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# Use of organic wastes of *Agave* processing for bioenergy production

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## Preliminary remarks

This thesis is based on manuscripts either published, accepted or prepared for publication in peer-reviewed journals and are referred to in the text by their chapter as shown below.

Chapter 3: López Velarde S.M., Ventura Ramos E.J., Rodriguez Morales J.A. and Hensel O. (2018). Inoculum adaptation for the anaerobic digestion of Mezcal vinasses. Rev. Int. Contam. Ambie. (Accepted).

Chapter 4: López Velarde S.M., Ventura Ramos E.J., Rodriguez Morales J.A. and Hensel O. (2108). Effect of inoculum source on the anaerobic digestion of Mezcal vinasses at different S:I-ratios. Rev. Int. Contam. Ambie. (Accepted).

Chapter 5*: López Velarde S.M., Ventura Ramos E.J., Rodriguez Morales J.A. and Hensel O. (2018). Comparison of bioreactor start-up for Mezcal vinasses anaerobic digestion using a low-cost biofilm carrier. (Draft manuscript to be submitted).

Chapter 6: López Velarde S.M., Rodríguez Valadez F.J., Mora Solís V., González Nava C., Cornejo Martell A.J. and Hensel O. (2017). Performance of a microbial fuel cell operated with vinasses using different COD concentrations. Rev. Int. Contam. Ambie. 33 (3) 521-528. DOI: 10.20937/RICA.2017.33.03.14

Chapter 7: López Velarde S.M., Ventura Ramos E.J., Rodriguez Morales J.A. and Hensel O. (2018). Simultaneous electricity and biogas generation of vinasses and cattle manure. (Draft manuscript to be submitted)

* Parts of this chapter has been presented in the Congreso Internacional de Ingeniería CONIN 2015 at the Autonomous University of Queretaro in Mexico, as a poster.

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## List of abbreviations and units used

| Abbreviations | |
|---|---|
| Airlift reactor | ALR |
| Ammonium | $NH_4^{+-}N$ |
| Ammonium/ammoniac | $H_4^+/NH_3$ |
| Anaerobic digestion | AD |
| Biochemical methane potential | BMP |
| Combined heat and power | CHP |
| Computer fluid dynamics | CFD |
| Continuous stirred tank reactor | CSTR |
| Fluidized bed reactor | FBR |
| Hydrogen ions | $H^+$-ions |
| Methane | $CH_4$ |
| Microbial fuel cell | MFC |
| Moving biofilm reactors | MBBR |
| Packed bed reactor | PBR |
| Polyethylene terephthalate | PET |
| Silver chloride electrode | Ag/AgCl |
| Sodium chloride | NaCl |
| Sodium hydroxide | NaOH |
| Upflow anaerobic sludge blanket | UASB |

| Units | |
|---|---|
| Biochemical oxygen demand | BOD (g/L) |
| Cumulative biogas production | L/kgVS |
| Cumulative methane production | L/kgVS |
| Chemical oxygen demand | COD (g/L) |
| Chemical oxygen demand:Nitrogen | COD:N |
| Conductivity | µS/cm |
| Contact surface | $m^2$ |
| Current | I (A) |
| Current density | $A/m^3$ |
| Density | $g/cm^3$ |
| Diameter | cm |
| Distance | cm |
| Electrical power | $kW_{el}$ |
| Energy | kWh |
| Euro | € |
| Inflow velocity | $cm^3/min$ |
| Kilogram | kg |
| Lag phase | λ (d) |
| Maximal specific growth rate | µm (L/kg d) |
| Mexican pesos | $ |
| Nitrate ion | $NO_3^-$ (g/L) |
| Organic loading rate | gVS/Ld |
| Phosphate ion | $PO_4^{3-}$(g/L) |
| Power density | $W/m^3$ |
| Reduction oxidation reactions | REDOX (mV) |
| Resistance | R (Ω) |
| Stirring time | min/d |

| | |
|---|---|
| Substrate to inoculum ratio | S:I-ratio |
| Sulphate ion | $SO_4^{2-}$(g/L) |
| Tons | T |
| Total dissolved solids | TDS (g/L) |
| Total inorganic carbon | TAC (g/L) |
| Total nitrogen | N (g/L) |
| Total phosphorous | P (g/L) |
| Total solids | TS (g/L,%) |
| Viscosity | Poise (P) |
| Volatile organic acids | FOS (g/L) |
| Volatile solids | VS (g/L,%) |
| Voltage | V (volts) |
| Volume | $m^3$ |
| Yearly operating hours | h/a |

# 1 Introduction

## 1.1 Mezcal and Tequila in Mexico

*Agave* is a natural resource of great importance for the Mexican agriculture. Many agricultural products are generated from this plant. Beverages, food and fibers are mainly produced and their commercialization is very important for the economy of the country. *Agave* is a genus of monocots, plants with only one embryonic leaf and are native especially to arid and tropical areas in North and South America. *Agaves* have many fleshy leaves and a big rosette. Mostly, *Agave* leaves ends with a sharp terminal spine. Each rosette grows during several years. A tall stem (quiote) grows beginning at the rosette center and bears many flowers. Afterwards, the plant dies (Godínez 2017).

Tequila and Mezcal are very important products for the Mexican economy and are produced from different kinds of *Agave*. When the stem begins to grow, many sugars are synthetized, so that it can continue growing. Afterwards the plant reproduce bearing flowers. Before this step occurs, for Tequila and Mezcal production, stem will be cut so that the sugars remain concentrated in the rosette. When the plant reaches maturity, the rosette is hackled, cooked, milled and fermented. The fermented juice is distilled for schnapps production. **Figure 1.1** shows the scheme of the Tequila and Mezcal production process (Godínez 2017). At the end of the process, vinasses remain as an agricultural residue.

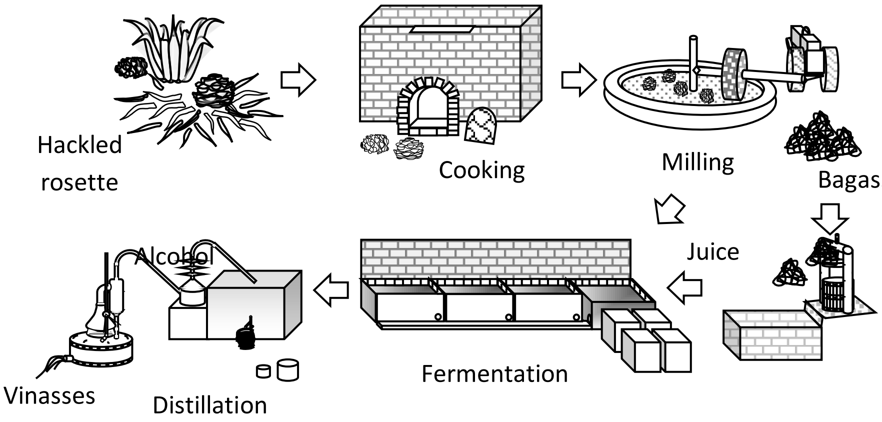

**FIGURE 1.1** Scheme of Tequila and Mezcal production from *Agave* (Godínez 2017)

Tequila is produced from the *Agave Tequilana weber* and Mezcal from other varieties of *Agave*, like *Agave salmiana*, *Agave cupreata*, *Agave potatorum* and *Agave angustifolia* among others (López-López *et al.* 2010, Zamora *et al.* 2010). Mezcal is produced in 22 states of the Mexican territory, its production is concentrated in the states of Guerrero, Oaxaca, Tamaulipas, Guanajuato, San Luis Potosi and Zacatecas (CRM 2015). The denomination of origin of Tequila includes the states of Jalisco, Nayarit, Guanajuato, Tamaulipas and Michoacán (CRT 2018).

Between years 2005 and 2009 Mezcal production arose up to 300 %, being Oaxaca the principal Mezcal producer with 54.4 % of the total production, followed by Zacatecas with 45.3 %, as well as Durango and Guerrero with 0.3 %. In 2009, 1.8 million liters Mezcal were produced, and the total export of this alcoholic beverage ascended to 7.7 million dollars. From the total of the exportations in 2009, 7.1 % were for Tequila and 6.5 % for Mezcal. From the exported Mezcal, 62.9 % goes to the USA, 8.1 % to Chile, 7.4 % to Spain, and 6.3 % to Australia (DGAPEAS 2011).

## 1.2 Vinasses characteristics

In the Mezcal and Tequila industries, vinasses are one of the main agricultural residues of fermentation and distillation of *Agave* to alcohol. Great volumes of vinasses with high pollutant charge remain in Mexico every year. From each liter Tequila produced, around 10 and 12 liters vinasses are being generated, and from the Mezcal production around 8 and 15 liters. The amount of produced vinasses and characteristics depends on the *Agave* species and schnapps production parameters. Vinasses from Tequila and Mezcal contain different organic substances like acetic and lactic acids, phenols, polyphenols, melanoidins or inorganic compounds like sulphates and phosphates salts. Vinasses are characterized because of their low pH in the range between 3 and 5, high organic matter content up to 50 g/L as BOD (biological oxygen demand), as well as 150 g/L as COD (chemical oxygen demand). Approximately 80 % of vinasses are discharged into water bodies and soils, what cause severe environmental problems. The high content of salts in vinasses can lead to soil sodicity and salinity, which can deteriorate the porosity, structure and fertility of the soils. Besides, accumulation of high suspended solid loads can lead to phytotoxicity and can inhibit seed germination. If vinasses are discharged after their production without cooling, at temperatures around 50 and 90 °C, they may rise water temperature so that dissolved oxygen shrinks under levels, where fish survival is no longer possible. The high contents of phosphor and nitrogen could also lead to eutrophication in water bodies (López-López *et al.* 2010, Robles-González *et al.* 2012). **Figure 1.2** shows an example of vinasses disposal in the Mezcal factory Laguna Seca in San Luis Potosi, Mexico. Vinasses are disposed in a lagoon, located nearby the factory. Locals reported that this waterbody showed high contamination levels.

**FIGURE 1.2** Vinasses disposal in Mezcal factory Laguna Seca in San Luis Potosi, Mexico

In order to avoid the disposal of vinasses in waterbodies or soils, vinasses could be used as substrate in anaerobic digesters, for the biogas and methane generation. Different techniques could be implemented in anaerobic digestion (AD), for biogas and methane yields enhancement. Biogas can be used as a biofuel for the electricity and heat generation, through a combined heat and power (CHP) system. In principle, all organic wastes can be disposed in a bioreactor for AD. Main goal is to achieve a high biogas production with high methane content. In the past years, several methods for AD enhancement have been developed and implemented, in order to make this technology more profitable and achieve better waste treatment rates (Taherzadeh and Karimi 2008, Ward *et al.* 2008, Valdez-Vázquez *et al.* 2010, Poornejad *et al.* 2012, Vancov *et al.* 2012).

Vinasses could also be used as input material for the direct voltage generation in microbial fuel cells (MFC). Due to high rates of electricity produced through AD, in comparison to microbial fuel cells, it is certainly improbable that microbial fuel cells replace AD technology. However MFCs could have a useful role when used to minimize the organic load and other contaminants, to reach the permissible levels in wastewater discharges. At the same time electricity would be produced (Higgins *et al.* 2013).

## 1.3 Research hypothesis and objectives

The hypothesis proposed at the beginning of this work, was that the organic wastes of *Agave* processing (vinasses) could be used for bioenergy production, instead of disposed in soils and water. Organic matter content can be thereby diminished. Bioenergy technologies to be tested are AD and MFC. Besides, it was proposed as secondary hypotheses, that AD efficiency could be enhanced when using a more suitable inoculum source, when using biofilms in the bioreactor, or when adjusting the substrate to inoculum ratio (S:I-ratio) for batch fermentation. It was also proposed that AD effluent, using vinasses as substrate, could be successfully used as influent in a MFC, generating simultaneously biogas and voltage.

Aim of this work was to evaluate the proposed hypotheses at labor scales. If the hypotheses are confirmed, results can be transferred to middle and large scale bioreactors. It was intended to improve the scientific knowledge regarding AD and MFC and to achieve a better understanding of the biological processes occurring in a bioreactor operated with vinasses. Literature research helped to know the most recent results of vinasses in MFC, vinasses AD, as well as AD enhancement through biofilms, S:I-ratios and inoculum sources. Mathematical modeling was carried out, to describe and compare the bacterial growth dynamics. CFD simulation was applied to evaluate the design of the small biorefinery with an AD-MFC configuration. Specific objectives of the research included:

1. To investigate if AD could be successfully carried out using vinasses as substrate and conventional anaerobic sludge as inoculum, in semi-continuous tests (**chapter 3**).
2. To demonstrate that the resulted AD could be enhanced using an alternative inoculum source (in batch and semi-continuous fermentation), adjusting S:I-ratio (in batch fermentation), and using biofilms in bioreactor (in semi-continuous fermentation) (**chapter 4** and **5**). The use of a biofilm carrier was not tested in the batch tests, because its use is more appropriate, when a continuous or semi-continuous substrate input can be guaranteed. Batch tests mean cell inactivity when lack of feeding (Qureshi *et al.* 2005).
3. To demonstrate that Mezcal vinasses could be successfully used for the first time in MFC technology for voltage production, with an optimal COD content in anolyte containing vinasses (**chapter 6**).
4. To demonstrate if a small biorefinery can be successfully implemented for the use of AD digestate as influent in a low-cost MFC, generating simultaneously biogas and electricity (**chapter 7**). The most efficient results obtained in the past chapters, regarding COD load and S:I-ratios, were used.

## 1.4 Thesis structure

First, a literature research was done, in order to have a deeper knowledge in relation to vinasses AD, their use in MFCs, and the removal of recalcitrant components found in this substrate. Mezcal and Tequila factories in Jalisco (Casa José Cuervo and Casa Sauza) and San Luis Potosi (Laguna Seca) were visited.

In order to confirm that vinasses could be successfully used as substrate for biogas production, the start-up of a small scale bioreactor was carried out according to the literature researched. The bioreactor operated semi-continuously, digesting Mezcal vinasses as substrate and anaerobic sludge as inoculum. For a better understanding of the microbial population dynamics, mathematical modelling was performed. The results of the start-up are found in **chapter 3**, article **“Inoculum adaptation for the anaerobic digestion of Mezcal vinasses”**.

Later, in order to enhance AD results obtained from the first start-up, BMP assays were carried out to compare the best available inoculum source that can improve AD efficiency. The role of the inoculum source for AD enhancement is gaining importance since the last years. A literature research regarding this topic was performed, and cattle manure was proposed due to the findings of the researched literature. As inoculum source, anaerobic sludge was compared with cattle manure. Not only the comparison of inocula was the objective of these assays, but also the determination of the best S:I-ratio, at which AD of vinasses and inoculum could be enhanced in batch tests. The correct S:I-ratio to be used for AD has been pointed out in the last years as important when designing and starting-up a bioreactor, or when operating a batch fermenter, which is one of the most AD technologies used in developing countries, such as Mexico. A review of existing literature regarding the effect of inoculum sources and S:I-ratios was performed before these assays were carried out. These results can be seen in **chapter 4**, article **“Effect of inoculum source on the anaerobic digestion of Mezcal vinasses at different S:I-ratios”**.

According to the results obtained in **chapter 4**, the start-up of a bioreactor was carried out using this time cattle manure as inoculum. Simultaneously, the effect of using a low-cost biofilm in bioreactor start-up and operation was investigated. Aim of the use of biofilms was to achieve higher biogas and methane production rates, as well as higher organic matter removal efficiencies. Literature regarding the use of biofilms in AD was performed. Results can be seen in **chapter 5**, article **“Comparison of bioreactor start-up for Mezcal vinasses anaerobic digestion using a low-cost biofilm carrier**”.

One of the hypothesis of this work was to determine if organic wastes of *Agave* processing (vinasses) could be used in a MFC for electricity production. Due to the fact that there is few information regarding vinasses in MFCs, vinasses were used for electricity generation in a conventional cell, operated in batch mode, with a proton exchange membrane and aerated cathode. A conventional MFC was used in order to obtain accurate first results, which were not influenced by undesirable boundary conditions. According to the literature research done at the beginning of this project, it was not clear which was the MFC tolerance regarding the organic load or COD content, which achieved an efficient voltage production. Therefore, different vinasses organic loads were tested in the conventional MFC. The results are found in **chapter 6**, article **“Performance of a microbial fuel cell operated with vinasses using different chemical oxygen demand concentrations”**.

The results obtained in **chapter 6** regarding tolerable organic loads for MFC, were used as basis to investigate if AD digestate could be used as influent in MFC, like a small biorefinery. Continuous tests for the simultaneous electricity and biogas generation were carried out. For this purpose, a new reactor was designed, in which AD effluent was used as input material for a low-cost microbial fuel cell. A computational fluid dynamics (CFD) simulation was performed, in order to find out the optimal inlet diameter and test the inflow velocity in reactor, for avoiding substrate sedimentation. Different S:I-ratios or COD contents were tested, digesting Mezcal vinasses with cattle manure. For AD, cattle manure was used, as determined in **chapter 4**. The results of this last assays are found in **chapter 7**, article **“Simultaneous electricity and biogas generation of vinasses and cattle manure”**.

In order to confirm the hypotheses proposed at the beginning of this study, results were evaluated in relation to the efficiency obtained when intending AD enhancement. The biological processes occurring in bioreactors were analyzed to have a deeper understanding of the reasons why AD efficiency was higher or lower. Also the MFC efficiency was evaluated in terms of appropriate COD content in anolyte, as well as the use of vinasses for the simultaneous biogas production and electricity generation.

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# 2 State of the art

Due to the population growth, the global warming, and the finite fossil fuel supply, the challenge to provide the population with enough food, energy, and resources has become an important topic for many countries. Reduction of green-house-gas-emissions and the sustainable development are hot topics in political debates. In order to overcome these problems, strategies should be conceptualized and established to develop technology for the efficient and sustainable resources utilization, as well as for food and energy production. The use of biomass is possible due to photosynthesis, where the pigments of the plants break down water through solar energy. Consequently, biomass is formed from the degraded hydrogen and the carbon dioxide of the atmosphere, whereby oxygen will be released. During the energetic use of biomass, carbon dioxide will be set free, though only the amount of carbon dioxide will be released, which the plant used from the atmosphere to be formed. That is why the biomass as an energy source stands for a climate neutral alternative for energy generation (Quaschning 2011).

