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Biogas Production from Anaerobic Treatment of Agro-Industrial Wastewater

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1. Introduction

Today, globally most energy is provided by burning oil and only a very small percentage is generated by nuclear power plants. The contribution of energy from renewable resources is almost negligible. But this will change in the future with increasing in environmental pollution and fossil fuel depletion, in addition to environmental problems generated by the Fukushima nuclear power plant.

One of the most attractive ways to obtain sources of alternative energy and the pollution control is the recover resource and energy from waste streams through bioconversion processes (Cantrell et al., 2008). In this respect, intensive studies have been conducted in the past few decades and various "green technologies" have been extensively reviewed (Kleerebezemand and Loosdrecht, 2007; Hallenbeck and Ghosh, 2009). For many years, anaerobic digestion has been a prevailing technology for biogas production, in which substrates are converted to methane and other products under a joint effort of several microbial groups in a reaction system (Sterling et al., 2001).

In this context biogas generated by agro-industrial wastewater will play a vital role in future. Biogas is a versatile renewable energy source, which can be used for replacement of fossil fuels in power and heat production, and it can be used also as gaseous vehicle fuel. Methane-rich biogas can replace also natural gas, as a feedstock in the production of chemicals and materials (Shin et al., 2010).

Sustainable development must be the foundation for economic growth in the twenty-first century. It is necessary redirect the efforts toward bioenergy production from renewable material, low-cost and locally available feedstock such as waste and wastewater agro-industrial. This effort will not only alleviate environmental pollution, but also reduce energy insecurity and demand for declining natural resources. The most cost-effective and sustainable approach is to employ a biotechnology option. Anaerobic treatment is a technology that generates renewable bioenergy necessary to replace the energy requirements around the world through the production of methane and hydrogen. However, it has also been employed for production of polyhydroxyalkanoates (PHA), these are linear polyesters generated by bacterial fermentation of sugar or lipids. They are produced by the bacteria to store carbon and energy. More than 150 different monomers can be combined within this family to give

materials with extremely different properties. These plastics are biodegradeable and are used in the production of bioplastics (Mu et al., 2006) and other biochemicals.

This chapter intends to bring together the knowledge obtained from different applications of anaerobic technology in the treatment of various kinds of agro-industrial wastewaters to generate biogas. The first part covers essential information on the fundamentals of anaerobic technology, to demonstrate how the anaerobic treatment is able to generate significant volumes of methane-rich biogas. The wastewaters used in this chapter to generate biogas, contribute significantly in the pollution of the water bodies. In this opportunity the wastewater from Tequila vinasses were treated by different microbial consortia with energy purpose. This chapter illustrates the basics concepts of microbiology and biochemistry involved in the wastewater anaerobic treatment. The remainder focuses on various anaerobic reactor configurations and operating conditions used for the treatment of agro-industrial wastewaters different, show some examples with technical viability and the potential benefits that would be obtained by the utilization of the biogas as source of energy to full scale.

2. Historical background

Very old sources indicate that using wastewater and so-called renewable resources for the energy supply it is not new, it was already known before the birth of Christ. Even around 3000 BC the Sumerians practiced the anaerobic cleansing of waste. The Roman scholar Pliny described around 50 BC some glimmering lights appearing underneath the surface of swamps (Lee et al., 2010).

In 1776 Alessandro Volta personally collected biogas from the Lake Como to examine it. His findings showed that the formation of gas depends on a fermentation process and that may form an explosive mixture with air. The English physicist Faraday also performed some experiments with marsh gas and identified hydrocarbons as part of this. Around the year 1800, Dalton, Henry and Davy first described the chemical structure of methane, however the final chemical structure of methane (CH_4), was first elucidated by Avogadro in 1821 (Horiuchi et al., 2002).

In the second half of 19th century, more systematic and scientific in-depth research was started in France to better understand the process of anaerobic fermentation. The objective was simply suppress the bad odor released by wastewater pools. During their investigations, researchers detected some of the microorganisms which today are known to be essential for the fermentation process. It was Béchamp who identified in 1868 that a mixed population of microorganism is required to convert ethanol into methane, since several end products were formed during the fermentation process, depending on the characteristic of substrate (Lee et al., 2010).

In 1876, Herter reported that acetate found in wastewater, stoichiometrically form methane and carbon dioxide in equal amounts. Louis Pasteur tried in 1884 to produce biogas from horse dung collected from Paris roads. Together with his student Gavon he managed to produce 100 L methane from 1 m^3 dung fermented at 35^oC. Pasteur claimed that this production rate should be sufficient to cover the energy requirements for the street lighting of Paris. The application of energy from renewable resources started from this time on (Deublein and Steinhauser, 2008).

3. Fundamentals of microbiology and biochemistry in anaerobic digestion

One of the key factors in the success of microbial-mediated processes is an adequate understanding of process microbial, more specifically the study of microscopic organisms involved in wastewater degradation and byproduct formation. The low growth rate, the specific nutrient and trace mineral requirements of methanogens, coupled with their susceptibility to changes in environmental conditions demand meticulous process control for stable operation (Khanal, 2008). The biochemistry mainly involves enzyme-mediated chemical changes (the chemical activities of microorganism), type of substrate (kind wastewater) microorganism can destroy or transform to new compounds, and the step-by-step pathway of degradation (Sachdeva et al., 2000).

3.1 Organics conversion in anaerobic systems

The anaerobic digestive process is a natural biological process in which an interlaced community of bacteria cooperates to obtain a stable and auto-regulated fermentation through assimilation, transformation and decomposition of the residual organic matter present in waste and wastewater into biogas. This is a complex multistep process in terms of chemistry and microbiology, where the organic material is degraded to basic constituents to obtain methane gas under the absence of an electron acceptor such as oxygen. The common metabolic pathway and process microbiology of anaerobic digestion is shown in Fig. 1 (Khanal, 2008).

Generally, the anaerobic digestion process consists of four stages; the first one is called hydrolysis (or liquefaction), it consists in the transformation of complex organic matter such as proteins, carbohydrates and lipids into simple soluble products like sugars, long-chain fatty acids, amino acids and glycerin, this stage is carried out by the action of extracellular enzymes excreted by the fermentative (group 1) (Khanal, 2008).

In the second step, called the acidogenic stage fermentative bacteria use the hydrolysis products to form intermediate compounds like organic acids, including volatile fatty acids (VFA). Theses VFA along with ethanol are converted to acetic acid, hydrogen and carbon dioxide by other group of bacteria known as hydrogen-producing acetogenic bacteria (group 2) (Khanal, 2008).

Organic acids are oxidized partially by bacteria called acetogenic in the third stage, which produce additional quantities of hydrogen and acetic acid. The acetogenesis is regarded as thermodynamically unfavorable unless the hydrogen partial pressure is kept below 10^{-3} atm, pathway efficient removal of hydrogen by the hydrogen-consuming organisms such as hydrogenotrophic methanogens and/or homoacetogens (Zinder, 1988).

