludge blanket (UASB) in Low-Anaerobic Digestion (Low-AD) [6]. In Low-AD, the process is operated with a TS content less than 20%, while the High-AD operated with TS content in the range 20-
Chapter 8. Biotechnological schemes for biogas production from milk whey and potato stem.

45% [7], [8]. Therefore, this study considers tree scenarios with Low-AD and four scenarios with High-AD. The proposed scenarios are presented in Table 8-2.

Additionally, for a better specification of equipment in Aspen Plus and taking into account the production of raw materials (see third chapter), the process scale was considered using 1000 m³/day for MW and 300 tonne/day for PS.

Table 8-2. Scenarios for heat and electricity production from milk-whey and potato stem.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Type</th>
<th>TS (%)</th>
<th>MW (tonne/day)</th>
<th>PS (tonne/day)</th>
<th>Water dilution (tonne/day)</th>
<th>Total (tonne/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>Low-AD</td>
<td>5.69</td>
<td>1000</td>
<td>0</td>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>PS</td>
<td>High-AD</td>
<td>27.79</td>
<td>0</td>
<td>300</td>
<td>700</td>
<td>1000</td>
</tr>
<tr>
<td>C1</td>
<td>High-AD</td>
<td>31.77</td>
<td>700</td>
<td>300</td>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>C2</td>
<td>High-AD</td>
<td>29.21</td>
<td>250</td>
<td>300</td>
<td>450</td>
<td>1000</td>
</tr>
<tr>
<td>C3</td>
<td>High-AD</td>
<td>30.63</td>
<td>500</td>
<td>300</td>
<td>200</td>
<td>1000</td>
</tr>
<tr>
<td>C4</td>
<td>Low-AD</td>
<td>9.44</td>
<td>956.9</td>
<td>43.1</td>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>C5</td>
<td>Low-AD</td>
<td>7.43</td>
<td>980</td>
<td>20</td>
<td>0</td>
<td>1000</td>
</tr>
</tbody>
</table>

* Low-AD: (TS<20%). High-AD: (TS: 20 - 45%).

8.1.3. Heat and electricity generation

The electricity and heat generation from biogas consisted of two sections: electricity production in a turbine and heat recovery steam generation. The flowsheet of the heat and electricity generation is presented in the Figure 8-2.

The first section was simulated with a stoichiometric reactor, for combustion reactions, connected to a turbine with an efficiency of 85% (commonly the polytrophic efficiency is between 85% and 95% [9]). Initially, the biogas produced in the anaerobic fermentation and preheated air are entered into a compression stage up to 24 bar. Then the compressed stream is carried out to a stoichiometric reactor, where the total combustion of methane occurs with an excess of 20% of air under a temperature of 1200-1260 °C. The combustion gases generated (mainly CO₂, H₂O) are expanded into an isentropic turbine which generates
the electricity. Sequentially, the exhaust gases are used to provide heat to a serial exchangers system. Therefore, the cooling of the gas results in heat being released, which is used to heat the incoming feed water and generate mid-pressure steam at 213 °C and 12 bar.

![Figure 8-2. Flowsheet of the heat and electricity generation using biogas as an energetic vector.](image)

On the other hand, the main characteristics of the simulated heat and electricity generation are presented in Table 8-3.

**Table 8-3.** Main characteristics of the simulated heat and electricity generation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compressor</td>
<td></td>
<td>Turbine</td>
<td>5</td>
</tr>
<tr>
<td>Discharge pressure</td>
<td>24 bar</td>
<td>Pressure ratio</td>
<td></td>
</tr>
<tr>
<td>Isentropic efficiency</td>
<td>85%</td>
<td>Isentropic efficiency</td>
<td>85%</td>
</tr>
<tr>
<td>Combustion chamber</td>
<td></td>
<td>Intercooler</td>
<td></td>
</tr>
<tr>
<td>Pressure</td>
<td>24 bar</td>
<td>Pressure drop</td>
<td>1%</td>
</tr>
<tr>
<td>Fractional conversion</td>
<td>100%</td>
<td>Pump</td>
<td></td>
</tr>
<tr>
<td>Air excess</td>
<td>20%</td>
<td>Discharge pressure</td>
<td>20 bar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Efficiency</td>
<td>80%</td>
</tr>
</tbody>
</table>
8.1.4. Economic assessment