The use of organic wastes offer many advantages regarding waste elimination, providing raw matter for the sustainable generation of energy and materials, as the case of a biorefinery (FNR 2012). In Mexico, many wastes are produced from the fermentation and distillation of *Agave* to Mezcal and Tequila. Vinasses produced from ethanol distillation are very aggressive to the environment, due to high content of recalcitrant and toxic organic substances. Thus, vinasses could be considered in Mexico as a raw material to be used for the transition of fossil energy sources to renewable energies. Besides, the problem of vinasses treatment and management can be attended.

## 2.1 Vinasses

The amount of Mezcal vinasses produced in Mexico ascend to 90 million liters a year (Robles-González *et al.* 2012). Few information is found out regarding the real disposal of these residues, and when visiting industrial distilleries, no deep information about their disposal is said. Méndez-Acosta *et al.* (2010) and López-López *et al.* (2010) reported that these residues are discharged untreated or partially untreated into surrounding crop fields or water systems. After visiting the small Mezcal fabric Laguna Seca in the Mexican state San Luis Potosi, it was found out that after the distillation of only one batch hackled hearts of *Agave*, more than 4000 L untreated vinasses are discharged in waterbodies near to the fabric. Locals point out, that water from some water bodies cannot be used anymore, for the normal activities like cooking or washing. An alternative to dispose these residues should be implemented, for both, waste management and bioenergy production.

## 2.2 Anaerobic digestion of vinasses

During anaerobic digestion (AD), bacteria degrade organic substance to its single molecules generating biogas containing methane, carbon dioxide and trace elements. AD occurs in four steps beginning with the hydrolysis, where high molecular structures are reduced to smaller ones. Carbohydrates, proteins and fats are converted respectively in sugar, amino acids and fatty acids. Acidogenesis is the next step of the AD, where bacteria degrades biomass to propionic acid, butyric acid, valeric acid and lactic acid. Acidogenic bacteria consume oxygen to degrade biomass, so that the next two steps of AD take place under anaerobic conditions. The next step is the acetogenesis, in which acetic acid, hydrogen and carbon dioxide are being formed. Hydrogen is used in the last step by methanogenic bacteria, for the methane production. Approximately 70 % of the methane is formed from acetic acid (a volatile organic acid) and 30 % from the hydrogen and carbon dioxide (KWS 2009, Friehe *et al.* 2013).

Different methods for Mezcal and Tequila vinasses treatment and bioenergy production have been reported (Robles-González *et al.* 2012, López-López *et al.* 2010). Because of its low operational costs, AD has been widely used for waste treatment on laboratory, pilot and industrial scales. Many researches have been performed regarding vinasses AD (Espinoza-Escalante *et al.* 2009, Buitrón and Carbajal 2010, Méndez-Acosta *et al.* 2010). Nunes Ferraz Júnior *et al* .(2016) compared the vinasses AD efficiency of one-stage and two-stage digesters, finding out that a two-stage system prodcued 27 % more methane. Méndez-Acosta *et al.* (2010) found out that the organic matter of vinasses, in terms of chemical oxygen demand (COD), could be diminished up to 95 % producing biogas, with 65 % methane content. AD for hydrogen production has also been reported in lab scales. Cruz-Salomón *et al.* (2017) redesign an upflow anaerobic sludge blanket (UASB) bioreactor to enhance the biogas production of vinasses. Espinoza-Escalante *et al.* (2009) found that pH, hydraulic retention time and temperature are very important factors for vinasses AD. Temperature of 55 °C is optimal for hydrogen generation and 35 °C for methane. The pH value must be adjusted for optimal AD conditions. López González *et al.* (2017) found out, that co-digesting vinasses with sugar cane press mod results in an improvement of the methane production. Nevertheless the recalcitrant compounds like the brown polymers, melanoidins or phenols can still remain after anaerobic treatment. In this effect some treatments, such as MFC, should be combined with AD to facilitate the removal of recalcitrant components (Zhang *et al.* 2009, Robles-Gonzalez *et al.* 2012).

## 2.3 Mathematical modelling of microbial growth

Mathematical modelling of AD curves is helpful to describe and study the growth rate of the microorganisms involved during biomass degradation. Growth curves are used to describe how a variable increase over a particular time interval, until saturation state. Normally, a bacterial growth curve begins at value cero and accelerates to an exponential phase, where a maximum growth rate (µm) is achieved, in a certain period of time called lag phase (λ). The final phase is achieved, when the growth rate decreases and reaches zero (point of saturation). In regards to AD, during the lag phase λ the initial degradation of insoluble substrate occurs through the hydrolytic bacteria, converting fat, protein and carbohydrates in fatty acids, amino acids and sugar. The exponential phase µm is achieved when the substrate becomes available for the acidogenic, acetogenic and methanogenic bacteria, where a rapid gas accumulation takes place. Different mathematical models, among others Gompertz model, transference function or logistic function, can be used to have a better understanding of the microbial dynamics in bioreactor (El-Mashad *et al.* 2013, Li *et al.* 2016, Ware and Power 2017).

## 2.4 Effect of different inoculum sources on anaerobic digestion

For long time, the focus of methane enhancement was to develop pretreatment methods to improve the substrate´s degradation degree. Recent research has demonstrated that the accessibility of the substrate for microorganisms depends not only on the substrate, but also on the microorganisms involved. For this reason, the effect of inoculum source for biogas production enhancement has gain importance in the past years. Liu *et al.* (2017b) reported that not only the substrate availability is important for AD enhancement, but also the microbial community contained in the inoculum should be understood. It was demonstrated that the initial hydrolysis step could be a rate-limiting step for degradation. Different substrates and inoculum sources has been tested by De Vrieze *et al.* (2015), Koch *et al.* (2017) and Mahdy *et al.* (2017), among others. Food waste, sewage sludge, crops, microalgae and manure, has been tested. It has been demonstrated that the microbial community in inoculum is important for AD start-up. Lignocellulolytic microbial communities are, for example, important when regulating the degradation rate of lignocellulose-rich biomass. Gu *et al.* (2014) evaluated the effect of different inoculum sources in dice straw AD. Digested manure, the most efficient of the inocula tested, showed to have a higher amount of micronutrients, than other inoculum sources. Liu *et al.* (2017b) showed that a high ammonia inoculum resulted in an inefficient AD, when digesting manure grass with different inocula. Li *et al.* (2010) recommended swine manure as the best inoculum source from four tested with corn stover. Brayan *et al.* (2017) evaluated the influence of trace elements (Ca, K, Fe, Zn, Al, Mg, Co, Ni, and Mo) present in different inoculum sources. It was demonstrated, that the inoculum source determines the content of macronutrients and trace elements, affecting methane production.

## 2.5 Substrate to inoculum ratios and chemical oxygen demand in anaerobic digestion

In order to make a correct design, start-up and operation of a bioreactor, accurate biochemical methane potential (BMP) tests of the specific material to be digested should be carried out (Yoon *et al.* 2014). BMP determination is necessary because AD process is defined by the characteristics of the inherent organic matter in substrate (vinasses). Proper substrate to inoculum ratios (S:I-ratios) should be tested in BMP assays before design and operation of a biogas plant. S:I-ratio can be expressed as the volatile solids amount contained in the substrate, per amount of volatile solids contained in inoculum. To achieve an optimal bioreactor start-up, the S:I-ratio is important, because it can significantly influence the biogas and methane yields. If this ratio is out of tolerance, AD could be significantly affected (Córdoba *et al.* 2018). According to VDI (2016), S:I-ratio should not exceed 0.5, nevertheless, depending on the substrate to be degraded and inoculum source, S:I-ratio could show different optimal values. Lawal *et al.* (2016) reported that this ratio had a significant effect on the biogas production rate. Chynoweth *et al.* (1993) reported that S:I-ratios between 0.5 and 1.0 resulted in higher methane yields, by the batch digestion of herbaceous, woody feedstock and municipal wastes. Raposo *et al.* (2009) reported a maximal methane yield of 250 L/kgVS when digesting sludge and maize at S:I-ratio of 1. S:I-ratios 0.5 and 0.66 showed 200 L/kgVS and S:I-ratio 0.33 produced only 170 L/kgVS. From this reason, each substrate to be digested indicate a different optimal S:I-ratio, depending on the inoculum source to be used for AD. Córdoba *et al.* (2018) demonstrated that a low inoculum amount resulted in a higher microorganism's adaptation time, delaying the methane production. Further investigation should be carried out to understand the inoculum activity and its relation with the substrate characteristics.

## 2.6 Biofilms in anaerobic digestion

Depending on the organic matter and inflow velocity, every bioreactor has a retention time, which could generate problems on the fermentation efficiency, if active bacteria flow continuously out of the bioreactor when extracting the treated biomass. This implies lower retention times, as well as wash out effect of the bioreactor or absence of active microorganisms. To overcome this problematic, the use of biofilm carriers has been successfully implemented inside AD bioreactors. Active microorganisms responsible of the biogas and methane production are attached to the biofilm surface, incrementing the capacity of biomass degradation, before the microbial population flows out of the bioreactor (Langer *et al.* 2014, Liu *et al.* 2017a).

Many research has been done regarding alternatives to overcome the problem of the microorganism's wash out effect, related to the hydraulic retention time. Carlos-Hernández *et al.* (2014) and Martí-Herrero *et al.* (2014) used a solid support to immobilize bacteria, so that the wash out effect could be hindered. The capacity of microbial population to degrade biomass and generate biogas and methane could be enhanced. Hydraulic retention time could be separated from solid retention time. The start-up of a bioreactor could be improved when using biofilms, due to the quick growth of active biofilm (Escudié *et al.* 2011). To favor biofilm growth, the increase of the organic loading rate should be coupled to a short hydraulic retention time. Biofilm-based reactors are effective in treating wastewater, but are not normally used for biogas production. Current research is focused on the bacterial affinity to be adhered to specific carrier materials. Langer *et al.* (2014) investigated the dynamics of biofilm forming at different organic loading rates (OLR). The higher the OLR, the higher the cell numbers found in the biofilm and thus the highest biogas production. Andersson and Björnsson (2007) found straw a suitable biofilm carrier, enhancing biogas production and organic matter removal in terms of COD. Gong *et al.* (2011) compared different biofilm carrier materials. Activated carbon fiber showed the highest methane and biogas yields. The start-up period of a biofilm bioreactor, using low-cost polyethylene terephthalate (PET) bottles was more efficient, when compared to a bioreactor without biofilms (Martí-Herrero *et al.* 2014). The use of low-cost biofilms made of PET should be deeper analyzed for AD enhancement.

## 2.7 Microbial fuel cells

Microbial fuel cells (MFC) are systems which use microorganisms as catalysts to oxidize organic matter, including acetate, glucose and volatile fatty acids, as well as inorganic matter such as sulphides or other salts, in order to generate current. At the anode, organic matter is being degraded by microorganisms, which release electrons and protons. Electrons flow to the cathode through an external circuit producing electricity, and protons migrate through a proton exchange membrane (PEM), also to the cathode. Protons react with oxygen on the cathode chamber producing water. Hereby the system generates electricity from chemical energy directly in one step (Logan *et al.* 2006, Poggi-Varaldo *et al.* 2009, Higgins *et al.* 2013).

MFC performance has been tested with different substrates, such as acetate, glucose, food processing wastewater, swine wastewater, domestic wastewater, starch processing wastewater, landfill leachate and distillery wastewater, to generate electricity and for wastewater treatment, among others (Zhang *et al.* 2009, Mohanakrishna *et al.* 2010). MFCs can take advantage of sulphide to generate electricity and oxidize it to elemental sulphur. Mohanakrishna *et al.* (2010) investigated the use of distillery wastewater, resulting in a high power generation for extended periods of times, when the carbon load was increased. This happened due to the fact that carbon contained in biomass functioned as electron donor. Also the substrate degradation was lower by lower organic charge and the removal of color and salts concentration was higher by higher organic loads. Nevertheless, some findings have pointed out that the MFC efficiency could be limited, if COD exceeds a specific concentration, whereas the electricity production could be hindered through the saturated state of the substrate (Vogl *et al.* 2016).

The development of this technology is challenging, especially because of the cost of membranes and electrodes, the potential of substrate-biofouling, and the high internal resistance that limits the power generation. New designs of open air cathodes and membrane-less MFCs have been developed in recent years, showing an effective biomass conversion to electricity. Hu (2008) designed and tested a membraneless MFC, improving the overall efficiency. A biofilm formation in the cathode was guaranteed to minimize oxygen intrusion to the cathode. Prashanth *et al.* (2010) reported that open air cathode MFC resulted in a higher power output, than aerated MFC. The performance of the MFC has been tested with different substrates, although few work has been reported regarding the use of vinasses for the electricity production through MFCs, as well as regarding the combination of both technologies, MFC and AD.

Taking all this aspects into consideration, the present work is intended to help in closing the gaps in knowledge regarding:

- The proposed alternative hypothesis, if the organic wastes of *Agave* processing can be used for energy production, through AD and MFC
- The enhancement of AD efficiency by means of using more efficient inoculum sources, biofilms in bioreactors, and an adequate S:I-ratio for batch fermentation
- The effectivity of using vinasses in MFC for voltage production, with an optimal organic load
- The effectivity of a small biorefinery, with an AD-MFC configuration, generating biogas and voltage at the same time

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# 3 Inoculum adaptation for the anaerobic digestion of Mezcal vinasses

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## 3.1 Abstract

Vinasses are a very harmful residue of the alcohol distillation, their discharge into soil and water can cause negative environmental impacts, if the appropriate treatments do not take place. Anaerobic digestion (AD) has shown to be the best technological and economical method to treat this residue, thus bioenergy can be generated as by-product of this process. Nevertheless, the slow adaptation of the microbial consortium in inoculum (anaerobic sludge) to the substrate (Mezcal vinasses) is very important to enhance the efficiency of the biogas and methane production, as well as organic matter removal. In this work, the adaptation process of Mezcal vinasses AD was carried out in a 30-day period. Inoculum (activates sludge) and vinasses were mixed initially at the ratio 7:3. The feeding steps were done every seven days replacing 30 % of the total volume with new vinasses. Biogas was quantified and qualified. Biogas production reached 217 L/kgVSvinasses generating a daily methane content between 50 and 55 % by the end of the adaptation period. The organic matter removal efficiency was almost seven times higher at the end of the adaptation, in comparison to the beginning. This suggests that a slow adaptation process enhance the organic matter removal and eventually other pollutants in vinasses. When comparing this results with the literature, biogas and methane production were similar, nevertheless AD digestion could be optimized, in order to increase the methane content in biogas and the removal rate of organic matter.

**Keywords:** biogas, chemical oxygen demand, FOS/TAC, methane

## 3.2 Introduction

Yearly, about eight million liters Mezcal and 271 million liters Tequila are produced in Mexico, from which almost 3400 million liters vinasses remain as residue after the distillation step (Robles-González *et al.* 2012, García-Depraect and León-Becerril 2018). These wastes are very aggressive to the environment due to the high organic matter content, high discharging temperature and low pH. If untreated vinasses are discharged into soils or water, the ecosystem could be seriously affected causing eutrophication in water bodies and contamination in soils and crops (Robles-González *et al.*2012). Mezcal and Tequila are Mexican alcoholic beverages generated after the distillation of broths produced from the fermentation of sugars contained in extracted *Agave* juice. Both beverages are produced in different regions of Mexico and each one possesses a distinctive sensory character (Villanueva-Rodríguez and Escalona-Buendía 2012).

Several methods for vinasses treatment have been researched and used in the recent years (Robles-González *et al.* 2012). One of the most suited method is the anaerobic digestion (AD) for biogas or hydrogen production (Jiménez *et al.* 2006, Espinoza-Escalante *et al.* 2007, López-López *et al.* 2010, Méndez-Acosta *et al.* 2010, Barrera *et al.* 2014). AD has found practical applications in industrialized countries through biogas plants for the production of heat and electricity, for the production of biomethane as biofuel, or as network supply. Nevertheless, the efficiency of the biogas production depends on the operational parameters of the bioreactor and the substrate characteristics such as pH, alkalinity, temperature, organic matter content, or toxic compounds (Espinoza- Escalante *et al.* 2009).

Through the vinasses AD, monosaccharides such as fructose and glucose are produced. These compounds contribute to the efficient biogas generation and the increase of methane content in biogas (Espinoza-Escalante *et al.* 2007). For this reason, vinasses are a very suitable substrate for the production of bioenergy. The centralized production of high amounts of vinasses in nearby locations make this substrate a suitable alternative for saving in transportation costs and for a cost-effective energy production. López-López *et al.* (2010) suggested that AD is a very competitive method regarding technical and economic advantages over aerobic processes.

The adaptation process of the inoculum microbial population to the substrate is a very important step to enhance the capacity of the inoculum to degrade higher amounts of substrate, increasing the methane production (Calabró *et al.* 2018). Different authors report different methods of microbial adaptation. Calabró *et al.* (2018) compared the methane production of olive oil mill wastewater (OMW) AD, when using adapted and non-adapted inoculum. Inoculum adaptation was done during 30 days batch tests digesting OMW. Methane production increased by 300 % in the tests with adapted inoculum. Méndez-Acosta *et al.* (2010) performed the efficient adaptation of anaerobic sludge under continuous operation of a bioreactor operated with vinasses. The bioreactor was started with a low organic loading rate (OLR) and high hydraulic retention time (HRT), thus the microbial consortium was stressed as less as possible. As a result, the adaptation granted the good microbial interaction by the continuous operation of the bioreactor. Eskicioglu *et al.* (2009) investigated the effect of the adaptation of the biogas production of microwave pretreated activated sludge. Inoculum adaptation did not only accelerate the biogas production, but also enhanced the biodegradation rate of the sludge. Rivera *et al.* (2002) achieved a successfully 20-day adaptation of a bioreactor digesting sludge, diluted manure and vinasses. The first two days no feeding took place, the next 10 days a daily feeding was carried out and the rest of the adaptation period, feedings were done every two days. After 20 days, the biogas production showed stable values (600 mL/d), and from this point a continuous operation of the reactor was done. Biofilms were used in order to enhance the adaptation process. Méndez-Acosta *et al.* (2010) achieved the sucessfully adaptation of a 5-liter bioreactor digesting sludge with vinasses. Methane content, biogas production and removal of chemical oxygen demand (COD), achieved stable values after the 50-days adaptation period.

In this work, a 30-day adaptation period of an 8-liter bioreactor operated with Mezcal vinasses and anaerobic sludge was carried out under anaerobic conditions. Biogas production was daily measured and characterized. The objective of this work was to start the adaptation process of a bioreactor in order to stress the microbial population as less as possible, and therefore achieve high biogas and methane yields, as well COD removal rate.