Finally, in the fourth stage, both acetic acid and hydrogen are the raw material for the growth of methanogenic bacteria, converting acetic acid and hydrogen to biogas composed mainly of methane, carbon dioxide and hydrogen sulfide (Khanal, 2008).

Acetate, H_2 and CO_2 are the primary substrate for methanogenesis. On chemical oxygen demand (COD) basis about 72% of methane production comes from the decarboxylation of acetate, while the remainder is from CO_2 reduction (McCarty, 1964). The groups of microorganisms involved in the generation of methane from acetate are known as acetotrophic or aceticlastic methanogens (group 3). The remaining methane is generated

from H_2 and CO_2 by the hydrogenotrophic methanogens (group 4). Since methane is largely generated from acetate, acetotrophic methanogenesis is the rate-limiting step in anaerobic wastewater treatment. The synthesis of acetate from H_2 and CO_2 by homoacetogens (group 5) has not been widely studied. Mackie and Bryant (1981) reported that acetate synthesis through this pathway accounts for only 1-2% of total acetate formation at 40°C and 3-4% total solids at 60°C in a cattle waste digester.

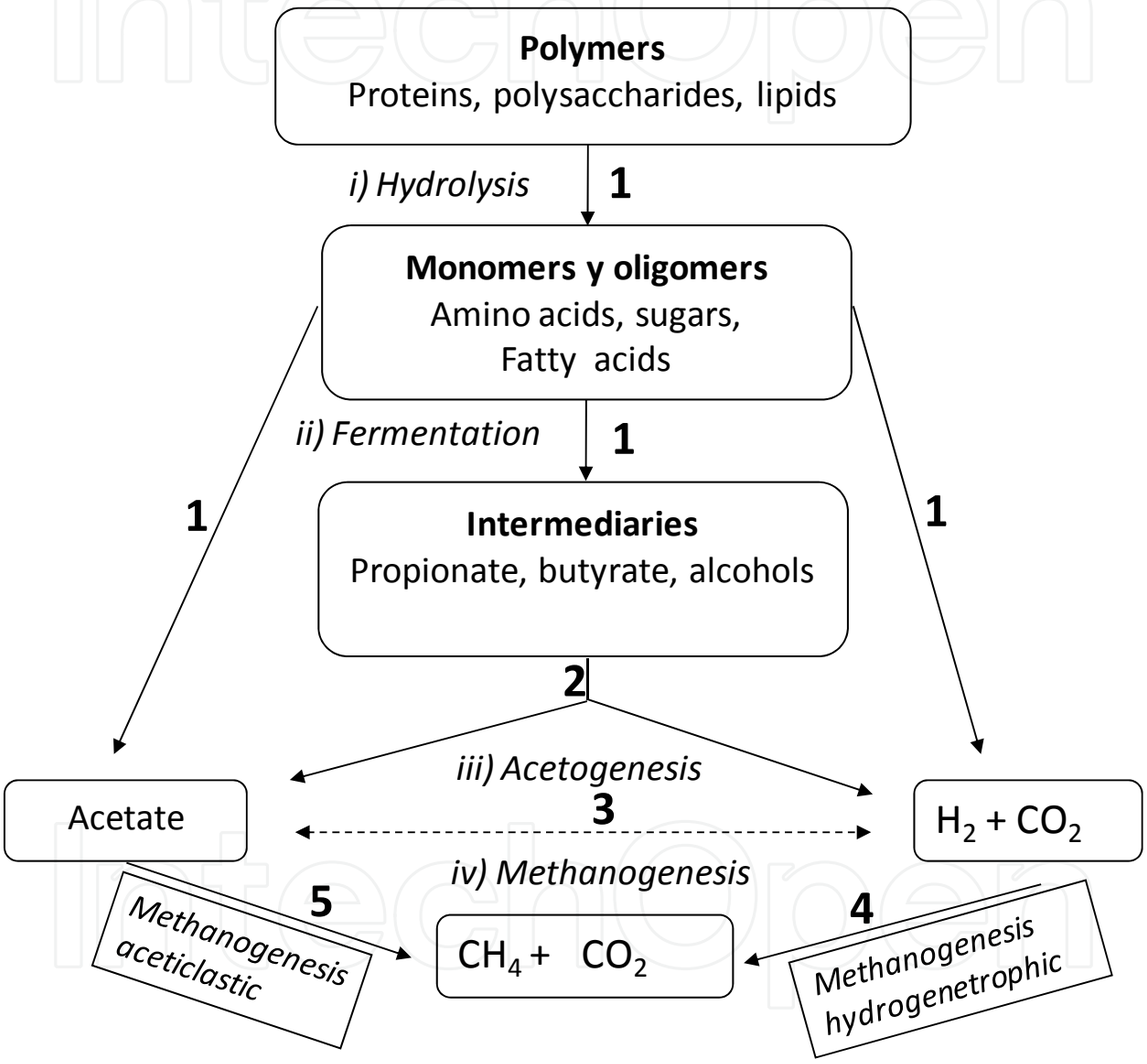

Fig. 1. Steps of anaerobic digestion of complex organic matter (the number indicate the group of bacteria involved in the process).

3.2 Process microbiology

The anaerobic degradation of complex organic matter is carried out by different groups of bacteria as indicated in Fig. 1. These exists a coordinated interaction among these bacteria. All process may fail if one group is inhibited (Khanal, 2008).

a. **Fermentative Bacteria (group 1):** This group of bacteria is responsible for the first stage of anaerobic processes. The anaerobic species belonging to the family of Streptococcaceae and Enterobacteriaceae and the genera of *Bacteroides, Clostridium, Butyrivibrio, Eubacterium, Bifidobacterium* and *Lactobacillus* are most commonly involved in this process (Novaes, 1986).
b. **Hydrogen-Producing Acetogenic Bacteria (group 2):** This group of bacteria metabolizes higher organic acids (propionate, butyrate, H_2, etc.), ethanol and certain aromatic compounds (i.e. benzoate) into acetate, H_2 and CO_2 (Zinder, 1998). The anaerobic oxidation of these compounds is not favorable thermodynamically by hydrogen-producing bacteria in a pure culture, however in a coculture of hydrogen-producing acetogenic bacteria and hydrogen-consuming methanogenic bacteria, these exists a symbiotic relationship between these two groups of bacteria. It is important to point out that during anaerobic treatment of complex wastewater such as vinasses or slaughterhouse, as many as 30% of the electrons is associated with propionate oxidation. Thus, these chemical appears to be more critical than oxidation of other organic acids and solvents (Deublein and Steinhaunser 2008).
c. **Homoacetogens Bacteria (group 3):** Homoacetogenesis has attracted much attention in recent years because of its final product acetate, an important precursor to methane generation. The responsible bacteria are either autotrophs or heterotrophs. The autotrophic homoacetogens utilize a mixture of hydrogen and carbon dioxide, with CO_2 serving as the carbon source for cell synthesis. The heterotrophics homoacetogens, on the other hand, use organic substrate such as formate and methanol as a carbon source while producing acetate as the end product (Eq. 1 to 4) (Khanal, 2008).