The capital costs (CAPEX) and operating costs (OPEX) were determined by an economic assessment. These costs were calculated using the tool Aspen Economic Analyzer based on the mass and energy balances from the simulation procedure and economic parameters of South America (Colombia). The economic parameters for analysis were: number of shifts, annual interest rate, return rate, utilities cost, feedstocks prices, operative charges, among others. The utilities cost was considered electricity, the heating and cooling requirements of equipment, such as heat exchangers, pumps, compressors, mills, and reactors, among others. The energy requirements were obtain from energy balance in the software Aspen Energy Analyzer (Aspen Technology, Inc., USA). The costs of raw materials were associated with transportation and recollection costs in Colombian context (depending on the location of the collection centers). The straight-line method for depreciation was defined with a project-life of 10 years, where a period of 8000 h was taken into account to perform the calculations. Subsequently, the Net Present Value (NPV) was used as an indicator of the economic feasibility in the single MW, PS cases and co-digestion of MW and PS. Net Present Value (NPV) indicates the potential benefits over the life of the project (10 years) based on the profit on the project, pay-off investment, and normal interest on the investment [10]. In this sense, the data used in the economic assessment are presented in Table 8-4.

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>Price</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Investment Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tax rate</td>
<td>%</td>
<td>25</td>
<td>[11]</td>
</tr>
<tr>
<td>Interest rate</td>
<td>%</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td><strong>Raw materials</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk-whey</td>
<td>USD/L</td>
<td>0.02</td>
<td>Average price&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potato Steam</td>
<td>USD/kg</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Dilution water</td>
<td>USD/m³</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td><strong>Utilities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Pressure steam</td>
<td>USD/ton</td>
<td>7.57</td>
<td>[12]</td>
</tr>
<tr>
<td>Mid Pressure steam</td>
<td>USD/ton</td>
<td>8.18</td>
<td></td>
</tr>
<tr>
<td>High Pressure steam</td>
<td>USD/ton</td>
<td>9.86</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 8. Biotechnological schemes for biogas production from milk whey and potato stem.

<table>
<thead>
<tr>
<th></th>
<th>USD/m³</th>
<th>1.25</th>
<th>Average price(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Process water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fuel</strong></td>
<td>USD/MMBTU</td>
<td>88.01</td>
<td></td>
</tr>
<tr>
<td><strong>Electricity</strong></td>
<td>USD/kWh</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td><strong>Operation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Operator</strong></td>
<td>USD/h</td>
<td>2.72</td>
<td></td>
</tr>
<tr>
<td><strong>Supervisor</strong></td>
<td>USD/h</td>
<td>5.00</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Average price in Colombian context

### 8.1.5. Environmental assessment

For the environmental analysis of the scenarios, it was considered the impact of energy requirements and impact by mass effluents. The software used for this purpose was the Waste Reduction Algorithm (WAR) developed by the National Risk Management Research Laboratory of United States Environmental Protection Agency [13]. This tool is divided into different impact categories, taking into account variables such as toxicity, global warming and acidification. In this sense, these variables cover eight categories: Acidification Potential (AP), Aquatic Toxicity potential (ATP), Global Warming Potential (PWP), Human Intake Toxicity Potential (HTPI), Human Toxicity Potential by Dermal Exposure and Inhalation (HTPE), Photochemical Oxidation Potential (PCOP), Potential Ozone of Depletion (ODP) and Terrestrial Toxicity Potential (TTP) [14]. The impact of each category is established with mass and energy balances, based on the rate of raw materials, products, by-products and waste [15], [16]. The software calculates the outlet impact (only considering the outlet streams) and the generated impact (the difference between the impact of outlet and inlet streams) [17]. Then the weighted sum of all impacts ends in the final impact per hour, giving the total (PEI). When the value is positive it means that the output currents generate more environmental impact that the input ones, while a negative value indicates that the environmental impact of the given index has been decreased.