## 3.3 Materials and methods

### 3.3.1 Inoculum and substrate

Anaerobic sludge from the wastewater treatment plant of the University was used as inoculum. As substrate, Mezcal vinasses were collected from the Mezcal factory Laguna Seca located in the Mexican state San Luis Potosi. Vinasses and sludge were collected, transported and kept at 4 °C prior to use. The characteristics of the vinasses and anaerobic sludge are listed in **table 3.1** and **table 3.2**.

**TABLE 3.1** Characteristics of Mezcal vinasses

| *Parameter* | *Value* | *Parameter* | *Value* |
|---|---|---|---|
| pH @ 27 °C | 4.77 | Sediment solids (mL/L,@60 min) | 102 |
| Chemical oxygen demand COD (g/L) | 59 | Total sugar content (g/L) | 51 |
| Sulphate ion $SO_4^{2-}$ (g/L) | 1.04 | Total solids TS (%) | 4.91 |
| Phosphate ion $PO_4^{3-}$ (g/L) | 0.3 | Total solids TS (g/L) | 49.1 |
| Nitrate ion $NO_3^-$ (g/L) | 0.48 | Volatile solids VS (%) | 3.52 |
| Total nitrogen (g/L) | 0.13 | Volatile solids VS (g/L) | 35.2 |
| Total phosphorous (g/L) | 0.02 | Turbidity NTU (Nephelometric Turbidity Units) | 55.4 |

**TABLE 3.2** Characteristics of anaerobic sludge

| *Parameter* | *Value* | *Parameter* | *Value* |
|---|---|---|---|
| pH @ 27 °C | 8.24 | Volatile solids VS (%) | 0.34 |
| Chemical oxygen demand (g/L) | 5.70 | Volatile solids VS (g/L) | 3.40 |
| Total solids TS (%) | 0.53 | Conductivity mS/cm | 15.66 |
| Total solids TS (g/L) | 5.34 | Total nitrogen (g/L) | 0.042 |

### 3.3.2 Bioreactor configuration

The bioreactor was made of polyvinyl chloride (PVC), 19 cm high, 36 cm long and 12 cm wide, with a volumetric capacity of 8.2 L and was 90 % filled with vinasses (7.4 L total of sludge and vinasses mixture). For pH adjustment, the bioreactor had an inlet at the top for the addition of sodium hydroxide (NaOH) (Espinoza-Escalante *et al.* 2007, Espinoza-Escalante *et al.* 2009, Méndez-Acosta *et al.* 2010). Also at the top a tedlar bag was connected for biogas storage. The reactor had a twist-off lid at the top and a tap at the bottom to replace every seven days 30 % of the total volume with new substrate. HRT was 7 days and OLR 5 gVS/Ld. A magnetic stirrer was placed inside the bioreactor so that it could be mixed 15 min/d.

### 3.3.3 Bioreactor start-up

The reactor adaptation consisted of filling 90 % of the total reactor volume with Mezcal vinasses as substrate and anaerobic sludge as inoculum, at a vinasses concentration of 42 % v/v. Four feeding steps were carried out by replacing 30 % of the total volume with new vinasses every seven days, which means by day 7, 14, 21 and 28. The amount of produced biogas and the content of methane, carbon dioxide and hydrogen sulphide were daily measured. Before every measurement was carried out, the bioreactor was mixed with the magnetic stirrer for 15 min, according to the norm VDI 4630 (VDI 2016) for the fermentation of organic materials for biogas production. The reactor was kept in a furnace at anaerobic conditions and mesophilic temperature (39 °C). The pH value of the reactor was daily measured and adjusted with NaOH. A second bioreactor under the same conditions was run out as control test digesting only inoculum. The biogas production was subtracted to the assay with inoculum, in order to determine the real biogas and methane production of the vinasses.

## 3.4 Measurements

The biogas quality (content of methane, carbon dioxide and hydrogen sulphide) was measured with a gas analyzer Multitec 540 from the German company SEWERIN GmbH. The pH value was daily measured with the pH-meter 110 from VWR and calibrated with buffer solutions prior to use. Biogas was collected in tedlar bags and the quantity was measured according to the water displacement principle with an Erlenmeyer flask and a graduated cylinder. Biogas and methane were reported in terms of L/kgVSvinasses. Biogas was calculated dividing the liters of biogas produced in one day by the kilograms of vinasses added at each feeding in terms of volatile solids. The methane volume was calculated by multiplying the gas volume by the methane content. COD was measured according to the norm DIN 38414-9:1986-09 (DIN 1986). COD removal (%) was calculated comparing the values measured at the beginning and at the end of the experiments. Sulphate ion ($SO_4^{2-}$), phosphate ion ($PO_4^{3-}$), nitrate ion ($NO_3^-$), total nitrogen (TN) and total phosphorous (TP) were measured with HACH vials. Total solids (TS) and volatile solids (VS) were measured according to the norm VDI 4630 (VDI 2016). FOS/TAC value, the quotient of the volatile organic acids and the total inorganic carbon, was measured to analzye the biochemical state of the fermentation sludge. FOS stands for Flüchtigen Organischen Säuren in German and TAC stands for Total Anorganischen Carbon (Voß *et al.* 2009, VDI 2016). FOS indicates the content of volatile organic acids or volatile fatty acids (VFA, mostly acetic acid) in terms of mgHAc/L and TAC shows the total inorganic carbon or buffer capacity in terms of mg$CaCO_3$/L. FOS/TAC is measured throughout the titration of sulphuric acid 0.05 M ($H_2SO_4$) in the vinasses:sludge solution, in order to change pH to 5 and then to 4.4 (Buchauer 1998, Mézes *et al.* 2011). In other words, the FOS/TAC value measures the relation between the acid concentration and the buffer capacity in the bioreactor. According to Burgot (2012) the quantity of strong acid or strong base required to modify the pH in a solution, determines its buffer capacity. If a high amount of acid or base is required, the buffer capacity of the system is also high. This buffer capacity (β) is indicated by the value TAC. Lossie and Pütz (2008) published that the FOS/TAC value should oscilate between 0.3 and 0.6, depending on the substrate to be fermented. If the fermentation substrate contains a high organic acid concentration and the pH drops below 6, the methanogenic bacteria will be inhibited.

The biodegradability index (BI) and competitiveness index (CI) were calculated according to Eq. 3.1 and 3.2 (Cruz-Salomón *et al.* 2017).

$$\mathrm{BI} = \frac{\mathrm{BMP}}{350\ \mathrm{mL\ CH4/gCOD}} \qquad \text{(Eq. 3.1),}$$

$$\mathrm{CI} = \frac{\mathrm{COD}}{\mathrm{SO_4^{2-}}} \qquad \text{(Eq. 3.2),}$$

whereas, BMP is the Biochemical Methane Potential or cumulative methane yield obtained and 350 is the theoretical volume of methane per gram COD removed at normal temperature and pressure (T = 273 °K; P = 1 atm). The BI indicates the amount of organic matter able to be degraded by the microbial population. If BI is lower than 0.3, the substrate is not suitable for AD or the microbial population conditions are not optimal for biodegradation. Regarding CI, if it shows values higher than 10, no competition between the sulphate-reducing and methanogenic bacteria takes place. Thus a good bacterial interaction can be determined.

## 3.5 Kinetic modelling

In order to understand the kinetics of methane and biogas production of AD vinasses and to predict the further biogas and methane yields, a mathematical model of sigmoidal bacterial growth curve was used. The curves of biogas and methane production were fitted to the curves generated from the modified Gompertz model (Eq. 3.3), in order to evaluate the specific growth rate and lag phase (first step to bacterial growth) of the biroeactor microbial population (Rolfe *et al.* 2012, Ware and Power 2017). A regression analysis of non-linear least-squares was carried out by means of the software Statistica 13. A 95 % confidence interval was used for the goodness-of-fit. The correlation coefficient r was calculated in order to determine the correlation between experimental and theoretical data.

$$y = N * \exp\left(-\exp\left((\exp(1) * \mu m)/No * (\lambda - t) + 1\right)\right) \qquad \text{(Eq. 3.3),}$$

from which:

y: cumulative gas yield ($L_{CH4}$/kgVS)

N: maximum production potential ($L_{CH4}$/kgVS)

No: start gas production ($L_{CH4}$/kgVS)

µm: maximum specific yield growth rate ($L_{CH4}$/kgVS*d)

λ: lag phase (days)

t: incubation time (days)

**Figure 3.1** (right) (Ware and Power 2017) shows the typical bacterial growth curve, which approaches a typical cumulative biogas and methane curve (**Fig. 3.1** left) VDI (2016). The bacterial growth begins with the dilatory gas generation and accelerates to a maximum growth rate µm in a certain time called lag phase λ. At this point the hydrolitic bacteria degrade polymers, carbohydrates, fat and protein, in monomers sugar, fatty acids and aminoacids. Afterwards a quick gas production can be apreciated, where methane is formed from the organic fatty acids, hydrogen and carbon dioxide. Latterly the stationary phase is reached, when the growth rate diminishes at the asymptote N (Ware and Power 2017).

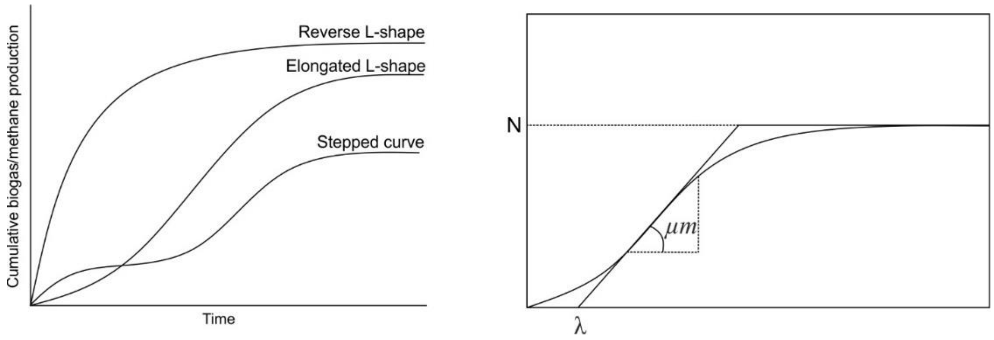

**FIGURE 3.1** Left: typical cumulative biogas/methane yields. Right: typical bacterial growth curves (VDI 2016, Ware and Power 2017)

Main objective of the kinetic study was to determine the maximum production potential, maximum specific gas yield growth rate and lag phase. By means of the Gompertz equation the further behavior of the methane and biogas yield curves was predicted.

## 3.6 Results and discussion

### 3.6.1 Biogas and methane production

COD of Mezcal vinasses and anaerobic sludge were 59 and 5.7 g/L, correspondingly. Sludge had a pH of 8.24 and vinasses 4.77. Total solids and volatile solids were 49.17 and 14.58 g/L for vinasses, as well was 5.34 and 1.97 g/L for anaerobic sludge. The COD determines the content of oxidizable compounds in the biomass, which contribute to the biogas production (VDI 2016). On the other hand, the biomass is composed of water and total solids. The total solids are composed of ash and volatile solids, from which biogas will be produced (SE 2015). The inoculum used at the beginning of the experiments showed to have a low amount of oxidizable matter, because COD and TS are very similar (5.7 and 5.3 g/L). This statement suggests that the microbial activity of the inoculum itself was not responsible for the biogas/methane production, but it was given from the vinasses, whose volatile solids and COD are much higher. Nitrogen content in Mezcal vinasses was 0.126 g/L and in anaerobic sludge 0.042 g/L. According to Friehe *et al.* (2013) a balanced content of essential nutrients like carbon and nitrogen is very important in order to achieve a stable AD process. Nitrogen is required for the activation of enzymes related to the metabolic activities in the bioreactor. When the C:N-ratio is high (content of carbon > content of nitrogen), the existing carbon can not be completely metabolized and the methane yield will be much lower than expected. If the C:N-ratio is low, the nitrogen content will be high. This can result in the generation of ammonia ($NH_3$), which can inhibit the bacterial growth, and thus the biogas and methane production. The COD:N-ratio for AD should lie by 800:5 (Moletta 2005). COD:N-ratios of the vinasses and anaerobic sludge were calculated according to the COD and TN measurements at the beginning of the experiments. The results of the calculations were 59000:126 or 2341:5 for Mezcal vinasses and 5700:42 or 679:5 for anaerobic sludge. In the case of vinasses, the content of carbon was three times higher than it should be. For the long term operation of the bireactor, a higher nitrogen content should be guaranteed, either using a substrate or an inoculum source rich in nitrogen (e.g. protein-rich), or directly adding nitrogen to the bioreactor (e.g. ammonium or urea addition) (Borja *et al.* 1996, Liu *et al.* 2015).

**Figure 3.2** shows the cumulative biogas and methane production of vinasses over a period of time of 30 days. AD reached a biogas yield of 217 L/kgVSvinasses and a methane yield of 85.5 L/kgVSvinasses. In terms of removed COD, the biogas and methane production resulted in 196 L/kgCOD removed with 55 % methane content in biogas, after 30 days of experiments. In agreement to this results, Méndez-Acosta *et al.* (2010) obtained 160 L/kg COD removed after 30 days start-up.

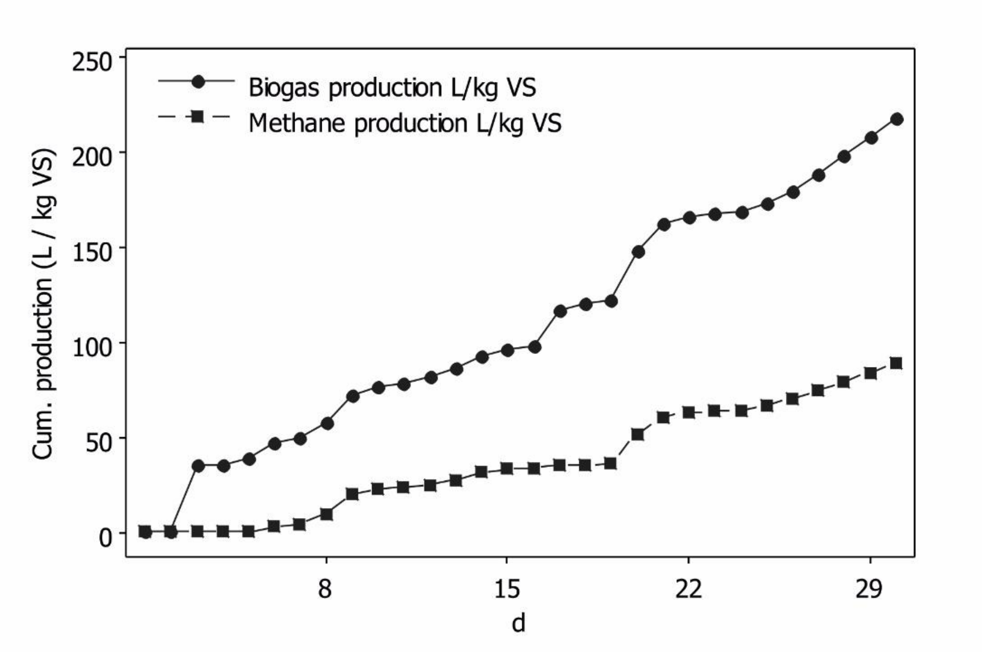

**FIGURE 3.2** Cumulative biogas and methane production in L/kgVS (volatile solids) vinasses

The further operation of the bioreactor could result in an efficient AD, this can be confirmed with the curves of the predicted biogas and methane yields (**Fig. 3.3**), which were obtained by means of the modified Gompertz equation. According to the mathematically predicted values, after 120 days of experiments, biogas and methane yields should achieve approx. 325 L/kgVS and 125 $L_{CH4}$/kgVS. By day 60, the curves should had achieved stable values around 300 L/kgVS and 120 $L_{CH4}$/kgVS.

Some authors suggest if all organic matter is anaerobically degradable and converted to methane, the expected methane production should be 350 L/kg COD removed in a long-term AD operation (Gallert *et al.* 1997, Koutrouli *et al.* 2009). Moletta (2005) reported a biogas production of 400-600 L/kg COD removed, in a long-term operation AD. Méndez-Acosta *et al.* (2010) reported a biogas production of 537 L/kg COD removed with 60 % methane, after 200 days of experiments.

The biogas production began the second day of the start-up process and the methane production the fifth day. AD occurs in four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The different inoculum bacteria groups related to each biogas production stage, reproduce at different rates to degrade polymers and generate acetic acid, hydrogen and carbon dioxide for the methane production (Friehe *et al.* 2013). The reproduction rate of the methanogenic bacteria is the most slowly of all the bacteria groups. Methanosarcina needs up to 360 hours for reproduction and the methanococcus 240 hours. In comparison, the hydrolytic and acidogenic bacteria need 24 - 36 hours and the acetogenic bacteria 40 - 132 hours for reproduction (KWS 2009). For this reason, the methanogenesis is the slowest step to methane production. This is reflected in the present work, where the methane production started five days after biogas generation started. The results of the kinetic study are shown in **table 3.3** and **figure 3.3**.