$$CO_2 + H_2 \rightarrow CH_3COOH + 2H_2O \quad (1)$$

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad (2)$$

$$4HCOOH \rightarrow CH_3COOH + 2CO_2 + 2H_2O \quad (3)$$

$$4CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2CO_2 \quad (4)$$

Acetobacterium woodii and *Clostridium aceticum* are the two mesophilic homoacetogenic bacteria isolated from sewage sludge (Novaes1986). Homoacetogenic bacteria have a high thermodynamic efficiency; as result there is no accumulation of H_2 and CO_2 during growth on multicarbon compounds (Zeikus 1981).

d. **Metanogenic Bacteria (group 4 and 5):** Methanogens are obligate anaerobes and considered as a rate-limiting specie in anaerobic treatment of wastewater. Abundant methanogens are found in anaerobic environments rich in organic matter such as swamps, marches, ponds, lake and marine sediments, and rumen of cattle. Most methanogens can grow by H_2 as a source of electrons via hydrogenase as shown in the follow reaction (Eq. 5) (Khanal, 2008):

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (5)$$

The source of H_2 is the catabolic product of other bacteria in the system, such as hydrogen-producing fermentative bacteria, especially *Clostridia* (group 1) and hydrogen-producing acetogenic bacteria (group 2). The hydrogenotrophic pathway contributes up to 28% of the

methane generation in an anaerobic treatment system. It bears mentioning that there are many H_2-using methanogens that can use formate as a source of electrons for the reduction de CO_2 to methane, as show in reaction (Eq. 6):

$$4HCOO^- + 2H^+ \rightarrow CH_4 + CO_2 + 2HCO_3^- \quad (6)$$

4. Factors affecting the generation of methane

Anaerobic microorganisms, especially methanogens are highly susceptible to changes in environmental conditions. Many researchers evaluate the performance of an anaerobic system based on its methane production rate because methanogenesis is regarded as a rate-limiting step in anaerobic treatment of wastewater. Methanogens are highly vulnerable and extremely low growth rate in an anaerobic treatment system require careful maintenance and monitoring of the environmental conditions. A temperature change in the substrates or substrates concentration can lead to shutdown of gas production (Novaes, 1986).

The microbial metabolism processes are dependent on many parameters, so that for an optimum fermenting process, numerous parameters must be taken into consideration and be controlled. Some of these environmental conditions are shown in the Table 1 (Deublein and Steinhauser, 2008). A brief discussion of the factors more reported in literature is shown follows.

Operation Parameters	Inhibitors
Hydrogen partial pressure	Oxygen (O_2)
Concentration of the microorganisms	Sulfur compounds
Type of substrate	Organic acids (fatty acids and amino acids)
Specific surface of material	Nitrate (NO_3^-)
Disintegration	Ammonium (NH_4^+) and ammonia (NH_3)
Cultivation, mixing and volume load	Heavy Metals
Light and Mixing	Tannins
Temperature	Disinfectants, herbicides and insecticides
Alkalinity and pH	Degree of decomposition of organic matter
Organic Loading Rate (OLR)	Foaming
Nutrients (C/N/P-ratio)	Scum
Trace elements	
Precipitants (calcium carbonate, MAP, apatite)	
Biogas removal	

Table 1. Environmental conditions and inhibitors in the degradation methanogenic (Deublein and Steinhauser, 2008).

4.1 Temperature

It is interesting to note that anaerobic digestion in the natural environments occurred in a wide range of temperatures between 4 ºC (lake sediment) to 60 ºC (thermophilic digestion process); however, for the industrial practices, the temperature range is limited to 20-55 ºC

(Fannin, 1987). In the natural environments, the optimum temperature for the growth of methane forming *archaea* is 5-25 °C for psychrophilic, 30-35 °C, for mesophilic, 50-60 °C, for thermophilic and >65 °C for hyprethermophilic (Tchobanoglous and Burton, 1996).

It is generally understood that higher temperature could produce higher rate of reaction and thus promoting higher application of organic loading rate (OLR) without affecting the organic removal efficiency (Chae et al., 2007; Choorit and Wisarnwan, 2007; Poh and Chong, 2009). Using palm oil mill effluent as the substrate, Choorit and Wisarnwan (2007) demonstrated that when the digester was operated at thermophilic temperature (55 °C), showed higher OLR application than the that of mesophilic (17.01 against 12.25 g COD/ m^3-d) and the methane productivity was also higher (4.66 against 3.73 L/L/d) (Choorit and Wisarnwan, 2007). A similarly study by Chae et al (2007), indicated that the higher temperature of 35 °C led to the highest methane yield as compared to 30 °C and 25 °C although the methane contents only changed slightly.

Using cheese whey, poultry waste and cattle dung as substrates, Desai et al. (1994) showed that when the temperature was increased from 20, 40 and 60 °C, the biogas production and methane percentage increased as well. The digestion rate temperature dependence can be expressed using Arrhenius expression:

$$r_t = r_{30}(1.11)^{(t-30)} \tag{7}$$

where t is temperature in °C, and r_t, r_{30} are digestion rates at temperature t and 30°C, respectively. Based in Eq. 7, the decrease in digestion rate for each 1 °C decreased in temperature below the optimum range is 11%. Similarly, the calculated rate at 25 °C y 5 °C are 59 and 7% respectively, relative to the rate at 30 °C (Dasai et al., 1994).

Although the thermophilic anaerobic process could increase the rate of reaction, the yield of methane that could be achieved over the specified organic amount is the same regardless of the mesophilic or thermophilic conditions. That value is 0.25 kg CH_4/kg COD removed or 0.35 m^3 CH_4/kg COD removed (0 °C, 1 atm) which is derived by balancing the following equation (Eq. 8), taking into account the different operating conditions worked, can be explained that the values obtained for methane production is different in many scientific reports:

$$CH_4+2O_2 \rightarrow CO_2+2H_2O \tag{8}$$

Although thermophilic condition could result in higher application of organic loading rates and better destruction of pathogens, at the same time it is more sensitive to toxicants and temperature control is more difficult (Gerardi, 2003; Choorit and Wisarnwan, 2007). Furthermore, biomass washout that could lead to volatile fatty acids accumulation and methanogenesis inhibition could also occur if the thermophilic temperature could not be controlled (Poh and Chong, 2009). As a result, in tropical regions mesophilic temperatures are the preferred choice for anaerobic treatment (Yacob et al., 2005, Sulaiman et al., 2009).