### 8.2. Final remarks

Technical, economic and environmental analysis are key elements in the development, implementation, and success of processes on an industrial scale. These determine the viability of the projects in a particular region. In the previous chapter are described the
Chapter 8. Biotechnological schemes for biogas production from milk whey and potato stem.

technical, economic and environmental considerations of the production and use of biogas from potato stem and milk whey in the Latin American context. This chapter shows and described the main technical parameters established to each of the simulation stages for the mono-digestion and co-digestion of the potato stem and milk whey. Additionally, the methodological considerations addressed for the analysis of the simulations with Aspen Plus, Aspen Economic Analyzer and Waste Reduction Algorithm software are described.

References


Chapter 8. Biotechnological schemes for biogas production from milk whey and potato stem.


9. Analysis of biogas production based on simulations.

The present chapter describes the results of the combined generation of heat and electricity from biogas as energy vector using potato stem and milk whey. This chapter is based mainly in the results of the published paper “Effect of co-digestion of milk-whey and potato stem on heat and power generation using biogas as an energy vector: Techno-economic assessment”. In this sense, the results of technical, economic and environmental assessment of the stand-alone processes and five scenarios with different load ratios for anaerobic co-digestion are presented.

9.1. Heat and electricity simulation results

The simulation results of the five scenarios of the co-digestion and two individual stand-alone processes are shown in the Figure 9-1 and Figure 9-2. The Figure 9-1 shows the mass balance of the seven scenarios, where it can be observed that production of biogas of High-AD is higher compared to Low-AD. In this context, the biogas production for scenarios PS, C1, C2, C3 was approximately fivefold than scenarios MW, C4, and C5, where the biogas production from one raw material processes for MW and PS were of 24201 m³/day and 104800 m³/day, respectively. The co-digestion of MW and PS produced between 30550 m³/day and 35917 m³/day of biogas for Low-AD. Meanwhile, High-AD for both materials produced between 122642 m³/day and 135874 m³/day of biogas. In terms of biogas production, the scenario C1 was better compared to all scenarios, increasing 6.8 and 1.2 times for MW and PS cases respectively.
Chapter 9. Analysis of biogas production based on simulations.

* The balance for HP-Stem in complemented with water for recovery heat.

- MW (tonne/day)
- PS (tonne/day)
- Dilution water (tonne/day)
- Biogas (tonne/day)
- Air (tonne/day)
- Digestate (tonne/day)
- Hexausted gas (tonne/day)
- MP-steam (tonne/day)
- Power electricity (kW)
Chapter 9. Analysis of biogas production based on simulations.

* The balance for HP-Stem in complemented with water for recovery heat.

- MW (tonne/day)  
- PS (tonne/day)  
- Dilution water (tonne/day)  
- Biogas (tonne/day)
- Air (tonne/day)  
- Digestate (tonne/day)  
- Hexausted gas (tonne/day)  
- MP-steam (tonne/day)  
- Power electricity (kW)
Chapter 9. Analysis of biogas production based on simulations.

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- MW (tonne/day)
- PS (tonne/day)
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- Biogas (tonne/day)
- Air (tonne/day)
- Digestate (tonne/day)
- Hexausted gas (tonne/day)
- MP-steam (tonne/day)
- Power electricity (kW)
Chapter 9. Analysis of biogas production based on simulations.

* The balance for HP-Stem in complemented with water for recovery heat.