**TABLE 3.3** Kinetic study parameters of cumulative biogas and methane curves

| | Biogas yield | Methane yield |
|---|---|---|
| Maximum production potential (L/kgVS) | 328.91 | 123.72 |
| Maximum specific gas growth rate µm (L/kgVS*d) | 7.77 | 3.81 |
| Lag phase λ (d) | 2 | 7 |
| Correlation coefficient r | 0.99 | 0.98 |

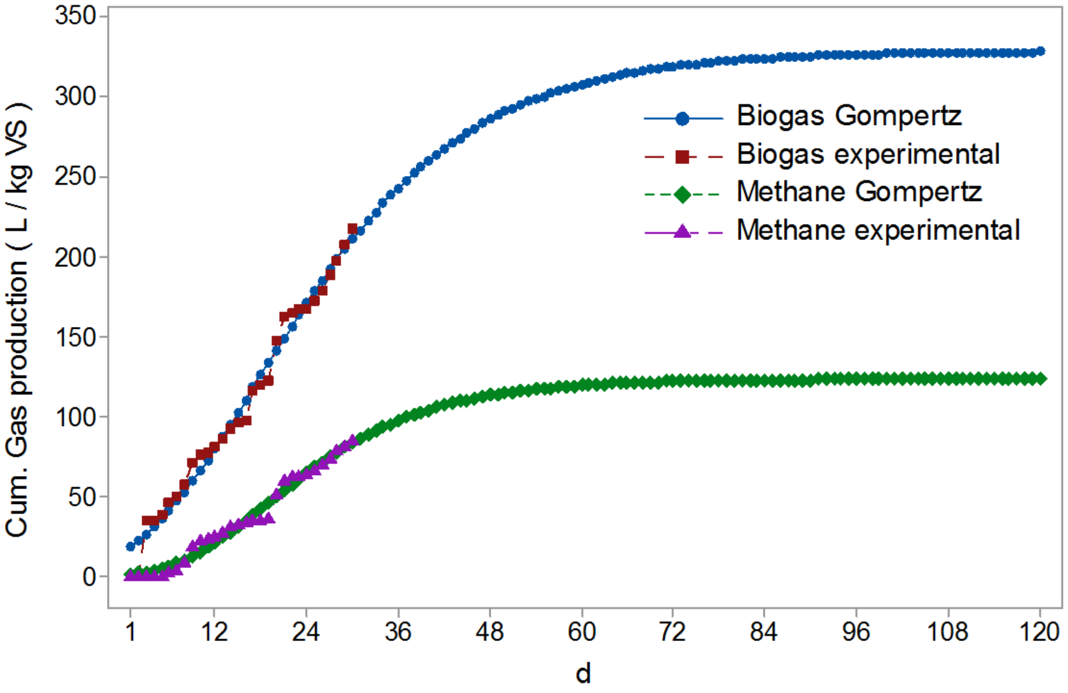

**FIGURE 3.3** Predicted cumulative biogas and methane yields in L/kg VS (volatile solids) vinasses

Both experimental curves showed good visual fits to the curves generated from the Gompertz equations. The correlation coefficient r shows also values near to 1, what indicates a positive correlation between experimental and theoretical curves. The maximum specific growth rates µm were 7.77 L/kgVS*d for biogas and 3.81 $L_{CH4}$/kgVS*d for methane. As expected according to the slow reproduction rate of the methanogenic bacteria, the lag phase or time to achieve µm, was achieved in 2 days for biogas and 7 days for methane.

The biogas rate in terms of liters biogas daily produced varied from 0 up to 2.75 L/d in the 30-days adaptation period (**Fig. 3.4**). The first feeding had positive effects in the biogas production. On the third day the highest recorded biogas production of 2.75 L/d was achieved, which according to the norm VDI 4630 (VDI 2016) is a normal behavior in AD assays. This result was also reflected on the kinetic study, whereas the maximum specific biogas growth rate, called lag phase λ, was achieved after two days. A second biogas peak was given after the first feeding, when the biogas production increased to more than 1 L/d. After this day, the biogas production diminished and remained low (0.1 - 0.5 L/d).

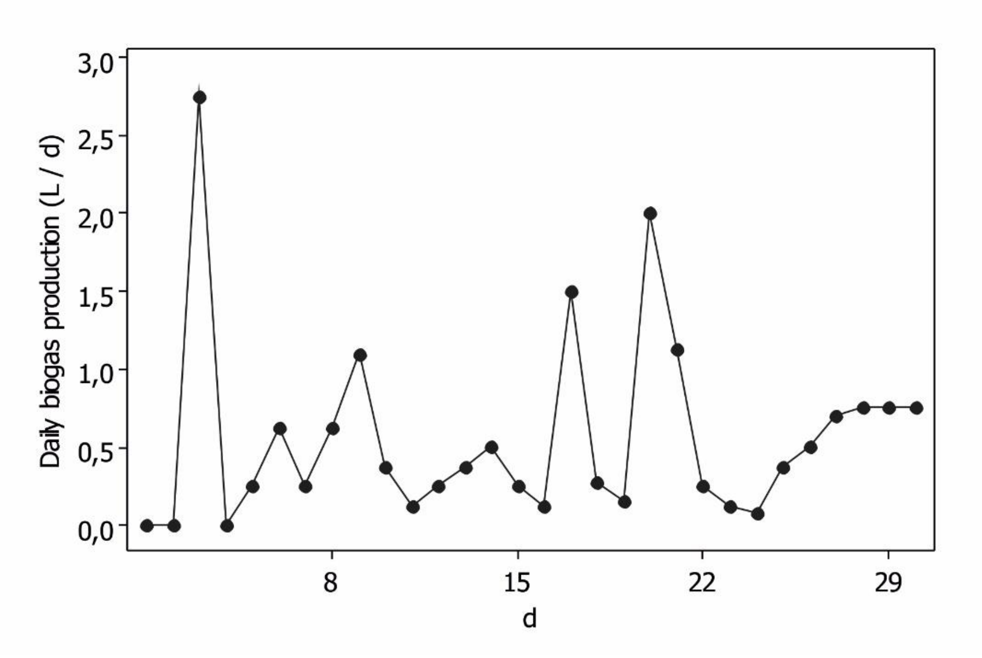

**FIGURE 3.4** Daily biogas production in L/d

According to Williams (1983) the organic acids content and COD content have a positive correlation with each other, the higher COD content, the higher organic acids content. The content of COD in vinasses is higher than in sludge, wastewater and other substrates, therefore the organic acids content in vinasses is also high. The organic acids measured in the bioreactor after the first feeding was 20 gFOS/L. This measured value was higher than recommended by Voß *et al.* (2009).

According to Mézes *et al.* (2011), if the amount of organic acids or FOS exceed 10000 mgFOS/L, an incomplete metabolism by high organic acid content might inhibit AD. At the same time the buffer capacity of the system was low, TAC value was 12 gTAC/L and increased with the time of the experiment up to 31 gTAC/L. In this study the adaptation process was done replacing 30 % of the total volume with new vinasses (Méndez-Acosta *et al.* 2010), nevertheless the results of the first parameters measured after first feeding suggest that the amount of vinasses in the bioreactor start-up should had been lower to achieve a better AD performance. The second feeding resulted at first in a reduction of the biogas production, two days later the biogas production increased to 1.6 L/d. By day 20 the biogas production increased almost to 2 L/d, which indicates that the high amount of organic matter was already degraded to simple sugars and carbohydrates, and the organic acids were converted to methane. At this point the amount of organic acids and the buffer capacity of the system increased. Before the third feeding took place, the conversion of VFA to methane decreased and by day 25 the biogas production increased again and gained stable values around 0.75 L/d. Although there was an increase in volatile organic acids (up to 45 g/L) after the last feeding, pH showed stable values around seven, as the buffer capacity of the system increased. These conditions allowed a good adaptation between the microbial system and the vinasses, already by day 30. Some authors have reported an adaptation period of more than two months, by AD of vinasses from wine distilleries (Méndez-Acosta *et al.* 2010).

**Figure 3.5** shows the methane content in the daily produced biogas and **figure 3.6** shows the pH measured every day.

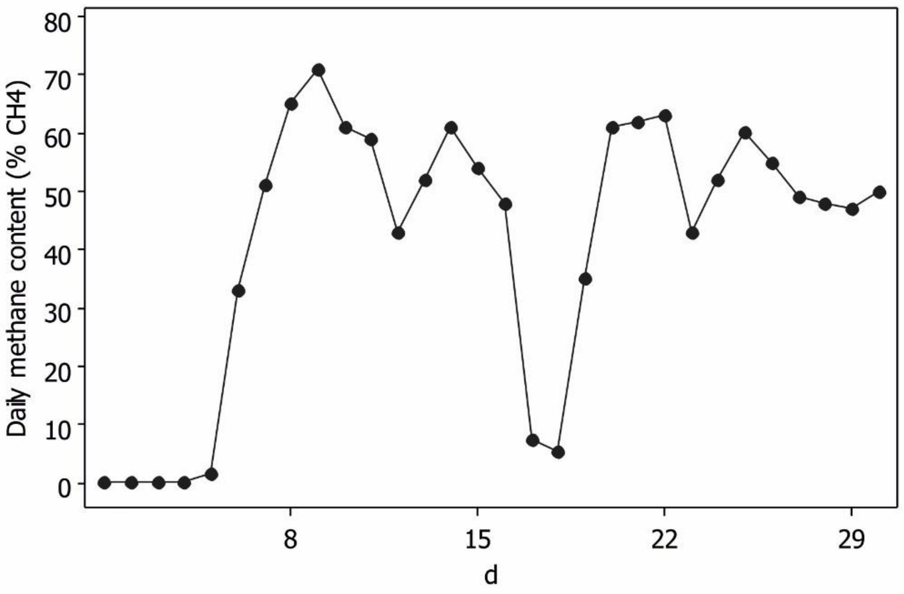

**FIGURE 3.5** Daily methane content in biogas in %

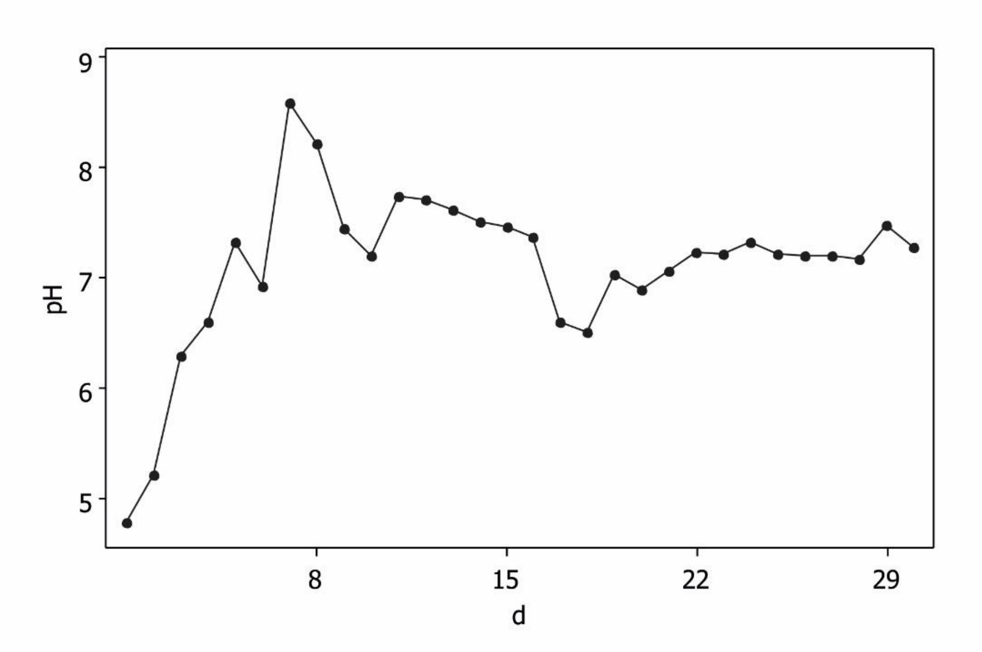

**FIGURE 3.6** Daily pH measured in bioreactor

The daily methane content fluctuates mostly between 40 and 60 %. The first five days, no methane production was shown and the first three days pH was lower than 6.5, which is the minimum recommended for methane production (Friehe *et al.* 2013). Even when adjusting pH to 7, it dropped after some hours of adjustment. This fact suggests a low buffer capacity of the system, which showed 12 gTAC/L. After the third day, pH adjustment could be successfully maintained by 7, whereas TAC value continued to increase. FOS/TAC value was 1.6, three times higher than recommended by Lossie and Pütz (2008), due to the amount of volatile organic acids (20 gFOS/L). After the first feeding, methane content began to increase to 33 % and by day nine the highest recorded methane content near to 70 % was achieved. This can also been appreciated in the kinetic study, when the maximum specific methane growth rate was achieved by the first feeding. At this point pH achieved also the highest value of 8.5, FOS/TAC remained the same and the amount of volatile organic acids continued to increase to 33 gFOS/L. After this and before the second feeding took place, methane content decreased up to 40 %, where pH remained stable around 7.5. The second feeding showed negative effects in the methane production, when the methane content decreased considerably up to 5 %, pH dropped to 6.3 and FOS/TAC met the highest recorded value (five times more than recommended). The amount of organic acids increased from 20 to 33 gFOS/L, resulting in a low efficiency in converting organic acids to methane. According to Franke-Whittle *et al.* (2014) the increase on volatile fatty acids reflects the imbalance between acid production and consumption by the microorganisms. It is related to the drop of pH and buffering capacity of the inoculum.

Three days prior to the third feeding, methane content increased again and achieved values around 60 %. After this point FOS/TAC dropped, pH continued to be stable (7 - 7.5), TAC continued increasing from 29 to 31 gTAC/L, and so the amount of organic acids (from 40 to 45 gFOS/L) until the end of the adaptation period. These findings indicate that the bioreactor achieved a high buffer capacity after the third feeding. A slightly drop of the methane content to 45 % was recorded by day 23 but after day 24, methane content remained around 50 and 55 %. The amount of volatile organic acids continued increasing, but pH value remained stable (around 7) due to the increase in the buffer capacity. For the long-term bioreactor operation, an enhancement in the methane content is expected, like the case of Méndez-Acosta *et al.* (2010) and Jáuregui-Jáuregui *et al.* (2014). Also the mathematical prediction of the methane and biogas curves suggests a succesfull long-term operation. In order to increase the biogas production and especially the methane content, the use of biofilms in bioreactor is recommended (Carlos-Hernández *et al.* 2014, Martí-Herrero *et al.* 2014).

Regarding the methane content, after day 25 a constant methane production around 50 and 55 % was achieved. Méndez-Acosta *et al.* (2010) reported a methane content of 60 % during a 50-days start-up period using a lower COD content of 10 g/L in the mixture wastewater vinasses. In the present study, during the 30-day adaptation period, COD content of the mixture anaerobic sludge and vinasses was 17 g/L. This comparison suggests that a higher COD content in fermenting mixture results in a lower methane content in biogas. A lower COD content should be used to stress the microbial population as less as possible, and achieve a higher AD efficiency. By the end of the experiment COD reduced to 11 g/L. In comparison to other authors, in this experiment a higher amount of vinasses could be used when feeding every seven days instead of every 15 days. The biogas quantity and methane content were similar, altough the organic matter removal was not especially high. Jáuregui-Jáuregui *et al.* (2014) reported an increase in the methane production from 60 to 70 % during the start-up period of 28 days of a bioreactor using a PVC support as biofilm to enhance AD and COD removal. Main target of biofilms is to avoid the wash out effect in bioreactor, which occurs when active bacteria flows out of the bioreactor before bacteria contribute to AD. When using biofilms, the hydraulic retention time is separated from the solid retention time, decreasing the bacteria wash out effect and increasing the biogas and methane production rates, as well as COD removal. Two kind of biofilms are found in industrial applications, biofilms that grow on supports (resin, concrete, pet) or granular biofilms (forming flocs or granulate formations). Different material have been tested, such as seashell, charcoal, break, gravel, plastic materials, ceramic, sintered glass, fire bricks, natural stones including limestone, gravel, pumice, clay, rocky aggregates, sand, granular activated carbon, saponite and synthetic plastic materials (Qureshi *et al.* 2005, Carlos-Hernández *et al.* 2014, Martí-Herrero *et al.* 2014). From a material comparison, charcoal showed the best COD removal, methane yield and lowest volatile fatty acids accumulation from whey treatment, while pumice showed the worst results (Patel *et al.* 1995).

Friehe *et al.* (2013) reported an optimal pH value between 5.2 and 6.3 for the hydrolytic and acidogenic bacteria to reproduce, nevertheless the most favorable pH for acetogenic and methanogenic bacteria lies around 6.5 to 8 pH. Due to the fact that most of the bioreactors work with one stage fermentation process, the optimal pH to be used in AD should lie around 6.5 and 8. According to Jayaraj *et al.* (2014) when comparing pH of 5, 6, 7, 8 and 9 for biogas production, the optimal pH showed to be 7 for more efficient biogas production.

### 3.6.2 Chemical oxygen demand removal

COD of the vinasses-sludge mixture was at the beginning of the experiment 17.37 g/L. Before the first feeding took place, COD removal was 2 %. After the second feeding COD removal was 5 %. Furthermore, COD removal continued to increase to 21 % by day 25 and up to 34 % at the end of the last feeding. COD removal increased to 34 % in 30 days, whereas the amount of organic acids increased and also the buffer capacity of the system. Accordingly, due to the high substrate availability, as the case of higher organic acid content, there is an increase in microorganism consumption of organic matter (Oliveira *et al.* 2013). Similar results were obtained by Méndez-Acosta *et al.* (2010) operating a biroeactor with vinasses with a low COD content. During the start-up period COD removal increased from 3 % to 85 % in 50 days. By day 30 the COD removal was around 70 %. In the present work, COD removal was rather poor, which can be explained because of the low C:N-ratio in bioreactor. A high C:N-ratio in bioreactor means that the carbon content is higher than the nitrogen content. In this case the existing carbon could not be completely metabolized and COD could not be efficiently removed. By increasing the nitrogen content in bioreactor, COD removal might be enhanced. Regarding BI, in this study the BI index was 0.22, what indicates that either vinasses are not suitable for AD or the microbial population conditions were not ideal. Some authors report vinasses as an adequate substrate for AD, due to the high carbon source and sugar content to be used by the microbial population (Robles-González *et al.* 2012, Cruz-Salomón *et al.* 2017). In the present assay, vinasses contained a total sugar content of 51 g/L. In this study CI index oscilated between 16 and 25, what indicates a good bacterial interaction when digesting vinasses with sludge in bioreactor (Cruz-Salomón *et al.* 2017). All this facts suggest that vinasses are an adequate substarte for AD, altough the microbial population conditions, such as a high FOS/TAC, were not ideal. On the one hand sludge might not be as efficient as other incoulum sources. According to Hidalgo and Martín-Marroquín (2014), the inoculum source is very important when digesting complex substrates with high organic content, like the case of vinasses. Gu *et al.* (2014) compared different inoculum sources for AD, finding out that manure showed better results than sludge. These findings open new alternatives to consider other inoculum sources for vinasses AD in further experiments. The use of biofilms for microorganisms immobilization could had enhanced also the COD removal, like the case of microbial fuel cells (MFCs) used for wastewater treatment and energy production (Revelo *et al.* 2013, Santoro *et al.* 2017).