4.2 Alkalinity and pH

As far as the anaerobic digestion process is concerned, it is more appropriate to discuss alkalinity and pH together because these parameters are related to each other and very

promising to ensure a suitable environment for successful methanogenesis process. Alkalinity is produced in the wastewaters as results of the hydroxides and carbonates of calcium, magnesium, sodium, potassium or ammonia and may also include borates, silicates and phosphates (Tchobanoglous and Burton, 1991). The alkalinity plays an important pH controlling role in the anaerobic treatment process by buffering the acidity derived from the acidogenesis process (Gerardi, 2003; Fannin, 1987).

Methane producing methanogens are known to be strongly affected by pH (Poh and Chong, 2009) and could only survive on a very narrow range of pH (Table 2) (Gerardi, 2003).

Genus	pH Range
Methanosphaera	6.8
Methanothermus	6.5
Methanogenium	7.0
Methanolacinia	6.6-7.2
Methanomicrobium	7.0-7.5
Methanosprillium	7.0-7.5
Methanococcoides	6.5-7.5
Methanohalobium	6.5-6.8
Methanolobus	6.5-6.8
Methanothrix	7.1-7.8
Methanosaeta	7.6

Table 2. The optimum pH range for selected methanogens (Gerardi, 2003; Steinhaus et al.2007, Tabatabaei et al., 2011)

As such, the methanogenic activity will be severely affected once the optimum pH range is not met. Steinhaus and coworker studied the optimum growth conditions of *Methanosaeta concilii* using a portable anaerobic microtank (Steinhaus et al., 2007). They reported an optimum pH level of 7.6 revealing that even little variations on both sides of the optimum pH suppressed the growth of the methanogens. Several studies have also reported reactor failure or underperformance simply due to pH reduction caused by accumulation of high volatile fatty acids in the anaerobic treatment system (Fabián and Gordon, 1999; Poh and Chong, 2009; Tabatabaei et al., 2011).

In a study using synthetic wastewater in the thermophilic temperature, was found that at the pH of above 8.0, the methanogenesis was strongly inhibited and the value recorded for acetotrophic methanogenic test was zero (Visser et al., 1993). When investigating the role of pH in anaerobic degradation test; Fabián and Gordon (1999), found out that the acidification led to the low performance of the anaerobic degradation, however the biodegradation was significantly increased once the wastewater when the pH was adjusted to above 6.5.

4.3 Organic Loading Rate (OLR)

The OLR variation can be derived from either variation in influent chemical oxygen demand (COD) or variation in flow rate with constant COD. An increase in OLR beyond the optimum level is followed by a decrease in the main process parameters such as COD removal, specific methane production. In addition, high amount of suspended solids

"known as biomass wash-out" are observed in the effluent, indicating that the reactor suffered a process imbalance and that biomass accumulated in the reactor (Converti et al., 1993; Fezzani and BenCheikh, 2007; Rincón et al., 2008). This could be ascribed to an increase in the concentrations of the VFA with a consequent decrease in pH (Tiwari et al., 2006) or to escalated levels of inhibitory or toxic compounds such as phenols, lignin and others.

Therefore, there is a maximal operational value for this parameter. For instance, Rizzi and coworkers in the year of 2006 reported a decrease in COD removal and specific methane production when OLR was increased from 10 to 15 kg COD/m^3-d. With the OLR increase to 20 kg COD/m^3-d the biomass excess started to wash out, followed by deterioration of the reactor performance. In a different study, stable reactor performance was observed when the OLR increased from 1.5 to 9.2 kg COD/m^3-d with the maximum methane production rate achieved for an OLR of 9.2 kg COD/m^3-d. However, a significant decrease in the pH value (from 7.5 to 5.3) was observed when OLR was further raised to 11.0 kg COD/m^3-d. In addition, the increase in the effluent COD with increased OLR was paralleled to a sharp increase in the effluent total volatile fatty acids (TVFA, g acetic acid/L) by about 400% (Rincón et al., 2008). This indicates that, at higher OLR the effluent total COD and mainly soluble COD is largely composed of the unused volatile acids produced in the reactor due to the inhibition of methanogenesis.

Methanobacteriaceae and *Methanosaeta* were found the main methanogens in a laboratory scale up-flow anaerobic digester treating olive mill wastewater (Rizzi et al., 2006). However, the authors also reported an interesting population shift by OLR variation. At lower OLR i.e. 6 kg COD/m^3-d, hydrogenotrophic Methanobacterium predominated in the reactor but the number of cells/g sludge showed a 1000 fold decrease from 10^{11} to 10^8 when the OLR was increased to 10 kg COD/m^3-d. In contrast, phylotypes belonging to the acetoclastic Methanosaeta were not affected by OLR variation and at 10 kg COD/m^3-d, dominated in the biofilm (10^9 cells/g sludge) (Rizzi et al., 2006).

Olive oil wastewater is characterized by high levels of inhibitory compounds such as tannins, and lipids. As a result, increased OLR leads to higher concentration of these substances and a consequent inhibition of methanogenic cells. However, acetoclastic *Methanosaeta* due to its high affinity for acetate is capable of occupying the deepest and thus more protected niches in the granule or biofilm with low concentrations of substrate (acetate) (Gonzales-Gil et al., 2001). Phylotypes belonging to the genus *Methanosaeta* were also dominant independent of different OLR in other anaerobic digesters (Rincón et al., 2008).

In a different study was investigated the microbial ecology of granules in UASB reactor fed by synthetic wastewater under various OLR. The authors showed that the predominant microbial biomass was *Methanosaeta*. However, increasing the OLR led to a substantial increase of *Methanosarcina* in the granules (Kalyuzhnyi et al., 1996). The increase of *Methanosarcina* in the studied synthetic wastewater (toxin-free) due to increasing OLR is explained by the low affinity of these methanogens for acetate in comparison with *Methanosaeta*. Hence, by increasing OLR and consequent VFA concentration, *Methanosarcina* is favored.

As reviewed earlier, under mesophlic conditions *Methanosaeta* plays a significant role in making cores of sludge granules (Sekiguchi et al., 2001) and thus their ratio seems to control the speed of granulation (Rincón et al., 2008). Higher OLR, result in consequent higher concentration of substrates (i.e. acetate) in the reactor. Morvai and coworkers in 1990

investigate the influence of organic load ranging from 0.5-3.0 g/L on granular sludge development in an acetate-fed system. They argued that in the range of feed acetate levels examined, higher concentrations of acetate caused faster granulation of the sludge bed and, presumably of the microbial population, and resulted in better sludge structure and improved sludge settleability.

Low OLR has been reported to cause acute mass transfer limitation leading to disintegration of the larger granules (Ahn et al., 2002). The disintegration begins at the core of the granules due to substrate limitation with a consequent loss of granules strength and stability. However, this was not in agreement with the studies reported, which low OLR (<1.5 kg COD/m^3-d) did not lead to disintegration of the granules in UASB reactors (Tiwari et al., 2005). This could be ascribed to the different experimental settings and wastewaters used in these studies. Teo and coworker (2000), treat a high iron bearing wastewater in a UASB reactor. Evidence shows that the presence of divalent and trivalent cations ions, such as Fe^{2+} and Fe^{3+}, helps bind negatively charged cells together to form microbial nuclei that promote further granulation.