- MW (tonne/day)  
- PS (tonne/day)  
- Dilution water (tonne/day)  
- Biogas (tonne/day)  
- Air (tonne/day)  
- Digestate (tonne/day)  
- Hexausted gas (tonne/day)  
- MP-steam (tonne/day)  
- Power electricity (kW)

**Figure 9-1.** Mass balances of energy production based on biogas from PS and MW.
The biogas yield was calculated for all scenarios, where the highest yields were for co-
digestion C1, C2, and C3 with 0.18, 0.16 and 0.16 m$^3$ of biogas per kg of feedstock. While
for the other scenarios (C4 and C5) the yields were 0.04 and 0.03 m$^3$/kg of feedstock. The
yield of biogas for scenarios MW and PS were 0.024 and 0.349 m$^3$/kg of feedstock,
respectively. These results showed that high-AD is most promising for utilization of MW and
PS. The above can be explained due to the high content of TS in the PS (Table 7-2), which
increases the available substrate. However, in order to allow a better comparison of the
yields obtained in the biogas simulation, these values were normalized in terms of the VS.
The results were comparable oscillating between 0.04 and 0.45 m$^3$/ kg VS for all scenarios.
Vivekanand et al. obtained a yield between 0.33 and 0.49 m$^3$/ kg VS for co-
digestion of MW and manure [1]. In the study by Tabassum et al. different seaweeds co-
digestions with other substrates obtaining yields close to 0.5 m$^3$/ kg VS [2]. These results are similar to that
obtained in this work for co-
digestions of PS and MW (≈ 0.45 m$^3$/ kg VS). Additionally, the
same results had been obtained from previous experimental work for cheese whey as a
substrate, where the yield for biogas production obtained by Viques et al. was between 0.02
and 0.04 m$^3$/kg MW [3] and by Yan et al. between 0.02 and 0.04 m$^3$/kg MW [4].

In the Figure 9-1 it can be also observed that the energy generation from co-digestion of
PS and MW (High-AD) is greater compared to single raw materials processes. The biogas
from anaerobic co-digestion generated a power electricity between 12790 and 13270 kW,
while the single processes (PS and MW) produced 10367 kW and 3540 kW, respectively.
C1 is the best scenario in terms of electricity production, generating 13277 kW of power,
21.92% and 73.34% respect to PS and MW. Likewise, the production of mid-pressure steam
(12 bar, 213 °C) for simulations of AD of PS, MW and co-
digestions (C1, C2, C3, C4, and
C5) were 329.59, 109.86, 399.51, 399.51, 381.53, 148.82 and 125.84 t/day, respectively. In
consequence, the greatest generation of energy was obtained with the integration of wastes
(scenario C1), where 13277 kW of power and heat in the shape of mid-pressure steam
(399.51 t/day, 213 °C, 12 bar) can be generated using 700 tonne of MW per day and 300
tonne of PS per day.
For comparing the biogas quality, the **Figure 9-2** shows the biogas composition for each scenario. **Figure 9-2** shows that the biogas composition is different for all scenarios. The biogas composition is mainly methane (44-65%), carbon dioxide (26-43%), water (5-6%) and hydrogen sulfide (0-1.4%). However, the scenarios with high amount of MW have a highest percent of methane than other scenarios (>12%). The biogas produced through AD of MW had a 65% of methane compared to biogas from PS with 44% methane composition. The biogas composition of all co-digestions is similar depending on the amount of substrate composition analyzed in this work.

These results indicate that there is a greater availability of methane when using only MW or Low-AD. Therefore, it is possible to generate higher energy per cubic meter of biogas. However, in this case the result can be affected by biogas yield per kg of feedstock that is lower in contrast to PS and High-AD. It should be emphasized that despite the advantages of the individual conversion of whey to methane, there is a high wastage of water in the process since only 5.59% is organic matter. The above can lead to high production costs due to a great amount of liquid wastewater. Nevertheless, co-digestion to generate energy emerged as an alternative to solve this problem, due to the integration of two residues with good availability in anaerobic co-digestion and use of wastewater. This advantage can be observed when calculating the process productivity in relation to the amount of feedstock. The volumetric methane productivity for stand-alone case with MW (15.82 m$^3$ CH$_4$/kg day)
is lower compared to the volumetric methane productivity for co-digestion of both materials which is 0.2-2.69 times higher.