### 3.6.3 FOS/TAC

The FOS/TAC value describes the condition of the bacteria in regards to acidification and the buffer capacity of the system. If the FOS/TAC value is more than 0.4, the substrate supply must be reduced. A low FOS/TAC value (0.2) indicates that more substrate needs to be fed in the bioreactor to increase the AD efficiency (Voß *et al.* 2009). In the present study, FOS/TAC values were always higher than recommended by Lossie and Pütz (2008). According to Buchauer (1998) and Mézes *et al.* (2011), the FOS or volatile organic acids indicate the VFA content, mostly acetic acid in terms of mgHAc/L. The FOS in the reactor increased after each feeding, it started by 20 gFOS/L and ended by 45 gFOS/L. The first days of operation the system showed to have a low buffer capacity. This could be verified with the pH drop (even adjusting with NaOH), the accumulation of organic volatile acids and the low existing TAC (12 gTAC/L). Later on, TAC values increased up to 31 gTAC/L, which indicates an enhancement of the buffer capacity of the system. By day 16, FOS/TAC achieved the highest value, where also the lowest methane content is recorded. At this point, the amount of organic acids contained in the bioreactor was two times higher than the buffer capacity of the system. These results suggest an accumulation of acids by the first two feeding steps, where the organic matter was high and the bacteria was still too stressed to degrade the substrate efficiently. That is why the methane content and pH value diminished by day 16. Afterwards the organic acids content continued to increase, although the FOS/TAC value continued to decrease. These indicates a higher organic acids availability for their further conversion in methane and a higher bioreactor buffer capacity, which suggests a successfully adaptation of the system where the organic acids did not affect the FOS/TAC or pH value anymore.

## 3.7 Conclusions

Due to their composition, vinasses are a very promising substrate for the biogas and methane production. The bioreactor adaptation is a very important step in order to achieve stable values of biogas and methane production, for the further long-term bioreactor operation. In this work the successfully 30-days adaptation of a bioreactor operating with anaerobic sludge as inoculum and Mezcal vinasses as substrate, at a vinasses concentration of 42 % v/v, was achieved. Four feeding steps were carried out replacing 30 % of the total volume with new vinasses every seven days. The adaptation step intends to stress the microbial population as less as possible, getting an efficient conversion of organic acids to methane. This was verified already after the third feeding took place, when pH and FOS/TAC remained constant, even when the amount of organic acids in the reactor increased. The buffer capacity increased at the same time, when TAC value increased. The constant biogas production as well as methane content showed also constant values after the third feeding, what suggests a successfully conversion of organic acids to methane.

An efficient adaptation of the anaerobic sludge to the Mezcal vinasses was achieved after three feedings, when the microbial population was better adapted to the environment showing a stable biogas production and methane content in biogas, as well as higher buffer capacity, although the organic acid content increased after every feeding. Biogas production reached 217 L/kgVSvinasses with an average of 50-55 % methane content. The highest achieved COD removal was 34 %, which took place by the last feeding step. This indicates that the adaptation process enhances the efficiency of organic matter removal and eventually other pollutants. COD removal was indeed high after the last feeding took place, however could had achieved higher values when taking in consideration the C:N-ratio for AD (increment of nitrogen content), or like suggested in the literature, when using biofilms in the bioreactor. The use of biofilms should separate the hydraulic retention time from the solid retention time in the reactor, decreasing the bacteria wash out effect and increasing the biogas and methane production rates, as well as COD removal.

## 3.8 References

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# 4 Effect of inoculum source on the anaerobic digestion of Mezcal vinasses at different S:I-ratios

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## 4.1 Abstract

Vinasses are a very harmful residue for the environment, if no treatment takes place before discharge. The present study focuses on the anaerobic digestion (AD) of Mezcal vinasses for treatment and energy generation. The effect of two inoculum sources, anaerobic sludge and cattle manure, were assessed by Biochemical Methane Potential (BMP) assays, testing different substrate to inoculum ratios (S:I-ratios). Mathematical modelling was performed using three sigmoidal bacterial growth curves, Gompertz, transference and logistic, in order to understand the kinetics of methane production. Anaerobic sludge was digested with vinasses at S:I-ratios 0.1, 0.3 and 0.4 and cattle manure at 0.3, 0.5 and 0.7. When using 0.3 S:I-ratio, the digestion of vinasses with manure showed the highest results regarding biogas (1025.44 ± 33.80 L/kgVS), methane (up to 81 %) and organic matter removal (54 % volatile solids removal). Manure indicated a higher specific methane yield growth, with a longer lag phase. Concentrations containing low vinasses content resulted in an inefficient AD due to the lack of organic matter. On the other hand, concentrations containing a high vinasses content resulted in AD inhibition. The present work shows that cattle manure is an alternative inoculum source to achieve a more efficient AD, in comparison to conventional sludge. Optimal S:I-ratio to be used for the digestion of vinasses is 0.3, at which the bacterial population has enough substrate to work efficiently and is not stressed due to high amounts of organic matter to be degraded.

**Keywords**: biogas, kinetic model, methane, organic matter removal, S:I-ratio

## 4.2 Introduction

Vinasses are a very aggressive residue from the distillation of alcoholic beverages, due to the high organic matter content (35-50 g/L as BOD and 100-150 g/L as COD), high discharging temperature and low pH. If no treatment takes place before their discharge, water and soils could be negatively affected causing eutrophication and crop contamination (Robles-González *et al.* 2012). In the recent years, anaerobic digestion (AD) has been a popular method for the simultaneous treatment of the recalcitrant content of vinasses, and bioenergy production. Key point for the efficient AD is the understanding of the biological processes ocurring in bioreactor.

### 4.2.1 Anaerobic digestion

Biogas is produced during AD of organic material. In principle, every organic material can be used, however not all organic material components can be degraded by the same bacterial strains at the same rate. According to the bacterial group contained in the inoculum, bacteria degrade substrate and multiply at different rates. The generation time of each bacterial group describes the capability to duplicate in cell number and accelerate the working speed. Methanogens have a slower generation time than hydrolytic and acidogenic bacteria. The generation time of the hydrolytic and acidogenic bacteria is about 24-36 hours, acetogenic bacteria 40-132 hours and methanogenic bacteria up to 240 hours (KWS 2009).

For the energy generation, one of the main targets of AD is to achieve a high methane content in biogas. A suitable method for determinig if the substrate to be digested is suitable for AD is the Biochemical Methane Potential test (BMP) (Strömberg *et al.* 2014). This test indicates the substrate degradation rate, as well as the methane potential. The cumulative methane yield is plotted against the digestion time. The kinetics of methane production is determined by methanogenic bacteria performance and substrate characteristics. **Figure 4.1** shows examples of typical cumulative biogas and methane yields. The evaluation of the BMP curves can be aided by mathematical models of methane production kinetics (El-Mashad 2013, Dong *et al.* 2016, Ware and Power 2017, Yangyang *et al.* 2018).

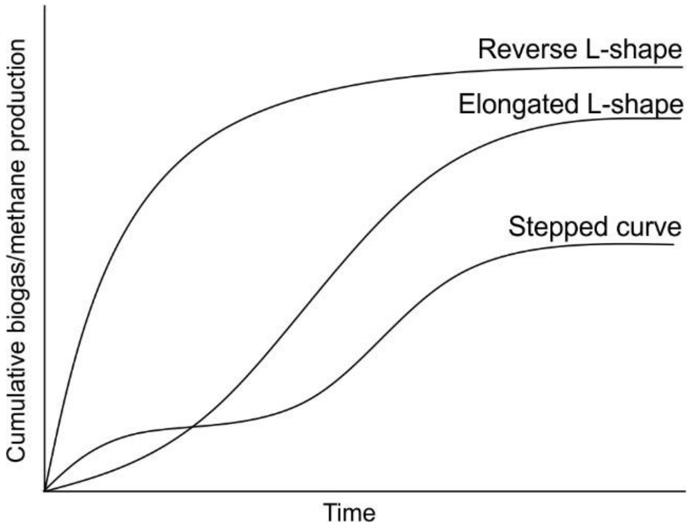

**FIGURE 4.1** Typical cumulative biogas and methane production curves (Ware and Power 2017, VDI 2016)

### 4.2.2 Kinetic modelling of methane production

Due to the similarity between the bacterial growth curves (**Fig. 4.2**) and the biogas/methane production curves (**Fig. 4.1**), authors suggest that AD curves obey a sigmoidal function (Altaş 2009, Syaichurrozi *et al.* 2013, Ware and Power 2017).

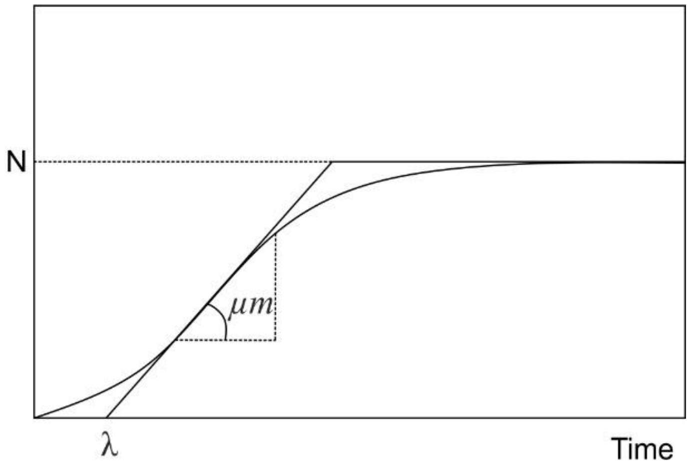

**FIGURE 4.2** Bacterial growth curve (Wave and Power 2017)

Matematical models of sigmoidal bacterial growth curves are normaly used to evaluate the specific growth rate and lag phase of a microbial population. The lag phase is the first phase of bacterial growth, where adaptation takes place and bacteria increase only in size but not in number (Rolfe *et al.* 2012). The growth curves generated from the matematical models describe bacterial growth over a period of time, until saturation state. As shown in **figure 4.2** (Zwietering *et al.* 1990), the specific bacterial growth begins at zero with a slow gas production and accelerates to a maximum growth rate (μm) in a specific time (lag phase λ). It continues with a rapid gas production (exponencial phase) and ends when the curve reaches a final phase at which the growth rate diminishes (asymptote N) up to zero, called point of saturation or stationary phase. When the lag phase takes place, hydrolitic bacteria degrade protein, carbohydrates and fat into aminoacids, sugar and fatty acids. Once the biomass is available for the acidogenic, acetogenic and methanogenic bacteria, the exponencial phase takes place. At this point organic fatty acids, hydrogen and carbon dioxide form methane. At the end, the nondegradable compounds of biomass remain and the stationary phase is reached (Friehe *et al.* 2013, Ware and Power 2017).

In the present study three sigmoidal bacterial growth curve equations (Gompertz model, logistic model and transference function) were used to determine the kinetics of vinasses methane production. When fitting the sigmoidal functions of the cumulative methane yield curves generated from BMP assays to the mathematical models, AD performance can be evaluated. The maximum methane production potential (N or y-axis intercept of highest curve point), the maximum specific methane yield growth rate (µm or slope of the exponencial phase) and the lag phase (λ or x-axis intercept of slope) can be determined. Some of these models were modified by Zwietering *et al.* (1990), so that the parameters have a biological meaning, rather than a mathematical. Therefore a better understanding of the microbiological processes can be achieved.

### 4.2.3 Effect of inoculum on anaerobic digestion

To achieve an efficient conversion to biogas and methane, the inoculum used for AD should contain a high concentration of active microbial communities. The source of inoculum play a crucial role, especially when digesting complex substrates with high organic content (Hidalgo and Martín-Marroquín 2014). The inoculum source affects the decomposition rate of the macromolecules such as proteins, fats and carbohydrates. The use of an adequate inoculum increases the substrate degradation rate, and enhances the enzymatic activity, as well as the process stability.

Since AD was subject of research during the last century, substrate and operation parameters played an important role for the efficient biogas generation. The source of the inoculum itself was though not studied deeply until the recent years (Gu *et al.* 2014). Córdoba *et al.* (2015) compared in batch experiments at mesophilic temperature, the methane production of swine wastewater using rumen, stabilized swine wastewater and sewage sludge, as inocula. Sewage sludge achieved not only the highest methane production of 250 $L_{CH4}$/kgVS, but also the highest organic matter removal near to 50 % in terms of volatile solids (VS) and chemical oxygen demand (COD). Facchin *et al.* (2012) tested the biogas production of food waste using two different inoculum sources in batch assays at mesophilic temperature. Inocula were obtained from a reactor digesting waste - activated sludge -food waste and only food waste. The biogas production using only food waste as inoculum produced 760 L/kgVS with 57% methane, whereas the mixture of waste - activated sludge - food waste resulted in 10% more methane production. Gu *et al.* (2014) evaluated the effect of different inoculum sources on rice straw AD. Digested manure, digester swine manure, digested chicken manure, municipal sludge, anaerobic granular sludge and paper mill sludge were compared. It was found out that digested manures were more effective than sludge, regarding biogas production and lignocellulose degradation. The reactors inoculated with digested manure achieved the highest biogas yield of 325.3 L/kgVS. Vinasses AD of Tequila/Mezcal production has been studied and reported by some authors, nevertheless the inoculum source used in AD is mostly harvested from brewery wastewater treatment. Methan yields of 210 up to 322 $L_{CH4}$/kg$COD_{removed}$ has beed reported (Espinoza-Escalante *et al.* 2008, Méndez-Acosta *et al.* 2010, Buitrón *et al.* 2014, Jáuregui-Jáuregui *et al.* 2014).

### 4.2.4 Effect of substrate inoculum ratio on anaerobic digestion

To prevent AD inhibition, the proportion of substrate should not exceed the proportion the inoculum. According to the VDI-4630 (VDI 2016), the substrate to inoculum ratio (S:I-ratio) should not exceed 0.5 in terms of VS (Eq. 4.1),

$$\frac{\text{VS Substrate}}{\text{VS Inoculum}} \leq 0.5 \qquad \text{(Eq. 4.1)}$$

Fagbohungbe *et al.* (2015) analyzed S:I-ratio effect on AD of human faecal. S:I-ratio 0.5 showed the highest methane production of 254.4 $L_{CH4}$/kgVS and highest pathogen removal, S:I-ratio 0.4 showed the lowest methane yield (110 $L_{CH4}$/kgVS) and lowest pathogen removal. Slimane *et al.* (2014) found out that AD of slaughterhouse wastewater increased with S:I-ratio 0.3, in comparison to S:I-ratios of 0.5 and 1. S:I-ratio 0.3 reached a biogas production of 864 mL, S:I-ratio 0.5 produced 856 mL and S:I-ratio 1 generated 504 mL biogas.

Only few literature is reported regarding the comparison of different inoculum sources for AD of vinasses from the Mezcal and Tequila production, or the effect of using different S:I-ratios. Aim of this study was to analyze in BMP assays the effect of two different inoculum sources. As inocula, anaerobic sludge and cattle manure were used and methane yield, as well as VS removal were compared. Different S:I-ratios ranging from 0.1 to 0.7 (0.1, 0.3 and 0.4 for sludge and 0.3, 0.5 and 0.7 for manure) were tested to determine the inoculum and vinasses ratio, which shows the highest efficiency of methane production, as well as organic matter removal in terms of VS.

## 4.3 Materials and methods

### 4.3.1 Substrate and inoculum

Vinasses generated from cooking, fermentation and distillation of *Agave salmiana* to Mezcal were used as substrate. Two different inocula were tested for AD. The first inoculum used was anaerobic sludge collected from a sequencing batch reactor (SBR) for wastewater treatment of the Faculty of Engineering at the University. The second inoculum used was cattle manure collected from local pasture-raised dairy. Cattle manure was filtered by passing it through a 0.5 mm sieve. **Table 4.1** and **4.2** show the vinasses and inocula characteristics measured prior to BMP assays. Inocula and substrate were collected, transported and stored in the refrigerator at 4°C prior to use.

**TABLE 4.1** Characteristics of vinasses

| *Parameter* | *Value* | *Parameter* | *Value* |
|---|---|---|---|
| pH @ 27°C | 4.77 | REDOX mV | -142 |
| Chemical oxygen demand COD | 59 | Sulphate ion $SO_4^{2-}$ (g/L) | 1.04 |
| Total sugar content (g/L) | 51 | Phosphate ion $PO_4^{3-}$(g/L) | 0.30 |
| Total solids TS (% FM) | 4.91 | Nitrate ion $NO_3^-$ (g/L) | 0.48 |
| Total solids TS (g/L) | 49.10 | Total nitrogen (g/L) | 0.13 |
| Volatile solids VS (% FM) | 3.52 | Total phosphorous (g/L) | 0.02 |
| Volatile solids VS (g/L) | 35.20 | Sediment solids (mL/L, @ 60 min) | 102 |
| Total dissolved solids TDS | 5.90 | Turbidity NTU (Nephelometric | 55.4 |
| Conductivity mS/cm | 11.76 | | |

**TABLE 4.2** Characteristics of inoculum sources

| *Parameter* | *Anaerobic sludge* | *Cattle manure* |
|---|---|---|
| pH @ 27°C | 7.32 | 8.10 |
| Chemical oxygen demand COD (g/L) | 31.75 | 24.39 |
| Total solids TS (% FM) | 3.19 | 5.31 |
| Total solids TS (g/L) | 33.19 | 53.10 |
| Volatile solids VS (% FM) | 2.95 | 4.40 |
| Volatile solids VS (g/L) | 29.5 | 44.00 |
| Total dissolved solids TDS (g/L) | 6.49 | 14.14 |
| Total nitrogen (g/L) | 0.33 | 1.50 |
| Conductivity mS/cm | 12.98 | 28.24 |
| REDOX mV | -313 | -352 |
| Volatile organic acids gHAc/L | 17.85 | 5.98 |
| Total inorganic carbon gCaCO3/L | 19.00 | 20.50 |
| FOS/TAC (volatile organic acids / total inorganic carbon) | 0.94 | 0.29 |

### 4.3.2 Anaerobic digestion tests

BMP assays were prepared in 250 mL Erlenmeyer flasks for batch tests at mesophilic temperatures, according to the German standard method VDI-4630 (VDI 2016). In the first assays, anaerobic sludge was tested as inoculum at S:I-ratios 0.1, 0.3 and 0.4. In the second assays cattle manure was tested as inoculum at S:I-ratios 0.3, 0.5 and 0.7. S:I-ratios were prepared according to Eq. 4.1, considering the % VS in **table 4.1** and **4.2**. The experimental setup was carried out for 26 and 30 days correspondingly, the criteria for test termination was when the daily gas production is equivalent to 1 % of the total volume produced over the total test duration. Control tests digesting only inoculum were performed to evaluate the microbial activity of the inoculum itself. Results were subtracted to the assays digesting vinasses and inoculum, in order not to confuse vinasses AD with inoculum AD (VDI 2016).