Tiwari et al. (2006) tried to enhance the granulation process by using natural ionic polymer additives. These may thus reduce the effect of low OLR (i.e. substrate limitation) on the granules and delayed the disintegration. Meanwhile was reported that COD removal rate, the COD specific removal rate (r_s) and methane production rate were not suppressed by increasing OLR when treating wine wastewater and sewage mixture (Converti et al., 1990). That indicated that no inhibition factor related to the organic content of the effluent was present in both wine wastewater and sewage mixture studied.

This was further supported by the cell mass concentration varied very little with increasing the OLR. However as completely noticed by the authors, even at the absence of inhibitory compounds in the initial part, the removal rate increased with the OLR, following a first order kinetic. In the second part, instead the removal rate tended to a constant maximum value, following a zero order kinetic. Afterwards, the removal efficiencies as well as the methane production yield gradually decreased with increasing influent COD due to increasing the OLR, which evidently showed a substrate inhibition occurrence (Converti et al., 1990).

This supports the idea that even at the absence of the inhibitory compounds in the wastewater, increasing influent COD by the means of increasing OLR could lead to substrate inhibition and consequent reduced removal efficiencies. In other study is described the dependence of the removal rate on the OLR by an empirical equation similar to Monod's model (Eq. 9) to compare the degradability of different effluents (Converti et al., 1990):

$$r_s = \frac{r_{s(max)} OLR}{(k + OLR)} \tag{9}$$

where $r_{s(max)}$ (kg COD/kg of vss d) is the maximum value of r_s, and k is a constant which physically is expressed in units of OLR, an increase of k indicates increased treatment ability of the studied effluent. The desired OLR is the function of the favorable effect of OLR on stimulating the growth of methanogens in the bioreactor by providing them with higher substrate concentrations, its reverse effect on elevating the concentration of inhibitory compounds and the buffering capacity of methanogenic community. In the other words, the maximal operational value of OLR is translated into the highest methane production

(indicating the highest conversion efficiency of the system) that the buffering capacity of methanogenic community is still capable of compensating for elevated concentrations of inhibitory compounds (Tabatabaei et al., 2011).

4.4 Mixing

There are only a limited number of studies found specifically focused on the effects of mixing on the treatment efficiency and biogas production using various types of agro-industrial wastewater including palm oil mill effluent, wash water of animal waste, lixiviate of municipal waste and fruit and vegetable wastes (Kaparaju et al., 2007; Sulaiman et al., 2009). Adequate mixing is very important in order to achieve successful anaerobic treatment of organic rich wastewater. In another word, it enhances the anaerobic process rate by preventing stratification of substrate, preventing the formation of surface crust, ensuring the remaining of solid particles in suspension, transferring heat throughout the digester, reducing particle size during the digestion process and releasing the biogas from the digester content (Kaparaju et al., 2007; Sulaiman et al., 2009).

Prior to 1950s, anaerobic digesters treating sewage sludge were not equipped with mechanical mixing and thus caused the formation of scum layer at the surface (Fannin, 1987). To overcome this problem, mixing was employed to disrupt scum formation and enhance contact between microorganisms and substrates. It has been reported that the acetate-forming bacteria and methane-forming bacteria are required to be in close contact to achieve continuous degradation of organic materials (Tabatabaei et al., 2011). In addition to the mentioned advantages, mixing also helps to eliminate thermal stratification inside the digesters, maintain digester sludge chemical and physical uniformity, rapid dispersion of metabolic products and toxic materials and prevent deposition of grit (Gerardi, 2003).

4.5 Heavy metals inhibition

Heavy metals are present in various types of wastewater, including agro-industrial wastewater, landfill leachate and cane vinasses (Del Real et al., 2009; Yusof et al., 2009). Although many metals are required in trace amounts to provide sufficient growth to methanogens, the methanogenic activity in anaerobic reactors is strongly affected by excess amounts of heavy metals (Colussi et al., 2009). The toxic effects of metals in biological process is particularly due to the inhibition of enzymes activity as a result of metals binding to the SH group of the enzyme. The inhibitory concentrations of four heavy metals on methane-producing granular sludge that caused 50% reduction in cumulative methane production was found to be 7.5 mg/L of Zn, 27 mg/L of Cr, 35 mg/L of Ni and 36 mg/L of Cd with an order of Zn>Cr>Ni≈Cd (Altas, 2009). Whereas a different study revealed that 50% reduction in methane production occurred at 6.4 mg/L of Cu (II), 4.4 mg/L of Cd(II) and 18.0 mg/L of Cr(VI) with an order of Cd(II)>Cu(II)>Cr(VI) in anaerobic digestion of cattail with rumen culture (Yue et al., 2007).

Yue and coworker in 2007, indicated that metals cause anaerobic system failures when they are in the form of free ions (in its soluble form) and above certain concentrations (Table 3). The differences reported in the metals inhibitory concentration might be due to the several factors including variation in sludge characteristics, chemical form of heavy metals and microbial resistance to metals (Altas, 2009). Various heavy metals presence in wastewater

also showed synergistic effects during anaerobic treatment process. For instance, the presence of chromium in the sludge results in higher toxicity of copper (Colussi et al., 2009).

Altas in 2009 showed that low concentrations of metals in the anaerobic reactor can be extremely toxic. Meanwhile, Cantrell and coworkers (2008) indicated that high concentrations of soluble metals have come to completely stop the production of biogas in an anaerobic system. To combat metal toxicity in the anaerobic degradation process, they can be precipitated as sulphate salts and carbonate salts, except iron and chromium.

Substance	Total Concentration (mg/L)	Soluble Concentration (mg/L)
Cu	200	0.5
Cr (VI)	50 – 70	3
Cr (III)	200 – 260	-
Ni	180 - 420	2
Zn	30	1

Table 3. Concentrations of inorganic compounds inhibitory of anaerobic process (Yue *et al.*, 2007).

5. Proprieties and volumetric compensation of the gases from anaerobic process

The physical and chemical proprieties of biogas, affect the choice of technology used for clean-up and combustion; therefore, knowledge of these proprieties is useful for optimization biogas utilization. Since biogas contains primarily methane and carbon dioxide, this section is focused on their respective physical characteristics (Table 4). Because others components (nitrogen, hydrogen sulfide, traces organics), are present in relatively small quantities are not considered in the table. The magnitude of CH_4 and CO_2 varies greatly and depends on the composition of the organic material digested in the wastewater.