As a transitory conclusion at this stage, in terms of biogas production, the scenario C1 is better compared to all scenarios, increasing 6.8 and 1.2 times for single MW and PS. However, these scenarios at High-AD obtained higher biogas yield (0.16 – 0.18 m$^3$/kg feedstock) compared to Low-AD (0.03 – 0.036 m$^3$/kg feedstock). Regarding energy generation, C1 is the best scenario in terms of electricity production generating 13277 kW of power that is higher in 21.92% respect to PS alone and 73.34% respect to MW alone.

9.2. Economic assessment

The economic evaluation of the seven scenarios was developed based on four parameters: capital costs (CAPEX), production costs (OPEX), product revenues (Revenues) and net present value (NPV). CAPEX refers to the initial investment to carry out the process on an industrial scale. OPEX refers to the production costs associated with the project for each year, which considers the cost of feedstocks, utilities cost, operating costs, maintenance of equipment, overhead plant and general and administrative expenses. The revenues refer to the annual net profits for the products sales (electricity). Finally, NPV represents the net economic benefits (profit or loss) at the end of the project.

9.2.1. NPV, CAPEX and OPEX

Figure 9-3, Figure 9-4 and Figure 9-5 shows the results of economic evaluation. In Figure 9-3 it can be observed the OPEX, where the main influence on this variable is the raw materials cost, which is the most representative cost in all scenarios (approx. 80%). Then, the high scale (1000 t/day) causes a decrease of the other parameters such as utilities (low in anaerobic digestion process), operating labor cost and maintenance cost. Additionally, the low utilities cost is explained by the use of just mid pressure stem for heating requirements in the all scenarios (e.g. biodigester heating).
Moreover, for High-AD processes the OPEX obtained is between 10.5 to 15.35 M.USD/year, which is higher, compared with scenario MW, C4 and C5 (7.96 M.USD/year, 8.20 M.USD/year and 8.61 M.USD/year). These results are explained by the influence of the raw materials costs on the global process, affecting directly the OPEX. On the other hand, the OPEX, for other scenarios are 10.25, 10.49, 15.35 and 13.85 USD/year for PS, C1, C2 and C3, respectively.

**Figure 9-3.** Production cost for electricity production using biogas as energy vector.

**Figure 9-4** shows the CAPEX for each scenario, where the processes of High-AD have the most prominent cost (about 33 M.USD for C1, and 33.95, 33.84 and 34.28 M.USD (2.2 times that MW) for C2 and C3 cases. These results were obtained due to the numbers of equipment (mixer and crusher) and the higher biogas flow to generate energy, which has a direct influence on the CAPEX where the number of generators increases. CAPEX for the MW (5.49 M.USD) is lower compared to the PS (31.52 M.USD) and co-digestion of PS and MW. This result occurs commonly in most biochemical processes, as reported in previous economic studies where the combination of materials increases the CAPEX [5],[6]. Therefore, despite capital and operational costs for the MW are low, the sales of products are lower. This product sales represents 200% less than the PS, 261% less for the High-AD (C1, C2 and C3) and 50% less for Low-AD (C4 and C5). Likewise, when comparing the High-AD (C1, C2 and C3) with PS there is a 23% increase of the first respect to the second.
While in the comparison of Low-AD (C4 and C5) with PS there is 50% less for the first respect to the second.

![Caption](Image)

**Figure 9-4.** Capital cost and revenues for electricity production using biogas as energy vector.

Finally, the economic profits evaluated with the NPV are showed in **Figure 9-5**, where a negative NPV was obtained in all scenarios. This means that at the end of the project (10 years) there is a net profit of -21.15, -24.00, -12.52, -33.47, -28.22, -31.09 and -45.21 M.USD for MW, PS, C1, C2, C3, C4 and C5, respectively.