### 4.3.3 Determination of methane yield

The biogas quantity produced in 24 hours was measured according to the water displacement principle. Grams of missing water were weighted daily and converted in litre biogas, considering the biogas density 1.2 $m^3/kg$ (Uni Bremen 2009). Biogas quality was measured with the gas analyzer Multitec 540 from Sewerin GmbH. Methane ($CH_4$), carbon dioxide ($CO_2$), oxygen ($O_2$), carbon monoxide (CO) and hydrogen sulphide ($H_2S$) were measured. The sensitivity ranges of the gas analyzer were:

- Test gas 100 Vol.-% $CH_4$, display 95-105 Vol.-%
- Test gas 100 Vol.-% $CO_2$, display 95-105 Vol.-%
- Test gas 20,9 Vol.-% $O_2$, display 20.4-21.4 Vol.-%
- Test gas 40 ppm $H_2S$, display 30-50 ppm
- Test gas 40 ppm CO, display 37-43 ppm

The biogas production was quantified daily in terms of liter per kilogram VS added initially. Methane production was calculated based on the daily methane content (%) in biogas. Methane yield of every assay was reported as the net volume of methane produced during the incubation period per VS contained at the beginning of the tests ($L_{CH4}$/kgVS).

### 4.3.4 Competitiveness and biodegradability indices

In order to compare the biodegradability of vinasses using both inocula, the biodegradability index (BI) was calculated according to Eq. 4.2. If BI is lower than 0.3, there is not sufficient biodegradable matter for anaerobic digestion. To evaluate the bacterial interactions, the competitiveness index (CI) was calculated according to Eq. 4.3. If CI is more than 10, there is no competition between methanogenic and sulphate-reducing bacteria (Cruz-Salomón *et al.* 2017).

$$\mathrm{BI} = \frac{\mathrm{BMP}}{350\ \mathrm{mL\ CH4/gCOD}} \qquad \text{(Eq. 4.2)}$$

$$\mathrm{CI} = \frac{\mathrm{COD}}{\mathrm{SO_4^{2-}}} \qquad \text{(Eq. 4.3)}$$

BMP means the for cumulative methane yield obtained, 350 is the theoretical volume of methane per gram COD removed at normal conditions (T = 273 °K; P = 1 atm).

### 4.3.5 Kinetic modelling

The regression analysis of non-linear least-squares was performed using the software Statistica 13. The cumulative methane yield curves of BMP assays were fitted to the non-linear equations of the Gompertz Model (Eq. 4.4), logistic model (Eq. 4.5) and transference function (Eq. 4.6),

$$M_T = N * \exp\left(-\exp\left((\exp(1) * \mu m)/No * (\lambda - t) + 1\right)\right) \qquad \text{(Eq. 4.4)}$$

$$M_T = N / \left(1 + \exp\left((4 * \mu m/No) * (\lambda - t) + 2\right)\right) \qquad \text{(Eq. 4.5)}$$

$$M_T = N * \left(1 - \exp\left(-\mu m * (\lambda - t)/No\right)\right) \qquad \text{(Eq. 4.6)}$$

whereas,

$M_T$: cumulative methane production ($L_{CH4}$/kgVS)

N: maximum methane production potential ($L_{CH4}$/kgVS)

No: start methane production ($L_{CH4}$/kgVS)

µm: maximum specific methane production growth rate ($L_{CH4}$/kgVS*d)

λ: lag phase (days) in which µm is achieved

t: incubation time (days)

These three mathematical methods determine N, µm and λ, minimising the sume of the squares of the discrepancy between experimental curves from BMP assays, and expected curves from model equations (Eq. 4.4 - 4.6) (Rolfe *et al.* 2012, Ware and Power 2017, and Li *et al.* 2018). In order to determine the correlation of the models to the experimental curves, the correlation coefficient r was also calculated. Confidence interval of 95% was stablished for the goodness-of-fit of the expected curves.

### 4.3.6 Analytical methods

Total Solids (TS) and VS of vinasses and inocula were measured according to VDI-4630 (VDI 2016). COD was measured according to the norm DIN 38414-9:1986-09 (DIN 1986). For sugar content analysis a digital refractometer from HANNA Instruments HI 96801 was used. Total Dissolved Solids (TDS) and conductivity were measured with a HI98311 waterproof tester from HANNA Instruments. The pH values were measured with VWR pH110, calibrated with buffer solutions prior to use. The FOS/TAC value was measured to analyze the inocula biochemical state (Moerschner 2015). This value was determined through titration of 0.1 N $H_2SO_4$ from start pH to pH 5.0 and 4.4. FOS/TAC is the quotient of the volatile organic acids, in German the Flüchtigen Organischen Säuren, and the total inorganic carbon or Total

Anorganischen Carbon. FOS/TAC shows the relation between the acid concentration and the buffer capacity of the bioreactor. FOS indicates in terms of g/L HAc the volatile organic acids or volatile fatty acids (VFA, mostly acetic acid) and TAC shows the total inorganic carbon in terms of mg$CaCO_3$/L (Buchauer 1998, Mézes *et al.* 2011). An optimal FOS/TAC value should oscillate between 0.3 and 0.6, depending on the fermentation substrate (Lossie and Pütz 2008).

### 4.3.7 Statistical analysis

All the experiments were carried out in triplicate. Biogas and methane yields were expressed as mean values with the correspondingly standard deviation. By means of the Minitab 15 software, a $2^k$ factorial design was performed at a 95% confidence level to analyze the effect of the interactions of three input variables on the cumulative methane yield. The variables or factors analyzed were vinasses content, daily methane content and daily biogas produced. Manure and sludge were analyzed separately, due to the different S:I-ratios used.

## 4.4 Results

### 4.4.1 Biogas and methane yields

**Table 4.3** shows the results of the cumulative biogas ($B_T$) and methane ($M_T$) yields using anaerobic sludge and cattle manure as inoculum, with the different S:I-ratios tested. The length of each assay (t) differs between each inoculum source, due to the test termination criteria (VDI 2016). **Table 4.3** shows also the required time in order to achieve 25, 50 and 75 % of $B_T$ and $M_T$.

**TABLE 4.3** Biogas yield ($B_T$) in L/kgVS (volatile solids), methane yield ($M_T$) in $L_{CH4}$/kgVS (volatile solids), and required days to achieve 25, 50 and 75 % of the total production, with different inocula and S:I-ratios (SI)

| | *t (d)* | *$B_T$ Biogas yield ($L_{biogas}$ /kgVS)* | *25 % of $B_T$ (d)* | *50 % of $B_T$ (d)* | *75 % of $B_T$ (d)* | *$M_T$ Methane yield ($L_{CH4}$/kgVS)* | *25 % of $M_T$ (d)* | *50 % of $M_T$ (d)* | *75 % of $M_T$ (d)* |
|---|---|---|---|---|---|---|---|---|---|
| Sludge SI 0.1 | 26 | 460.87 ± 65.48 | 2 | 2 | 4 | 87.83 ± 5.06 | 2 | 2 | 4 |
| Sludge SI 0.3 | 26 | 523.02 ± 16.00 | 2 | 4 | 10 | 188.46 ± 10.34 | 2 | 4 | 9 |
| Sludge SI 0.4 | 26 | 72.19 ± 7.45 | 2 | 4 | 7 | 28.16 ± 0.34 | 2 | 5 | 10 |
| Manure SI 0.3 | 30 | 1025.44 ± 33.80 | 8 | 11 | 17 | 598.92 ± 33.34 | 9 | 12 | 18 |
| Manure SI 0.5 | 30 | 377.05 ± 5.82 | 2 | 26 | 28 | 205.94 ± 10.18 | 24 | 26 | 28 |
| Manure SI 0.7 | 30 | 192.81 ± 21.56 | 2 | 3 | 29 | 32.19 ± 2.44 | 30 | 30 | 30 |

In both cases, sludge and manure as inoculum, the highest biogas and methane yields were produced using S:I-ratio 0.3. In comparison to sludge, manure produced twice more biogas (1025.44 ± 33.80 against 523.02 ± 16.00 L/kgVS) and three times more methane (598.92 ± 33.34 against 188.46 ± 10.34 $L_{CH4}$/kgVS). The highest S:I-ratio, 0.4 for sludge and 0.7 for manure, generated the lowest biogas and methane yields. S:I-ratios of 0.5 and 0.7 showed a lower AD effiiency, in comparison to control tests, with only inoculum. By day 4, control assays produced 56 % more methane than S:I-ratio 0.7, and by day 7 produced 10 % more methane than S:I-ratio 0.5. After day 24, control assays showed again lower values than S:I-ratios 0.5 and 0.7.

Regarding the requiered time to achieve 25, 50 and 75 % of the total biogas $B_T$ and methane $M_T$ yields, even though sludge showed lower methane yields, it showed also a faster digestion time. All sludge assays showed 25 % of $B_T$ and $M_T$ already by the second day and 50 % by the 4th or 5th day. In the case of manure, only $B_T$ of 0.5 and 0.7 ratios showed 25 % by the second day. $M_T$ was much slower, whereas 25 % was achieved by day 24 and 30, correspondingly. The highest achieved $B_T$ and $M_T$ (manure S:I-ratio 0.3) showed a slow degradation time, 25 % was achieved by day 8 - 9, 50 % by day 11 - 12 and 75 % by day 17 - 18.

When analyzing the cumulative methane yield of sludge in **figure 4.3**, methane production of S:I-ratio 0.3 was twice as much as S:I-ratio 0.1 and six times higher than S:I-ratio 0.4. For all the ratios tested, the highest increase in methane production can be seen between the first and second days. The curve for ratio 0.1 showed a remarked increment during the first eight days. Afterwards only a slightly increment can be appreciated. S:I-ratio 0.3 and 0.4 showed an increment until day 19, afterwards the increment was not high. Regarding the daily methane content in biogas, the highest content for S:I-ratio 0.3 was 46.8 % by the third day. S:I-ratio 0.1 reached the highest methane content by day six producing 24.4 % and S:I-ratio 0.4 produced 28.7 % by day three.

In regards to the assays with manure, the highest methane production achieved was 598.92 ± 33.34 $L_{CH4}$/kgVS with S:I-ratio 0.3, increasing the methane content significantly after the sixth day (**Fig. 4.4**). In order to have a better appreciation of the curves behavior, y-axis in figures **4.3** and **4.4** are shown in different relation among each other.

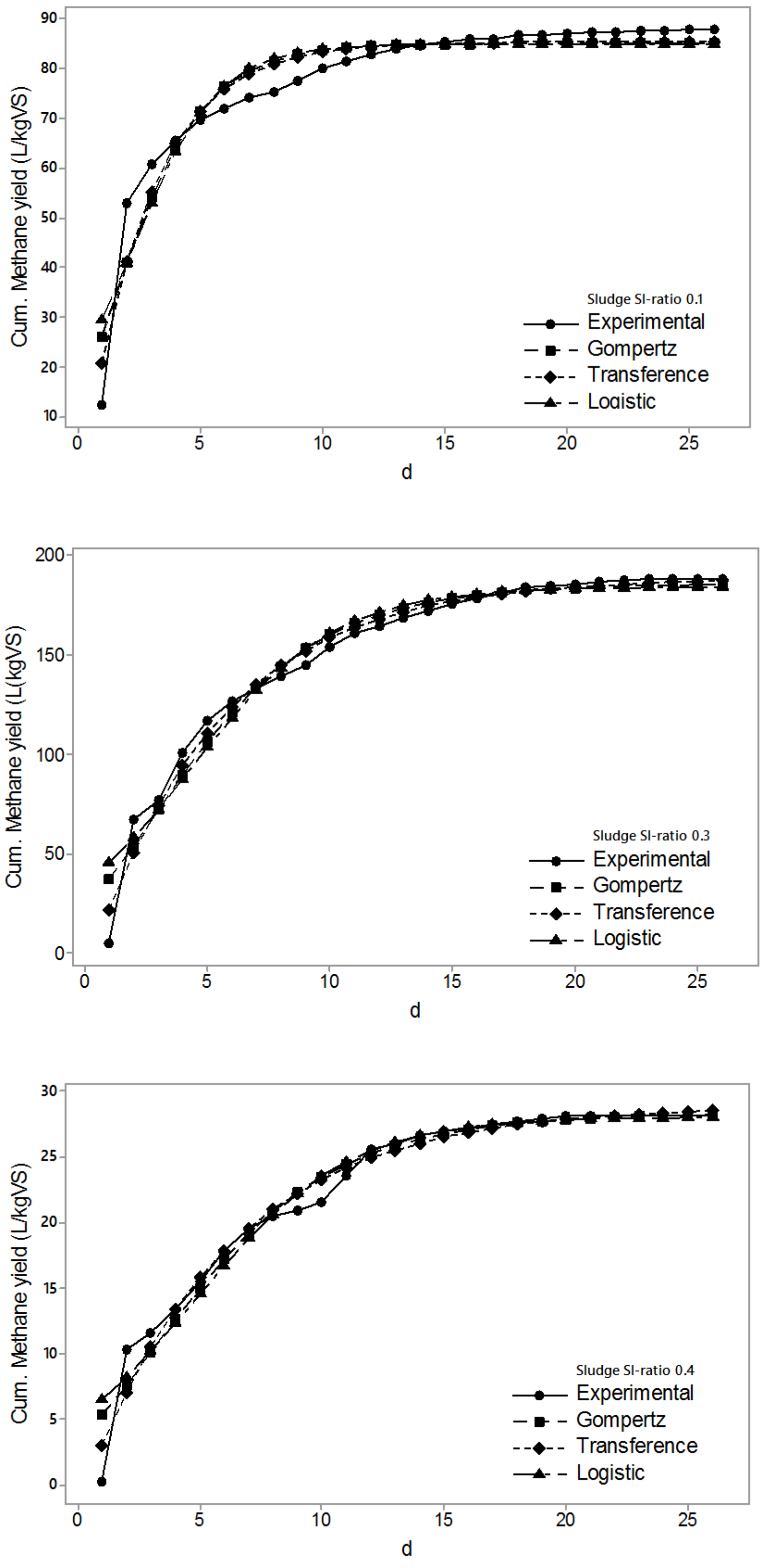

**FIGURE 4.3** Cumulative methane yield in L/kgVS (volatile solids), using sludge as inoculum, experimental data and data fitted to mathematical models

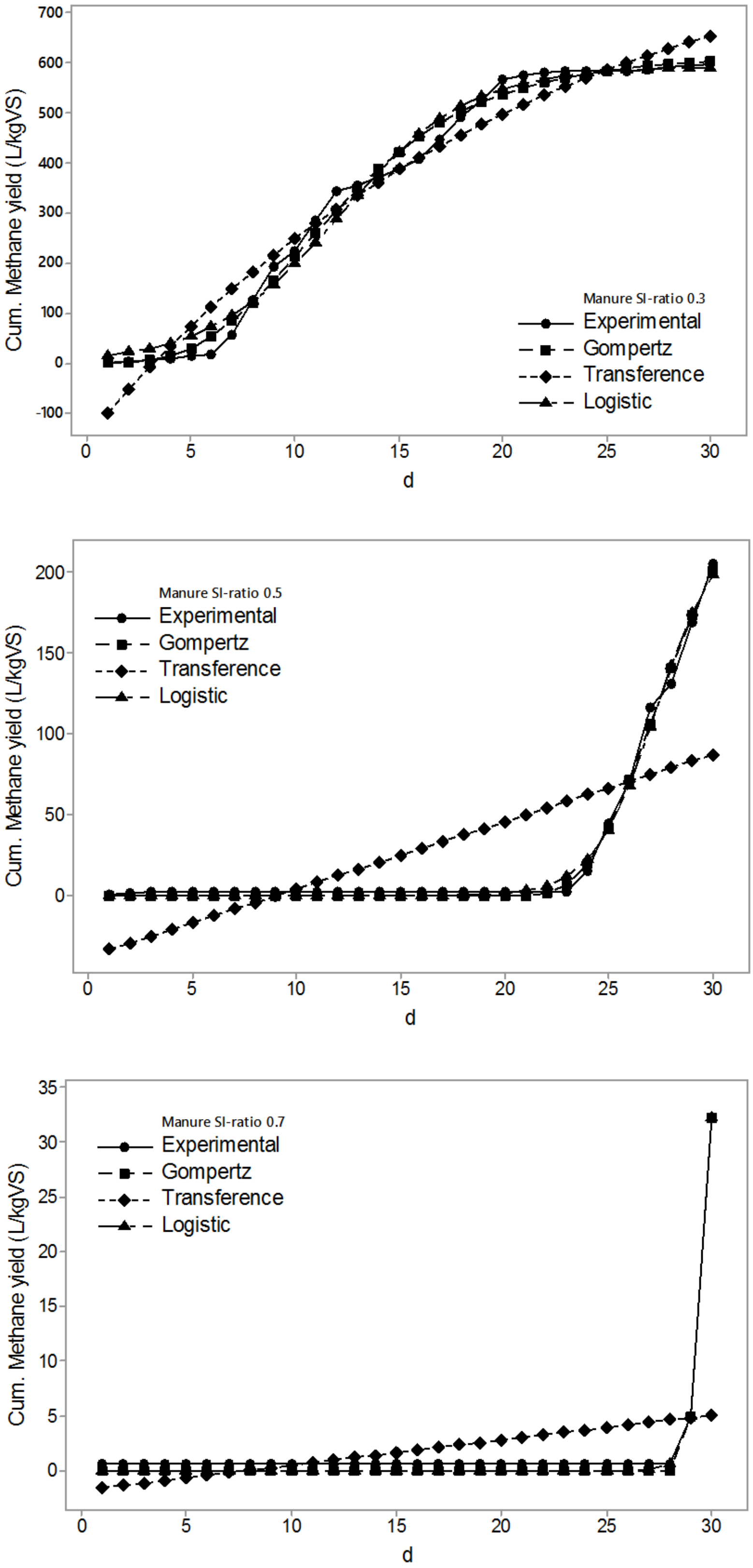

**FIGURE 4.4** Cumulative methane yield in L/kgVS (volatile solids), using manure as inoculum, experimental data and data fitted to mathematical models

S:I-ratios 0.5 and 0.7 showed a lower AD efficiency than control tests, where only manure was digested. S:I-ratio 0.5 did not produce a significant methane amount until day 24 and S:I-ratio 0.7 until day 28. By the end of the BMP assays, the total methane production achieved were 205.94 ± 10.18 $L_{CH4}$/kgVS for S:I-ratio 0.5 and 32.19 ± 2.44 $L_{CH4}$/kgVS for S:I-ratio 0.7. In terms of percentage, the daily methane content in biogas was 81 % by the 10th day, when digesting S:I-ratio 0.3, further measurements varied between 70 and 75 %. Tests with S:I-ratio 0.5 showed the highest methane content of 77.4 % by day 26 and S:I-ratio 0.7 showed 73.6 % methane by day 28.