Proprieties	Methane (CH_4)	Carbon dioxide (CO_2)[a]
Molecular weight (g/mol)	16.04	44.01
Specific gravity, (air =1[c])	0.554	1.52
Boiling point, (14.7 psia)	126.0 °C	43.0 °C [b]
Freezing point, (14.7 psia)	-182.4 °C	- 56.55 °C
Specific volume (m^3/kg)	1.51	$7.03x10^{-3}$
Critical temperature (^oC)	46.62	31.08
Critical pressure (kPa)	4,640.1	7391.15
Heat capacity (kJ/kg K)	2.26	0.858
Ratio Cp/Cv	1012	
Heat of combustion (kJ/m^3)	377	
Limit of inflammability	5-15% by volume	
Stoichiometry in air [c]	0.0947 by volume 0.0581 by mass	

a: pure gas given at 77 ^oF and 101.3 kPa; b: sublimes; c: Air at 101.3 kPa and 15.54 °C

Table 4. Physical constants of methane and carbon dioxide

The volumetric measurement of biogas must compensate the pressure and temperature differences. The equation 10, illustrate a simple method of gas volume compensation for a saturated gas taking into account the adjustment by pressure and temperature (Salisbury, 1950):

$$V_s = V * 17.626 * \frac{(H-A)}{(459.6+T)} \quad (10)$$

where V is the observed volume, V_s volume at standard conditions (60°F and 30 inches Hg), H is absolute gas pressure (inches Hg), A water vapor pressure (inches Hg), and T temperature of gas (°F). Pure methane at standard temperature and pressure has a lower heating value of 912 BTU/ft³ (34 kJ/L). Typical biogas of 65% methane has a heating value of approximately 600 BTU/ft³ (22.36 kJ/L), since only the methane portion will burn, approximate equivalents of biogas to others fuels are presented in the table 5.

Biogas with 65% of methane (1000 L)
600 L of natural gas
25.0 L of propane
22.3 L of Butane
17.79 L of gasoline
16.28 L of diesel

Table 5. Equivalents of biogas others fuels (Palmer, 1981)

6. Reactor types

Many reactor configurations are used for the anaerobic treatment of agro-industrial wastewaters. Among them, the most common types are discussed and illustrated in Fig. 2.

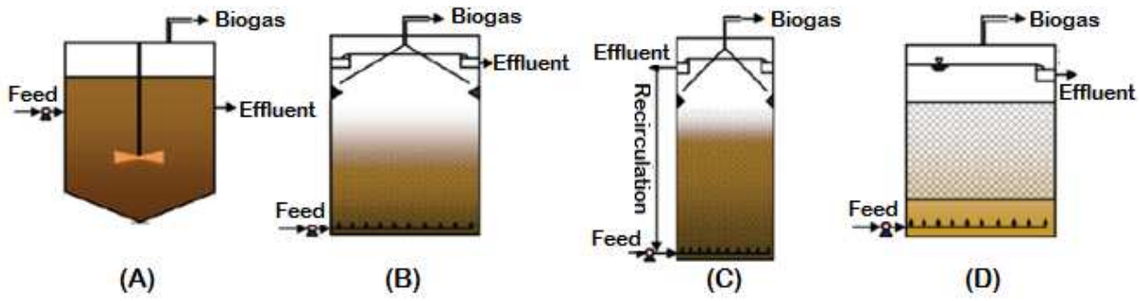

Fig. 2. Most commonly used anaerobic reactors types: (A) Completely mixed anaerobic digester, (B) UASB reactor, (C) AFB reactor, (D) Upflow AF reactor (Ersahin et al., 2011)

6.1 Completely stirred anaerobic digester

The completely stirred anaerobic digester (CSTR) is the basic anaerobic treatment system with an equal hydraulic retention time (HRT) and solids retention time (SRT) in the range of 15-40 days in order to provide sufficient retention time for both operation and process stability. Completely mixed anaerobic digesters without recycle are more suitable for wastes with high solids concentrations (Tchobanoglous et al., 2003). A disadvantage of this system

is that a high volumetric loading rate is only obtained with quite concentrated waste streams with a biodegradable COD content between 8,000 and 50,000 mg/L. However, many waste streams are much dilute (Rittmann and McCarty, 2001). Thus, COD loading per unit volume may be very low with the detention times of this system which eliminates the cost advantage of anaerobic treatment technology. Typical the OLR for this digester is between 1-5 kg COD/m^3-d (Tchobanoglous et al., 2003).

6.2 Upflow anaerobic sludge blanket reactor

One of the most notable developments in anaerobic treatment process technology is the upflow anaerobic sludge blanket (UASB) reactor invented by Lettinga and coworkers (Lettinga et al., 1980) with its wide applications in relatively dilute municipal wastewater treatment and over 500 installations in a wide range of industrial wastewater treatment including food-processing, paper and agro-industrial process (Tchobanoglous et al., 2003).

Influent flow distributed at the bottom of the UASB reactor travels in an upflow mode through the sludge blanket and passes out around the edges of a funnel which provides a greater area for the effluent with the reduction in the upflow velocity, enhancement in the solids retention in the reactor and efficiency in the solids separation from the outward flowing wastewater. Granules which naturally form after several weeks of the reactor operation consist primarily of a dense mixed population of bacteria that is responsible for the overall methane fermentation of substrates (Rittmann and McCarty, 2001). Good settleability, low retention times, elimination of the packing material cost, high biomass concentrations (30,000-80,000 mg/L), excellent solids/liquid separation and operation at very high loading rates can be achieved by UASB systems (Speece, 1996). The only limitation of this process is related to the wastewaters having high solid content which prevents the dense granular sludge development (Tchobanoglous et al., 2003). Designed for OLR is typically in the range of 4 to 15 kg COD/m^3-d (Rittmann and McCarty, 2001).

6.3 Fluidized and expanded bed reactors

The anaerobic fluidized bed (AFB) reactor comprises small media, such as sand or granular activated carbon, to which bacteria attach. Good mass transfer resulting from the high flow rate around the particles, less clogging and short-circuiting due to the large pore spaces formed through bed expansion and high specific surface area of the carriers due to their small size make fluidized bed reactors highly efficient. However, difficulty in developing strongly attached biofilm containing the correct blend of methanogens, detachment risks of microorganisms, negative effects of the dilution near the inlet as a result of high recycle rate and high energy costs due to the high recycle rate are the main drawbacks of this system. The expanded granular sludge bed (EGSB) reactor is a modification of the AFB reactor with a difference in the fluid's upward flow velocity. The upflow velocity is not as high as in the fluidized bed which results in partial bed fluidization. (Rittmann and McCarty, 2001). OLR of 10-50 kg kg COD/m^3-d can be applied in AFB reactors (Ozturk, 2007; Ersahin et al., 2011).

6.4 Anaerobic filters

The anaerobic filter (AF) has been widely applied in the beverage, food-processing, pharmaceutical and chemical industries due to its high capability of biosolids retention. In

fact clogging by biosolids, influent suspended solids, and precipitated minerals is the main problem for this system. Applications of both upflow and downflow packed bed processes can be observed. Prevention of methanogens found at the lower levels of the reactor from the toxicity of hydrogen sulfide by stripping sulfide in the upper part of the column and solids removal from the top by gas recirculation can easily be achieved in downflow systems in comparison to upflow systems. However, there is a higher risk of losing biosolids to the effluent in the downflow systems. Design OLR is often in the range of 8-16 kg COD/m^3-d which is more than tenfold higher than the design loading rates for aerobic processes (Rittmann and McCarty, 2001).