Otherwise, these results showed that all scenarios become economically not viable. However, the main variable is raw material costs that affect 80% of OPEX. For these reasons, an alternative evaluation was proposed taking into account the cost of feedstocks and valorization of subproducts such as digestate. This type of analysis is very important to understand the real industrial scale possibilities that can offer the anaerobic digestion and co-digestion projects if considering what are the limits to get feasibility in raw materials costs and digestate selling prices (as fertilizer).
9.2.2. Influence of raw materials cost

Given the great influence of the raw material cost on the economic feasibility of each scenario, three different raw material costs were evaluated for PS and MW separately and together. In this sense, the NPV calculation was carried out reducing the cost to 50% of the initial value for each feedstock and increasing 50% the initial value. Additionally, a point of inflection was established between gains and losses when the NPV is zero. The results are presented in Figure 9-6, Figure 9-7 and Figure 9-8. The Figure 9-6 showed that economic feasibility in electricity production becomes less feasible compared to the initial price (0.08 USD/kg) when the cost of PS increases. Last leads to a negative NPV value.

Otherwise, when the price is reduced (-50%) the scenarios in the framework of the High-AD have a higher NPV compared to the price of reference over the life of the project (10 years). The NPV for these scenarios (PS, C1, C2, and C3) were of -5.04, -5.44, -15.40, and -11.24 M.USD, respectively. However, for all scenarios, the NPV is negative considering a value of 0.04 USD/kg of PS. It should be noted that the value of NPV is zero for PS, C1 and C3 when
a cost of PS is of 0.028, 0.009 and 0.012 USD/kg. From this point of view, there is not economic feasibility where PS is used as a raw material.

Figure 9-6. Influence of PS cost on NPV for electricity production from MW and PS.

Figure 9-7. Influence of MW cost on NPV for electricity production from MW and PS.
The influence of MW cost in each scenario can be seen in Figure 9-7. The same behavior was presented as in the case of PS. The NPV for each scenario was negative compared to the initial cost (0.02 UD/L). A NPV between -6.98 and -61.91 M.USD was obtained. Here the minimum cost of MW to obtain a NPV equal to zero was of 0.005 USD/L for the MW and 0.0061 USD/L for the C1. This cannot be possible because although the MW is a waste, the costs associated with transport at least must be considered (raw material value cannot be zero). On the other hand, the influence of PS and MW costs on each scenario can be observed in Figure 9-8. As a result, it was obtained that reducing the cost of both raw materials has more favorable NPV values.

![Figure 9-8. Influence of PS and MW cost on NPV for electricity production.](image)

**9.2.3. Biodigestate valorization as biofertilizer**

Digestate is commonly applied directly to crops since it consists of mineral compounds such as phosphorus, potassium and nitrogen [7]. In 2015, 95% of the digestate produced in biogas plants in Europe was used for agricultural crops mostly with direct application, replacing chemical fertilizers [8]. Additionally, Pellets and pearls can be priced at 0-20 €/t, and in some cases up to 100 €/t [8]. Using these approaches, Abubaker et al. demonstrated that the digestate from biogas production improves soil for wheat cultivation [9].
To analyze the digestate valorization, it was considered the same initial price of the feedstocks and a biofertilizer (digestate) price between 0 to 30 USD/tonne. The above considers that biofertilizer production is developed mainly through solar drying, that considers a low operation cost (trending to cero).