### 4.4.2 Kinetic modelling

By means of the modified Gompertz model, logistic model and transference function, the variables were determined in regards to the maximum methane production potential ($M_T$ or y-axis intercept of highest curve point), maximum specific methane yield growth (µm or slope of the exponencial phase) and lag phase (λ or x-axis intercept of slope). The results of these three matematical models of sigmoidal bacterial growth were plotted against the average cumulative methane yields from the BMP tests, for sludge and manure (**Fig. 4.3** and **4.4**, correspondingly). The parameters calculated from the nonlinear regression, as well as the coefficient r and the difference between experimental $M_T$ and predicted $M_T$ (% error), are shown in **table 4.4**.

Regarding the use of sludge as inoculum, S:I-ratios 0.3 and 0.4 showed visually good fits between the three mathematical models and the experimental data, with r > 0.90. Gompertz and logistic models showed minimal variances between the parameters $M_T$, µm and λ. The highest % error between $M_T$ experimental and $M_T$ theoretical was 3.56 for S:I-ratio 0.1. In regards to the curves, there was no visual difference between the slopes of each S:I-ratio, but comparing the parameter µm, which increments with a steeper slop, the highest value was shown with S:I-ratio 0.3. The lowest µm was given by S:I-ratio 0.4. The lag phase (λ) was almost cero for all three S:I-ratios.

When analyzing data for manure, both Gompertz and logistic curves provided accurate visual fits to the experimental data showing a r of 0.99. Transference function did not show good fits, especially for S:I-ratios 0.5 and 0.7, which demostrated also very low r of 0.45 and 0.12, correspondingly.

**TABLE 4.4** Kinetic parameters of cumulative methane production curves

| | *Models* | *$M_T$ experimental ($L_{CH4}$ / kgVS)* | *$M_T$ theoretical ($L_{CH4}$ / kgVS)* | *µm ($L_{CH4}$ / kgVS*d)* | *λ (d)* | *r* | *% error $M_T$ exp / $M_T$ theo.* |
|---|---|---|---|---|---|---|---|
| Sludge S:I-ratio 0.1 | Experimental | 87.83 ± 5.06 | | | | | |
| | Gompertz | | 85.00 | 14.98 | -0.74 | 0.91 | 3.32 |
| | Transference | | 85.36 | 32.63 | 0.27 | 0.94 | 2.89 |
| | Logistics | | 84.81 | 12.14 | -1.38 | 0.90 | 3.56 |
| Sludge S:I-ratio 0.3 | Experimental | 188.46 ± 10.34 | | | | | |
| | Gompertz | | 185.65 | 18.13 | -0.98 | 0.97 | 1.51 |
| | Transference | | 188.80 | 35.84 | 0.35 | 0.98 | -0.18 |
| | Logistics | | 184.09 | 15.68 | -1.60 | 0.95 | 2.37 |
| Sludge S:I-ratio 0.4 | Experimental | 28.16 ± 0.34 | | | | | |
| | Gompertz | | 28.31 | 2.53 | -0.99 | 0.97 | -0.52 |
| | Transference | | 28.89 | 4.90 | 0.34 | 0.98 | -2.52 |
| | Logistics | | 28.03 | 2.23 | -1.52 | 0.96 | 0.46 |
| Manure S:I-ratio 0.3 | Experimental | 598.92 ± 33.34 | | | | | |
| | Gompertz | | 611.71 | 47.30 | 5.51 | 0.99 | -2.09 |
| | Transference | | 923.14 | 42.59 | 3.20 | 0.96 | -35.12 |
| | Logistics | | 592.92 | 47.34 | 5.90 | 0.99 | 1.01 |
| Manure S:I-ratio 0.5 | Experimental | 205.94 ± 10.18 | | | | | |
| | Gompertz | | 298.31 | 35.41 | 24.01 | 0.99 | -30.96 |
| | Transference | | 2396326289.63 | 4.17 | 8.99 | 0.45 | -99.99 |
| | Logistics | | 231.17 | 39.00 | 24.33 | 0.99 | -10.91 |
| Manure S:I-ratio 0.7 | Experimental | 32.19 ± 2.44 | | | | | |
| | Gompertz | | 40.10 | 33.23 | 28.88 | 0.99 | -19.72 |
| | Transference | | 322504388.03 | 0.23 | 7.82 | 0.12 | -99.99 |
| | Logistics | | 97.63 | 54.00 | 29.42 | 0.99 | -67.02 |

In regards to the % error between $M_T$ experimental and theoretical curves, S:I-ratio 0.3 evaluated with Gompertz and logistic models showed low values of 1.01 and 2.09, which are aqurate and are comparable with the errors found using sludge. Transference function showed much higher errors of 35.12 % for S:I-ratio 0.3 and almost 100 % for S:I-ratio 0.5 and 0.7. Gompertz model indicated errors of 19.72 and 30.96 % for S:I-ratio 0.7 and 0.5, and logistic model showed errors of 67.02 and 10.91. Therefore, Gompertz indicated more accurate results for S:I-ratio 0.7, and logistic for S:I-ratios 0.3 and 0.5. If the slope of the curve is evaluated according to the models which show the lowest % error, it can be infered that the steepest slope was given by S:I-ratio of 0.3 with a µm of 47.34 L/kgVS*d in comparison to 39.00 L/kgVS*d and 33.23 L/kgVS*d for S:I-ratios 0.5 and 0.7. It is important to notice that this information can not be cleary seen from the cumulative curves, but can be infered due to µm in **table 4.4**. The smallest value of $\lambda$ was shown by S:I-ratio 0.3, which showed also the highest $M_T$. The lag phase for S:I-ratio 0.5 and 0.7 were around 24 and 29 days, correspondingly. The best fits to a normal bacterial growth curve (**Fig. 4.2**) was generated by the assays with manure at S:I-ratio 0.3, which shows also the highest AD efficiency.

### 4.4.3 Total and volatile solids

The results regarding TS and VS removal are shown in **table 4.5**. In both cases, the highest removals were achieved with S:I-ratio 0.3, which generated the highest biogas and methane yield.

**TABLE 4.5** Removal of total solids (TS) and volatile solids (VS)

| | *Initial* | *Final* | *TS removal* | *Initial* | *Final* | *VS removal* |
|---|---|---|---|---|---|---|
| | *Total Solids* | | | *Volatile Solids* | | |
| | % | | % | % | | % |
| Sludge S:I-ratio 0.1 | 2.60 | 2.50 | 3.85 | 1.87 | 1.68 | 10.16 |
| Sludge S:I-ratio 0.3 | 2.80 | 2.09 | 25.36 | 2.00 | 1.42 | 29.00 |
| Sludge S:I-ratio 0.4 | 2.65 | 2.49 | 6.04 | 1.89 | 1.64 | 13.23 |
| Manure S:I-ratio 0.3 | 4.87 | 3.00 | 38.40 | 3.64 | 1.67 | 54.12 |
| Manure S:I-ratio 0.5 | 5.52 | 3.49 | 36.78 | 3.61 | 1.74 | 51.80 |
| Manure S:I-ratio 0.7 | 6.29 | 4.08 | 35.14 | 4.05 | 2.00 | 50.62 |

The digestion of vinasses and manure achieved TS and VS removals of 35-38 % and 50-54 %, correspondingly. When digesting vinasses and sludge, TS and VS removals differed much more between every concentration. TS and VS removal were around 3.85 and 10.16 % for sludge S:I-ratio 0.1, 25.36 and 29 % for sludge S:I-ratio 0.3, as well as 6.04 and 13.23 % for sludge S:I-ratio 0.4.

### 4.4.4 Determination of pH and FOS/TAC

**Table 4.6** shows the pH, single FOS and TAC, as well as the calculated FOS/TAC values of the assays with manure and sludge. FOS/TAC values began much higher than recommended by Lossie and Pütz (2008) and, as expected, achieved lower values by the end of the assays. FOS/TAC shows the relation between the acid concentration and the buffer capacity. FOS indicates the fraction of organic acids and TAC the total inorganic carbon.

**TABLE 4.6** FOS/TAC (volatile organic acids / total inorganic carbon) and pH values of substrate, inocula and S:I-ratios measured at the beginning and end of assays

| | *pH* | *FOS mg/L* | *TAC mg/L* | *FOS/TAC* | *pH* | *FOS mg/L* | *TAC mg/L* | *FOS/TAC* |
|---|---|---|---|---|---|---|---|---|
| | *initial* | | | | *final* | | | |
| Sludge | 7.32 | 17853 | 19000 | 0.94 | 7.75 | 257 | 1902 | 0.13 |
| Manure | 8.1 | 5984 | 20500 | 0.29 | 7.99 | 72 | 1495 | 0.04 |
| Sludge S:I-ratio 0.1 | 7.1 | 19845 | 20300 | 0.98 | 8.69 | 423 | 1851 | 0.23 |
| Sludge S:I-ratio 0.3 | 6.7 | 23497 | 19900 | 1.18 | 8.66 | 589 | 2402 | 0.25 |
| Sludge S:I-ratio 0.4 | 6.46 | 25157 | 13700 | 1.84 | 8.04 | 755 | 2252 | 0.34 |
| Manure S:I-ratio 0.3 | 7.83 | 4739 | 9300 | 0.51 | 8.03 | 81 | 744 | 0.10 |
| Manure S:I-ratio 0.5 | 7.75 | 5320 | 9675 | 0.55 | 8.02 | 144 | 977 | 0.15 |
| Manure S:I-ratio 0.7 | 7.7 | 6482 | 9950 | 0.65 | 8.75 | 284 | 1672 | 0.17 |

Regarding the assays with sludge, at the beginning the amount of organic acids increased proportionally to the increase of vinasses content and FOS/TAC value, at the same time the pH value decreased and so the amount of inorganic carbon and thus the buffer capacity. At the end of the assays the buffer capacity, organic acid content as well as FOS/TAC increased with increased vinasses content, meanwhile the pH value decreased. In regards to the assays with manure, a similar behavior of vinasses content, FOS and FOS/TAC can be appreciated. The more vinasses content, the more organic acids diminishing pH value. Nevertheless TAC increased with the increased vinasses content, what suggests a higher buffer capacity. By the end of the experiments this behavior remained, like the case of sludge.

## 4.5 Discussion

### 4.5.1 Anaerobic digestion

Vinasses AD is a very suitable alternative to treat these residues, while generating energy. Jáuregui-Jáuregui *et al.* (2014) reported 65 % methane content in biogas when digesting vinasses and brewery sludge. Méndez-Acosta *et al.* (2010) obtained 60 % methane and Buitrón *et al.* (2014) obtained 64 %. In the present study, the highest achieved methane content was 81 %, with a further constant value between 70 and 75 %. The highest methane yield achieved in this study was 598.92 ± 33.34 $L_{CH4}$/kgVS. In comparison, Fu *et al.* (2017) generated 274 $L_{CH4}$/kgVS and López González *et al.* (2017) obtained 365-368 $L_{CH4}$/kgVS, when digesting vinasses and sludge. Friehe *et al.* (2013) published a list regarding biogas yields tested for 32 different biomass sources such as sugar beet, maize silage, organic waste bin or ruminal contents, among others. From the list, only the amniotic fluid and the process water showed to generate a higher biogas yield of 1500-2000 L/kgVS and 3000-4500 L/kgVS correspondingly, in comparison to the biogas generation obtained from the vinasses in the present assays (1025.44 ± 33.80 L/kgVS). According to the list, flotation sludge showed to achieve a biogas yield of 900-1200 L/kgVS, all other substrates reported between 200 and 850 L/kgVS biogas. Regarding methane content, the highest value in the list of substrates is 75 %. This was achieved by two sugar-rich substrates; molasses and pressed pulp. In the present study, methane content in biogas (manure S:I-ratio 0.3) was 70-75 % and reached a peak of 81 %, which is higher than reported when digesting other sugar-rich substrates. This found states that the digestion of cattle manure with vinasses is very suitable for AD. According to the Friehe *et al.* (2013) carbohydrates are a very effective source for AD, due to the fact that sugar, in comparison to fat or protein, is more accessible for the bacteria to be biodegraded. Robles-González *et al.* (2012) reported that vinasses contain high amounts of dissolved solids, from which 50% are reducing sugars (4 – 5 g/L), being originated by the condensation of the fermented *Agave* juice. As a consequence, more biogas and methane can be produced. Vinasses in the present assays contained a total sugar content of 51 g/L.

Regarding AD using manure as inoculum, methane production started some days after biogas production. This can be explained because AD occurs in four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The bacteria groups of each stage reproduce at different rates in order to produce acetic acid, hydrogen and carbon dioxide for the methane formation (Friehe *et al.* 2013). Methanogenic bacteria have the slowest reproduction rate of all, up to 360 hours for methanosarcina, or 240 hours for methanococcus. Hydrolytic and acidogenic bacteria need between 24 and 36 hours to reproduce, while acetogenic bacteria 40 up to 132 hours (KWS 2009). Methanogenesis is the slowest step to methane generation.

The most favorable pH value for AD should lie between 6.5 and 7.5. If pH lies under 6.5, the methanogenic bacteria metabolism is inhibited and methanogens cannot degrade biomass at the same rate as hydrolytic and acidogenic bacteria. An accumulation of acids from the acidogenic stage takes place and pH value can continue to drop, moving the $NH_4^+/NH_3$-balance to $NH_3$, which could have an inhibition effect on AD (Friehe *et al.* 2013). If pH lies over 8, the methane yield will be slower (Mézes *et al.* 2011). This is the case at the end of the assays, when a low methane production is given. If the puffer capacity is not high enough, change in pH will be significant. FOS/TAC (**table 4.6**) at the end of the assays showed lower values than recommended by Lossie and Pütz (2008), this indicates that the biomass input was far too low, whereby an increase in biomass input should reactivate the system, in order to maintain an efficient biogas and methane production. By the end of the assays the amount of organic acids decreased considerably (97-99 %), what indicates a successful conversion of organic acids in methane.

When comparing indices BI and CI, both showed much higher values than recommended by Salomón-Cruz *et al.* (2017). Assays with cattle manure indicated that more organic material was able to be biodegraded, in comparison to sludge. This suggests a better suitability of the microbial population in cattle manure to degrade vinasses. CI was higher for the assays with sludge, indicating that the competition between sulphate reducing bacteria and methanogenic bacteria could had been a little higher, though CI values showed that there was not competition.

### 4.5.2 S:I-ratios evaluation

The results of BMP assays indicate that the highest biogas and methane yield comparing S:I-ratios tested, were achieved using a S:I-ratio of 0.3, for both manure and sludge. Manure showed the highest AD efficiency. S:I-ratio 0.3 showed also the highest organic matter removal, especially using manure. Budiyono *et al.* (2013) found out by vinasses AD that a very high organic matter content affects the organic removal rate. Microorganisms experience difficulties in degrading high contents of organic material, especially because the methanogenic bacteria does not reproduce at the same rate as the hydrolytic or acidogenic bacteria, creating a bottleneck for material degradation. This was the case of the highest S:I-ratios tested. The highest S:I-ratios, 0.4 for sludge and 0.7 for manure, showed the lowest biogas and methane yields. Between the 5th and the 20th day, S:I-ratios of 0.5 and 0.7 showed a lower AD efficiency, in comparison to the control tests with only inoculum. The high amount of organic matter might lead to organic acids accumulation, which could affect the capacity of the microorganisms to degrade organic material. As a consequence, AD and the removal of organic matter is negatively affected. According to Fagbohungbe *et al.* (2015) if the organic loading rate increase beyond the degradation capacity of the microbial population, the volatile fatty acids (VFAs) accumulate and pH drops, reducing the methanogenic activity. VFAs (organic acids) are intermediate products in AD, from which 70 % of the total methane is produced. When increasing the organic loading rate, the organic acid concentration increases causing methanogenesis inhibition. Similarly, this can explain the results of AD using S:I-ratios 0.4 and 0.7, which showed lower biogas and methane yields, in comparison to smaller S:I-ratios. In **table 4.6** can be seen, that the amount of organic acids FOS increased with the increased vinasses content, at higher S:I-ratios. On the other hand, when using S:I-ratio 0.3, the organic load was slightly lower than the microorganism's degradation capacity, preventing an accumulation of VFAs. Zhou *et al.* (2011) reported higher methanogenic activity by AD of bean curd when using S:I-ratios between 0.3 and 0.6, rather than S:I-ratios between 0.7 and 3.

Methane production decreased when substrate load increased. Liu and Sung (2002) reported a significant decrease in the methane conversion efficiency using algal residue as a substrate, when S:I-ratio were higher than 1. S:I-ratio 0.3 tend to be more promising than S:I-ratio 0.5, which is recommended by VDI-4630 (VDI 2016) in Eq. 4.1.

### 4.5.3 Effect of inoculum sources

The inoculum source play a crucial role on the degradation efficiency of polymers and molecules contained in complex substrates, such as vinasses. Furthermore, the micronutrients contained in the inoculum could enhance the enzymatic activity and thus methane production (Gu *et al.* 2014).

In the present study, BMP assays with sludge showed a lower methane production than manure, being the highest methane yield of sludge 188.46 ± 10.34, against 598.92 ± 33.34 $L_{CH4}$/kgVS with manure (**table 4.3**). At the beginning and end of the assays the amount of organic acids (FOS), buffer capacity (TAC) and FOS/TAC values incremented proportionally to the vinasses content (higher S:I-ratios), except when starting the sludge assays, which showed a decreased buffer capacity (TAC). Assays with manure showed at the beginning of the tests a higher TAC value with increased vinasses content (higher S:I-ratio). It can be inferred, that manure has a higher buffer capacity than sludge, which suggests a higher balance between ammonium and ammoniac $NH_4^+/NH_3$. According to Moerschner (2015) the conductivity increase with the increase of salts content, such as ammonium content. It can be said that 10 µS/cm conductivity corresponds to 1 g/L $NH_4^+$-N. Manure shows in **table 4.2** a higher conductivity than sludge. Besides, with pH increase, the concentration of $H^+$-ions might increase and the $NH_4^+/NH_3$–balance could had moved to $NH_4^+$. Furthermore, FOS/TAC values of sludge assays showed higher values than manure assays. The relation between the acid concentration and the buffer capacity of sludge assays were much higher than recommended in the literature and practice (Lossie and Pütz 2008, Mézes *et al.* 2011, Moerschner 2015).