7. Bioenergy production from different kinds of wastewaters

Methanogenic anaerobic digestion is a classical anaerobic bioconversion process that has been practiced for over a century and used in full-scale facilities worldwide. This is a complicated process that involves a mixture of population of microorganisms and several gasses and liquid products, thus strict process control and product purification are required. Biogas production have been demonstrated in numerous studies with great success like can see in the Table 6 (Gavrilescu, 2005).

Wastewater	Reactor Type	HRT (days)	OLR (kg COD/m^3-d)	COD removal (%)	CH_4 Yield (m^3/kg COD)	Reference
Brewage distillery	UASB	--	16.5-44.0	80	16.5	Shin et al., (1992)
Cane-molasses stillage	AFB	5.6-32	4.65-20	85	0.168	Yeoh (1997)
Cheese whey and dairy	Hybrid reactor	--	10	98	--	Malaspina et al (1996)
Cheese whey and dairy	Hybrid reactor	--	0.97-2.82	91-97	0.28-0.35	Strydom et al (1997)
Cheese whey and dairy	CSTR	2.0	5	90	--	Ince (1998)
Cheese whey and dairy	CSTR	4-7	--	--	0.55	Yilmazer y Yeningüm (1999)
Landfill leachate	AFB	4.7-16	2.41-7.98	>90	---	Lin (1990)

Table 6. Typical performance of anaerobic reactor used for wastewater treatment (Gavrilescu, 2005)

8. Biogas production from agro-industrial wastewaters

8.1 Case vinasses of tequila

Tequila is a Mexican regional alcoholic beverage obtained from the fermentation of sugars from the cooked stems of blue agave (Agave tequilana Weber var. azul). Its production and

commercialization is verified and certified by the Mexican Tequila Regulatory Council (CRT) (NOM-006-SCFI-2005, 2006). In 2008 the CRT registered 139 producers and 1,018 brands of Tequila (bottled in Mexico and in foreign countries, CRT 2008). Based on the number of employees, only 7% are large factories and the rest are small and medium factories, with a grand total of around 30,500 direct employees (National Tequila Industry Chamber, CNIT 2009). Therefore, this industry represents an important economic activity for the 180 Mexican municipalities within the *appellation d'origine contrôlée* granted in 1995 for Tequila.

Tequila production has had an important increase from 2004 to 2008, as it is shown in Fig. 3. In 2010 about 187.3 million liters of Tequila (55% Alc. Vol.) has been produced with a projection for annual growth of at least 10% (CNIT 2010); there is also a decrease in production of Tequila between 2000 and 2003, due to the agave crisis (Dalton 2005). Although exhaustive reviews regarding the treatment of different distillery wastewaters are published elsewhere (Satyawali and Balakrishnan 2008; Mohana et al. 2009), it is considered that special attention should be paid to distillery effluents from the Tequila industry due to their complex composition. This section present the potential generation of energy from wastewater treatments to generate biogas from the Tequila industry.

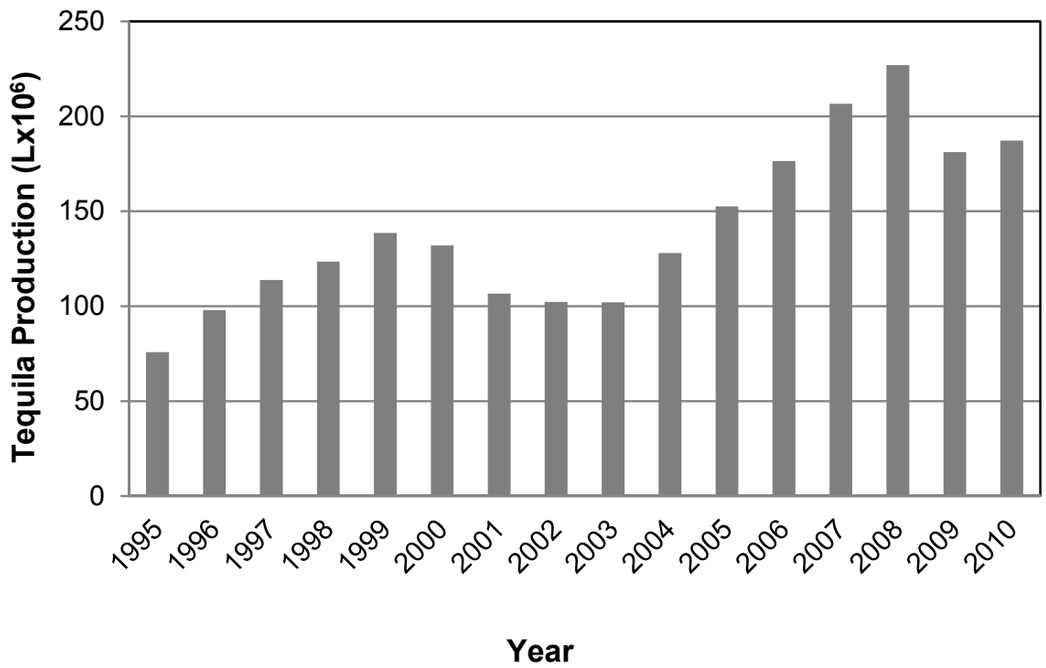

Fig. 3. Dynamics of Tequila production (55% Alc. Vol.). (calculated from CNIT 2010)

The production of Tequila generates large quantities of bagasse and vinasses. Bagasse is a residual solid; it is generated in the elaboration of Tequila and is produced during the extraction of juice from the cooked heads of agave. Vinasses are the liquid residues that are generated and remain in the bottom of the still after the distillation of the must of fermented agave.

Vinasses are dark brown in color, because they contain phenolics (tannic and humic acids), melanoidins that are low and high molecular weight polymers formed as one of the final products of Maillard reaction (Satyawali and Balakrishnan 2008). It is known that for each

liter of Tequila produced, 1.4 kg of bagasse and 10–12 L of vinasses are generated. Under this basis of calculation, it is estimated that the production of Tequila in 2010 generated 262.2 million kilograms of bagasse and 1,873.0 million liters of vinasses.

In the majority of the Tequila factories, bagasse is converted into compost, which is also done in the agave plantations. However, approximately 80% of the vinasses are discharged directly into water bodies (rivers, streams, lakes, reservoirs) and municipal sewer systems or directly onto the soil without receiving adequate treatment for discharge. This common practice causes a deterioration of different degrees to the water bodies receiving the discharges due to low pH, high temperature and elevated concentrations of both BOD and COD of these effluents. On the contrary, if the vinasses receive appropriate treatment and management, they can be used as a source of nutrients and organic matter in agricultural activities; they can also be a potential source of renewable energy. A summary of the physicochemical characteristics of the vinasses generated from the process of producing traditional Tequila (100% agave) is shown in Table 7 (Lopez-Lopez, 2010).