For each scenario was determined the minimum sale price where the NPV is zero. Figure 9-9 presented the results of this analysis. As a result, none of the scenarios had a positive NPV value at a sale price of 10 USD/tonne of digestate. However, Figure 9-9 showed that economic feasibility improves with the increase of biofertilizer cost, which can lead to a positive NPV value when biofertilizer cost is 20 USD/t and 30 USD/t. Accordingly, when the price is changed to 20 USD/t, the scenarios PS, MW, C1 and C2 have a positive NPV at the end of 10 years. The NPV for these scenarios were of 0.28, 4.12, 7.69 and 0.65 M.USD, respectively. The scenarios C3, C4 and C5 present a negative NPV. For the other price (30 USD/t), the NPV have a positive value in six of the seven scenarios. These scenarios are PS, MW, C1, C2, C3 and C4, which present a NPV of 10.76, 15.83, 17.80, 16.36, 4.66 and 6.64 M.USD, respectively. The NPV is equal to zero for fertilizer prices equal to 19.74, 16.48, 12.39, 19.8, 25.58, 24.2 and 28.5 USD/t for PS, MW, C1, C2, C3, C4 and C5, respectively. This means that it is possible to invest in these types of processes when the revenues are high based on the valorization of the by-products and/or the decrease of the raw materials costs.

Figure 9-9. Influence of digestate price on NPV for electricity production from milk whey and potato stem.
Finally, an economic analysis in terms of the NPV was carried out in order to determine the scenarios with greater potential under favorable conditions. In this way, it was established that the collecting and transport for raw materials have a low value (assume that raw materials cost is fifty percent more economical that initial value) and a maximum fertilizer price of 30 USD/tonne. In this context, the results in terms of the NPV are shown in Figure 9-10. In red the processes operated at Low-TS content (Low_AD) and in blue the processes operated at High-TS content (High-AD). It is worth stressing that all scenarios have a NPV positive, however, the most outstanding scenarios to generate heat and electricity are C1 and C2 with a NPV of 33.93 M.USD and 41.05 M.USD. Due to the above, it should be highlighted that the co-digestion of PS and MW is promising for heat and electricity production. However, the process scale, logistics and transport cost of the feedstocks was not explored fully in this investigation. This can represent an economic deficit when operating on a small scale since the initial investment increases causing a displacement of the NPV and generating a negative economic benefit [10].

![Figure 9-10. NPV for electricity production from milk whey and potato stem.](image)

In accordance with the analysis developed above, it was obtained that in terms of biogas production the scenarios with high organic load were the best (TS content > 20%). The use of a liquid waste such as whey, in some scenarios, decreased the dilution water requirement...
and improved biogas production. In other words, biogas production from anaerobic digesters could potentially be boosted via co-digestion of solid organic wastes such as potato stem and liquid waste such as whey. A similar result was obtained by O-Thong et al. In that study, co-digestion of oil palm empty fruit bunches (solid waste) with palm oil mill effluent (liquid waste) enhanced microbial biodegradability and resulted in 25–32% higher methane production at different mixing ratios than digesting oil palm empty fruit bunches alone [11]. Also Dai et al., studied the system stability and performance of high-solids anaerobic co-digestion of dewatered sludge (liquid waste) and food waste (solid waste) in comparison with mono-digestions. As a result, the addition of food waste improved the stability of the process and enhanced in volumetric production of biogas for high organic loads [12]. In the present thesis through the sensitivity analysis carried out, it was possible to verify the great influence of the cost of the raw material on the NPV of the project (negative values at high cost, less negative values at low cost). However, the valorization of the digestate as biofertilizer was necessary in order to obtain economic viability in the proposed scenarios. This topic has been discussed in the literature: Monlau et al. [13] present a complete review of alternative valorization routes of the digestate to reduce its environmental impact and to improve the economic profitability of anaerobic digestion plants. Alburquerque et al. [14] developed an assessment of the fertilizer potential of digestates from farm and agroindustrial residues, due to the fact that sustainability of biogas production systems depends greatly on the appropriate disposal of the digestate produced. In other study Monlau et al. [15], investigated the feasibility to combine anaerobic digestion and pyrolysis of digestates in order to increase the energy recovery from agricultural residues and the sustainability of the anaerobic digestion plants. As final remarks, sensitivity analysis carried out is a suitable tool to find the combination of parameters that makes the process viable. This information is very valuable for future experimental works and validation of the obtained simulation results, in terms of biogas production.