Parameter	Value
pH	3.4-4.5
Oils and fats (mg/L)	10-100
Total COD (mg/L)	60,000-100,000
Soluble COD (mg/L)	40,000-80,000
Total BOD (mg/L)	35,000-60,000
Soluble BOD (mg/L)	25,000-50,000
Total solids (mg/L)	25,000-50,000
Total suspended solids (mg/L)	2,000-8,000
Fixed suspended solids (mg/L)	10-500
Volatile suspended solids (mg/L)	1,990-7,500
Total dissolved solids (mg/L)	23,000-42,000
Settleable solids (mL/L)	10-900
Total alkalinity (mg/L)	< 6.00
Total acidity (mg/L)	1,500-6,000
Fixed acidity (mg/L)	1,480-5,800
Volatile acidity (mg/L)	20-200
Ca (mg/L)	200-1,100
Mg (mg/L)	100-300
K (mg/L)	150-650
Phosphates (mg/L)	100-700
Total nitrogen (mg/L)	20-50
NH_4^+-nitrogen (mg/L)	15-40
Organic nitrogen (mg/L)	5.0-10
Total reducing sugars (% w)	0.5-2.0
Direct sugars (% w)	0.4-1.0
Cu (mg/L)	< 3.0
Fe (mg/L)	< 45
Ni (mg/L)	< 0.02
Zn (mg/L)	< 1.0

Table 7. Physicochemical characteristics of Tequila vinasses (Lopez-Lopez 2010)

The anaerobic biological process has been utilized for treating Tequila vinasses on laboratory, pilot and industrial scales due to technical and economical advantages over aerobic processes (Linerio and Guzman 2004; Mendez, et al. 2009). On a laboratory scale, Lopez-Lopez and coworkers (2011), Mendez and coworkers (2009) showed an anaerobic digester capable of removing 90–95% of organic material as COD; generating significant amounts of biogas rich in methane. The most common system found at an industrial level in treating Tequila vinasses is of anaerobic type. Fig.4 shows the amount of energy that can be generated if the entire volume of vinasses is treated.

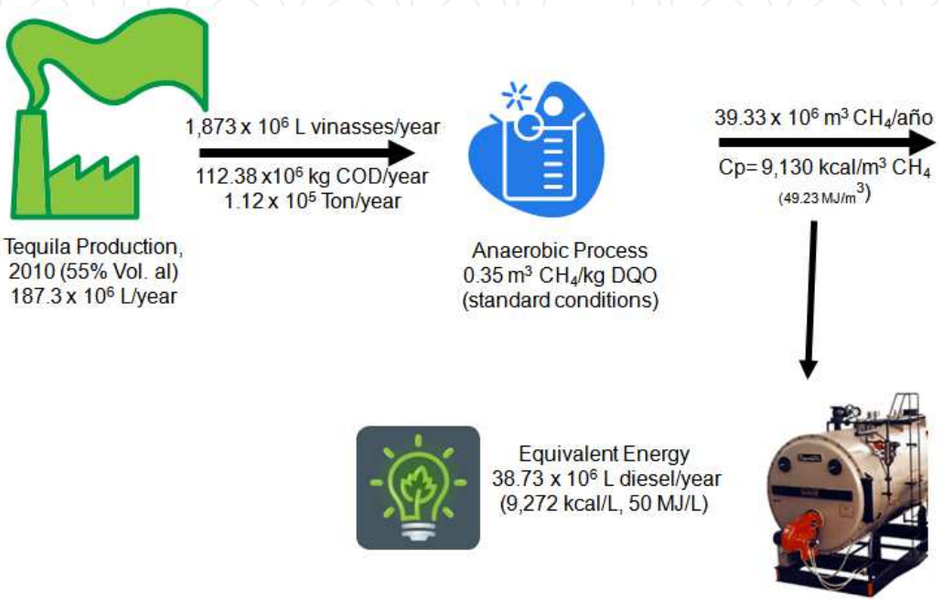

Fig. 4. Production of biogas from Tequila vinasses as a source of energy

8.2 Others cases

In general, all types of wastewater can be used as substrates as long as they contain carbohydrates, proteins, fats, cellulose and hemicelluloses as main components. It is important that the following points are taken into consideration when selecting the wastewater industrial.

The content of organic substance should be appropriate for the microorganisms selected in anaerobic process.

- The high nutritional value of the organic substance for the microorganism, hence the potential for gas formation and should be as high as possible.
- The substrate should be free of pathogens and others organism which would need to be made innocuous prior to the anaerobic process.
- The content of harmful substances and trash should be low to allow the fermentation process to take place smoothly.
- The composition of the fermentation residue should be such that it can be used, e.g. as fertilizer.

In this section some of agro-industrial wastewater employed like organic substrates is shows, because the degree to which the organic substances in the wastewater is decomposed

in the bioreactor depends on the origin of the liquid. In the Table 8, is shows the methane production rate from wastewater of different types.

Wastewater type	Reactor Type	HRT (days)	OLR (kg COD/m³-d)	Temperature (°C)	COD removed (%)	MPR (m^3CH_4/kg COD)	Ref.
Slaughter-house	Anaerobic filter	0.6-3.0	3.7 -16.5	25	50-81	0.41	[1]*
Slaughter-house	CSTR	20-30	0.2-0.3	37	70-80	0.45	[2]*
Tequila vinasses	UASB	2.0-2.5	2.0-12.0	37	50-85	0.46	[3]*
Cane vinasses	CSTR	20-30	2.5-12.7	35	50-75	0.42	[4]*
Pulping coffee	CSTR	20-30	0.2-0.4	35	60-75	0.37	[5]*

Table 8. Methane production rate from wastewater of different type

In all previous cases, the wastewaters are discharged directly into the body of water, causing several environmental pollution in addition to the loss of the energetic potential contained in the effluents.

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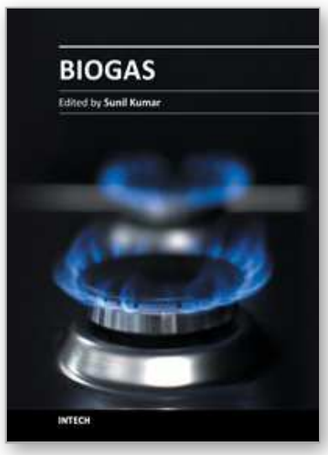

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This book contains research on the chemistry of each step of biogas generation, along with engineering principles and practices, feasibility of biogas production in processing technologies, especially anaerobic digestion of waste and gas production system, its modeling, kinetics along with other associated aspects, utilization and purification of biogas, economy and energy issues, pipe design for biogas energy, microbiological aspects, phyto-fermentation, biogas plant constructions, assessment of ecological potential, biogas generation from sludge, rheological characterization, etc.

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