9.3. Environmental assessment

The environmental assessment was carried out to obtain the process viability, which allows estimation of the environmental impact of the heat and electricity generation using biogas from milk whey and potato stem. The results of the environmental assessment for biogas production through eight different scenarios are shown in Figure 9-11.
Only 6 categories are presented since the Global Warming Potential (PWP) and Potential Ozone of Depletion (ODP) categories have not a decisive influence on the result of the analysis and proposed scenarios (their impact is zero in all scenarios). The figure below shows that Aquatic Toxicity potential (ATP), Photochemical Oxidation Potential (PCOP) and Acidification Potential (AP) are the categories that have an impact on all scenarios. The results of acidification are partially explained by the presence of some acidic compounds generated in the digestion hydrolysis stage, which are present in the output flows. In addition, other volatile organic compounds and nitrogen compounds (present in output flows) can react with UV rays and generate the PCOP impact [16]. In contrast, in all the proposed scenarios decreases the environmental impact of the categories: Human Intake Toxicity Potential (HTPI), Human Toxicity Potential by Dermal Exposure and Inhalation (HTPE) and Terrestrial Toxicity Potential (TTP). This information is very interesting since it shows good environmental performance. The above could be due to the use of the two residues that avoid a considerable effect on human and terrestrial toxicity.

Figure 9-11. Environmental Potential Impact (PEI) of the heat and electricity generation.
Conclusions

On other hand, there is a considerable gap between all scenarios. Thus, the C1, C2, and C3 co-digestions are the most promising because of to decrease the environmental impact on the last three categories listed. Additionally, these scenarios showed better results with respect the ATP, PCOP, and AP categories. It should be noted that the potential environmental impact (PEI) is affected by waste composition and process energy requirements. In this way, the scenario C2 is one the most promising in terms of environmental impact.

9.4. Final remarks

Simulations based on the use of agroindustrial residues as feedstocks shows that the production of biogas can be a good alternative to expand the energy portfolio of every country, avoiding negative environmental impact and adding value to the national agriculture and industry sectors. However, this statement should be demonstrated for every project under design stage. In this sense, the integration of potato stem and milk whey can be an innovative alternative to recover energy, reduce the dependence on fossil fuels and potentially obtain economic benefits. Heat and electricity co-generation using biogas as an energy vector was analyzed through several potato stem and milk whey digestions and co-digestions demonstrating the best technical and environmental configurations. From an economic point of view, good indicators of economic feasibility were obtained in scenarios C1 and C2 with a NPV of 33.93 and 41.05 M.USD respectively when at least the raw materials prices are possible (0.01 USD/L for milk whey and 0.04 USD/kg for potato stem) and a fertilizer selling market price of 30 USD/t is reached. In this way, the integration of potato stem and milk whey to generate heat and electricity is a promising alternative if the logistics and other government policies can be applied to reduce the residues costs. Another alternative is the valorization of the digestate that represents a challenge for a well-developed supply chain and participation of producers through government policies support as some European countries have for example already done.

References

Conclusions


Conclusions.

- Milk whey and potato stem generated in the Colombian and Chilean context are able to be used as substrates for biotechnological process such as biogas production through anaerobic digestion.

- From the chemical characterization that the potato stem can be concluded that these are an interesting resource for biotechnological applications such as fermentable sugars obtain, syngas production thought thermochemical process and biogas production from anaerobic digestion.

- Potato stem and milk whey are capable of generating biogas through mono-digestion or co-digestion. However, the co-digestions have different synergistic effects and its can be modeled employed the Gompertz equation.

- The co-generation of heat and electricity through co-digestion of potato stem and milk whey generates a positive net economic profit but with limits in raw material costs and digestate selling prices.