A high organic acids content (> 10 g/L) could result in an incomplete bacterial metabolism, which might lead to process inhibition. If at the same time the buffer capacity of the system is adequate, the inhibition will not be evidenced (Mézes *et al.* 2011). Moerschner (2015) suggested TAC values between 8.5 and 13 g/L. As the case of sludge, FOS was at the beginning of the assays, higher than 10 g/L and total inorganic carbon was higher than 13 g/L. This was not the case of the assays with manure, which showed a higher $M_{T.}$

Also the removal rate of organic material achieve better results when using manure, in comparison to sludge. When comparing 0.3 S:I-ratios, the digestion of manure removed 10 % more TS and 20 % more VS than sludge. These results suggest that manure has a better adaptability in vinasses digestion, maybe because it contains microorganisms that produce enzymes which hydrolyze the vinasses for the efficient AD. Another reason is that the content of volatile organic acids is much higher in sludge than in manure (**table 4.2**), causing inhibition.

Gu *et al.* (2014) reported similar results when comparing different inoculum sources (digested manure, swine manure, chicken manure, anaerobic granular sludge, municipal sludge and paper mill sludge) for biogas and methane production using rice straw as substrate. The highest methane production was obtained using manure, especially when using digested manure. It was reported that the anaerobic digesters inoculated with manure showed higher and more stable biogas production in comparison to sludge. Córdoba *et al.* (2015) showed contrary results when comparing sludge to manure as inoculum. Bacteria in manure was not able to consume the available volatiles fatty acids and showed a lower methane generation. Sludge was reported to have more VFAs than manure, 1509 mg$CaCO_3$/L against 1476 mg$CaCO_3$/L. The adequate inoculum and S:I-ratio promote VFAs consumption and methane production, otherwise there is an accumulation that could inhibit the methanogenic activity. The efficient AD process requires a large diversity of methane-forming population and active microbial communities (Gerardi 2003).

According to **table 4.3** biogas and methane production started earlier using sludge, in comparison to manure. Regarding the assays with sludge, 25, 50 and 75 % of the biogas and methane were generated already around days 2, 4 and 9, correspondingly. Manure took much longer to digest, even though the end results showed lower cumulative biogas and especially methane yields. The faster digestion time by sludge might had occurred due to the lower COD, VS and TS content, compared to manure (**table 4.2**).

Regarding the statistical analysis, assays with sludge indicated a significant effect between all interactions tested (daily biogas in L, daily methane % and vinasses content) on the cumulative methane yield. Assays with manure indicated that only the interaction between the daily biogas produced and the vinasses content had a significant effect on the cumulative methane production. This results can be explained due to the fact that the daily methane content for manure assays showed similar values in all S:I-ratios tested (73.6 - 81 %). In the case of sludge a higher methane content variation was appreciated between S:I-ratios (24.4 - 46.8 %).

### 4.5.4 Kinetic modelling

Modelling the methane production kinetics provided information regarding the maximum methane production potential ($L_{CH4}$/kgVS), maximum specific methane yield growth µm ($L_{CH4}$/kgVS*d) and the lag phase λ (days), in which µm is achieved. When comparing the three matematical models of BMP assays, Gompertz and logistic models showed the best visual fits to the curve, highest r, lowest % error experimental/expected, and similarity of parameteres $M_T$, µm and λ.

A high µm indicates a steeper slop and thus higher specific methane production growth rate. In general, sludge assays showed a lower µm, eventhough the lag phase was given right at the beginning of the experiments (**table 4.6**). In both cases, manure and sludge, S:I-ratio 0.3 showed the highest specific methane growth rate. The highest S:I-ratios (0.4 for sludge and 0.7

for manure) indicates the slowest growth rate.

The % of error obtained in the assays with all sludge S:I-ratios and manure S:I-ratio 0.3 (< 3.5 %) was low, in comparison to the assays with manure S:I-ratio 0.5 and 0.7 (> 20 %). Errors up to 8.7 or 10 % has been reported when digesting water hyacinth or sun flower oil cake (Raposo *et al.* 2009, Patil *et al.* 2012).

The lag phase (λ) is almost cero for all three sludge S:I-ratios. In the case of manure, the smallest value of λ (aprox. 5) is shown by S:I-ratio 0.3. The small lag phase of the assays with sludge indicates that the time to achieve the maximum methane production growth rate was shorter than the assays with manure. This could had happened due to the lower amount of organic matter in sludge, in terms of % VS and TS, as well as COD (**table 4.2**). The lag phase for S:I-ratio 0.5 and 0.7 were around 24 and 29 days, correspondingly. As reported by Ware and Power (2017), the lag phase cero indicates a high bioavailability of organic degradable compounds. This can be supported with **table 4.3** where the biogas and methane production started faster with sludge, than with manure. This is also confirmed when comparing the % error $M_T$ experimental and theoretical. A good fitting within the theoretical and experimental methane production curves implies an uncomplicated digestion of the substrate, i.e. without AD inhibitions (Ware and Power 2017). This was not the case of the use of manure with S:I-ratios 0.5 and 0.7, which had a high vinasses content and showed inhibition.

The correlation coefficient r measures how strong is the relationship between experimental and predicted methane curves. If r approaches one, the correlation is stronger, approaching cero, no correlation can be determined. The coefficient r was > 0.9 in almost all the cases, except for manure S:I-ratios 0.5 and 0.7 evaluated by the transference function. This suggests the inadecuacy of using this mathematical model to describe the methane production kinetics of this S:I-ratios.

The best visual fits to the mathematical models are shown by assay resulting in the highest AD (S:I-ratio 0.3). For manure S:I-ratio 0.5 and 0.7, the elevated errors, long λ and high μm might indicate the complicated vinasses digestion, due to the high amount of organic matter, where the microorganisms experience difficulties to degrade the biomass, resulting in a low methane production potential.

## 4.6 Conclusions

Vinasses as substrate for AD are more efficient than other substrates, due to the amount of soluble sugars contained. Inocula was digested with vinasses at S:I-ratios of 0.1, 0.3 and 0.4 for anaerobic sludge and 0.3, 0.5 and 0.7 for cattle manure. From all S:I-ratios tested, S:I-ratio 0.3 for sludge and S:I-ratio 0.3 for manure produced the highest biogas and methane yield, as well as organic matter removal (% TS and % VS). At the end of the assays, the amount of volatile organic acids was reduced almost 99 %, which suggests an efficient conversion of organic acids to methane. The highest S:I-ratios tested (0.4 and 0.7) showed the lowest biogas and methane production. When analyzing the FOS/TAC value, these two S:I-ratios showed the highest organic acid content in comparison to lower S:I-ratios using the same inoculum. On the other hand, FOS/TAC values of assays with sludge were much higher than assays with manure. This fact indicates that the relation between the acid concentration and the buffer capacity of assays with sludge is higher than recommended in the literature and practice. Manure S:I-ratio 0.3 resulted in the highest biogas yield of 1025.44 ± 33.80 L/kgVS, obtaining also the highest methane content of 81 %, and further measurements around 70 and 75 %. Manure showed to have a higher buffer capacity than sludge, suggesting a higher balance between ammonium and ammoniac ($NH_4^+/NH_3$). The conductivity of manure was 28.24 mS/cm in comparison to 12.98 mS/cm of sludge, what indicates a higher $NH_4^+$-N content in manure. Regarding mathematical modelling, for sludge and manure, S:I-ratio 0.3 showed better visual fits within the mathematical model and the experimental curves. In comparison to sludge, manure indicated a steeper slope, with higher $\mu m$ values and higher $\lambda$. It can be inferred that the specific methane growth rate is higher for manure, though the methane production rate was achieved much later than sludge, which showed a lag phase of cero. A small lag phase indicates a high bioavailability of organic matter for digestion. When using a higher vinasses content (manure 0.5 and 0.7), the % of error between experimental and expected methane curves was much higher. This indicates a difficult AD when digesting high organic contents. The best visual fits to the sigmoidal curves resulted with the assay having the highest AD efficiency (manure S:I-ratio 0.3). The present work opens new perspectives for digestion of vinasses with cattle manure, in comparison to conventional use of sludge for AD. The digestion of vinasses with manure as inoculum is suggested at S:I-ratio 0.3, to enhance the methane and biogas production, organic matter removal, and so the effectivity of the system.

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# 5 Comparison of bioreactor start-up for Mezcal vinasses anaerobic digestion using a low-cost biofilm carrier

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## 5.1 Abstract

Vinasses are residues of the production of Mezcal and Tequila. These residues have many compounds which could have toxic effects to the environment, if discharged in soils or water bodies without treatment. Anaerobic digestion (AD) is one of the most used methods for vinasses treatment and simultaneous production of electricity and heat. AD could be enhanced and the recalcitrant content could be better removed using biofilms inside the bioreactor. Nevertheless, the use of biofilm carriers could also mean an increase in operative costs, due to the nature of the biofilm material source. In this work, a low cost biofilm made of polyethylene terephthalate (PET) was used in a 6 L bioreactor digesting Mezcal vinasses and cattle manure. Biogas and methane production, as well as organic matter removal, buffer capacity, and organic acids content were compared. The bioreactor with the biofilm resulted in 40 % and 70 % more biogas and methane production, as well as a lower hydrogen sulphide content in biogas. A higher buffer capacity using the biofilm and a higher organic matter removal could also be demonstrated. Organic acids were not accumulated in the biofilm bioreactor, in comparison to the bioreactor without biofilm, which showed an accumulation of organic acids with higher FOS/TAC values. This work presents an alternative of using low cost and reusable biofilm carriers, enhancing the AD efficiency.

**Keywords**: biogas, COD removal, FOS/TAC, kinetic modelling, methane

## 5.2 Introduction

Vinasses are one of the main wastes produced from the Tequila and Mezcal industries in Mexico, what means contamination for soil and water if discharged without any treatment. AD is one of the most used methods for wastewater and organic wastes treatment, due to the low operation costs and the generation of byproducts, such as methane or fertilizer. Much research has been done in regards to this technology (Ilangovan *et al.* 2000, Espinoza-Escalante *et al.* 2009, Buitrón and Carvajal 2010, Méndez-Acosta *et al.* 2010). Through AD, not only methane and fertilizer can be produced, but also the removal of organic matter and other recalcitrant agents can be achieved. Biogas and methane production could be more efficient and the removal of contaminants could be higher implementing biofilm carriers inside the bioreactor. Biofilms are composed of microorganisms, which can better degrade organic matter and chemical bonds in the bioreactor. This paper aims the comparison of the start-up of two bioreactors digesting Mezcal vinasses with cattle manure as inoculum. In bioreactor BF, a low cost polyethylene terephthalate (PET) biofilm was placed, and in bioreactor B0, no biofilm was placed. The efficiency of biogas and methane production, as well as organic matter removal, buffer capacity and organic acids content was compared.

### 5.2.1 Biofilms in anaerobic digestion

Every bioreactor under continuous AD operation has a retention time, according to the organic matter content and inflow velocity. Sometimes this could cause problems because active bacteria can continuously flow out of the bioreactor due to the extraction of treated substrate. This implies higher retention times, microorganisms wash out effect or decrease of active microbial population. An alternative to solve this problem is the bacteria immobilization on a solid support, incrementing the capacity to degrade biomass (Carlos-Hernández *et al.* 2014). With the use of biofilms, the hydraulic retention time can be separated from the solid retention time, diminishing the wash out effect in the bioreactor, and increasing the biogas yield and methane content (Martí-Herrero *et al.* 2014). Besides, the use of biofilms in bioreactors can contribute to the degradation of organic matter, which could be difficult by conventional AD process (Bertin *et al.* 2004).

### 5.2.2 Biofilm types

Biofilms are formed of microbial communities adhered to a specific material or support. Two kind of biofilms are used on industrial applications. On the one hand, there are biofilms that grow on supports such as charcoal, resin, concrete, clay or sand. Biomass is formed all around the supports, and the biofilm size particles grow with the time. On the other hand, there are biofilms called granular biofilms, which form aggregate formations and flocs, like the ones used on up-flow anaerobic sludge blanket reactors (UASB). Extracellular polymeric substances are being produced by the bacterial cells, and are adhered to the cells forming flocs. Depending on the material, biomass and nutrients, biofilm may be formed in several days or months (Qureshi *et al.* 2005).

### 5.2.3 Biofilm materials

Different kind of biofilm bioreactors have been used for wastes AD. Dairy wastewater treatment was carried out in these bioreactors using different biofilm materials such as seashell, charcoal, break, gravel, plastic materials, ceramic, sintered glass, fire bricks, natural stones including limestone, gravel, pumice, clay, rocky aggregates, sand, granular activated carbon, saponite and synthetic plastic materials (Patel *et al.* 1994, Qureshi *et al.* 2005). Patel *et al.* (1994) compared different materials for whey AD. Charcoal showed the best organic matter removal, in terms of chemical oxygen demand (COD), the highest methane production was achieved, as well as the lowest volatile fatty acids (VFA) accumulation. Pumice showed the worst results. The low surface area of pumice could had been the reason of these results. Clay showed also a good performance by COD removal and methane production, which was related to surface morphology and adsorption capacity. Porosity plays an important role at high organic loading rates. Magnetoactive supports have also shown a good performance by the two first AD steps, especially because of the reaction of hydrogen sulphide ($H_2S$) with iron ions. Plastic material was coated with iron and copper powder, showing effective results in AD (Karadag *et al.* 2014). Martí-Herrero *et al.* (2014) used strips of PET bottles as biofilm carrier to test the AD of cattle manure at psychrophile temperature. Biogas production was enhanced.

### 5.2.4 Typical biofilm reactor configuration

The construction of biofilms in bioreactors can be done as a batch reactor, continuous stirred tank reactor (CSTR), packed bed reactor (PBR), fluidized bed reactor (FBR), airlift reactor (ALR) and up-flow anaerobic sludge blanket reactor (UASB), among others. Batch reactors are nevertheless not very appropriate for biofilm use because of cell inactivity when lack of feeding (Qureshi *et al.* 2005). When stirring a bioreactor like CSTR, only fibrous bed support can be used, granular activated carbon is not worth considering. Up-flow anaerobic filter showed the best performance in methane production and COD removal, when using high organic loading rates, compared to other feeding systems. Biofilms in up-flow filters show to have a better surface contact with biomass. Fluidized bed reactors (FBR) and moving biofilm reactors (MBBR) were used for treating high organic loads, showing to be very efficient in terms of COD removal and methane production (Karadag *et al.* 2014). PBR and FBR bioreactors are very similar, thus PBR is fed at the bottom and FBR at the top. When feeding PBR at the bottom, the whole tank will be fed up with biomass and an excessive cell growth can take place and inhibit the process. When feeding FBR at the top, biofilm may not have full contact with the biomass, although no risk of excessive cell growth is seen (Qureshi *et al.* 2005).

## 5.3 Materials and methods

### 5.3.1 Bioreactor configuration

Two bioreactors made of polyvinyl chloride (PVC) with a volumetric capacity of 6 L were filled with 4 L cattle manure and 1.1 L Mezcal vinasses. This ratio corresponds to a substrate to inoculum ratio (S:I-ratio) of 0.3. At the top of the bioreactor, a tedlar bag was connected in order to collect the biogas produced daily. In bioreactor BF, a biofilm made of six sanded and stacked PET bottles was put inside. The contact surface of the complete biofilm placed in the bioreactor was 0.212 $m^2$. The bottles were sanded in order to achieve a porous surface, which can eventually enhance the adsorption capacity of the material, and so its ability to retain the microorganisms forming the biofilm (Patel *et al.* 1994). In bioreactor B0 no biofilm was placed. Vinasses pH was adjusted to 7 with sodium hydroxide (NaOH) prior to start-up, and prior to each feeding (Espinoza-Escalante *et al.* 2009, Méndez-Acosta *et al.* 2010). BF and B0 were fed simultaneously. The first feedings were done every seven days during 60 days of experiments. After day 60, when the methane content and FOS/TAC (ratio of the volatile organic acids and total inorganic carbon) achieved stable values for BF, feeding took place every two days. Experiments were carried out for a period of 80 days. Before every feeding took place, the same amount of influent substrate was taken out of the bioreactor, to maintain the same volume of 5.1 L. The bioreactor was kept in a furnace at 39 °C and was shacked for 5 min/d, according to the norm VDI 4630 (VDI 2016).

### 5.3.2 Inoculum and substrate

As inoculum, cattle manure was collected from a local pasture-raised dairy and filtered by passing it through a 0.5 mm sieve. Mezcal vinasses were collected from the Mezcal factory Laguna Seca located in the Mexican state San Luis Potosi. Inoculum and substrate were stored in the refrigerator at 4 °C prior to use. **Table 5.1** shows the characteristics measured in the cattle manure and Mezcal vinasses.

**TABLE 5.1** Characteristics of cattle manure and Mezcal vinasses

| *Parameters* | *Cattle manure* | *Mezcal vinasses* |
|---|---|---|
| pH @ 27°C | 8.29 | 4.41 |
| Chemical oxygen demand COD (g/L) | 31.10 | 63.73 |
| Total solids TS (% FM) | 3.07 | 5.26 |
| Total solids TS (g/L) | 30.70 | 52.68 |
| Volatile solids VS (% FM) | 1.80 | 2.88 |
| Volatile solids VS (g/L) | 18.02 | 28.80 |
| Total dissolved solids TDS (g/L) | 14.14 | 5.87 |
| Total nitrogen (g/L) | 1.50 | 0.13 |
| Conductivity mS/cm | 28.24 | 11.75 |
| REDOX mV | -352 | -142 |
| Volatile organic acids mgHAc/L | 1585 | N/A |
| Total inorganic carbon mgCaCO3/L | 9525 | N/A |
| FOS/TAC (volatile organic acids/total inorganic carbon) | 0.17 | N/A |

### 5.3.3 Bioreactor start-up

The start-up consisted of filling BF and B0 at a substrate to inoculum ratio (S:I-ratio) of 0.3, with 4 L cattle manure and 1.1 L Mezcal vinasses. After seven days, 0.1 L vinasses were fed increasing weekly the amount of influent to 0.15, 0.25, 0.35, 0.45, 0.55, 0.75, 0.85, 1.05, 1.15 and 1.5 L. OLRs increased weekly from 0.4, 0.6, 1.0, 1.4, 1.9, 2.3, 3.1, 3.5, 4.3 and 6.2 gVS/Ld. Aim of the stepwise increase was to compare the performance of both bioreactors in regards to effluent FOS/TAC, total dissolved solids (TDS) and conductivity, as well as biogas productions and methane yield. After stepwise increase, a stable methane content above 60 % and stable FOS/TAC values could be appreciated in BF. After this point only 0.05 L of vinasses (1 % v/v) were added every two days.

## 5.4 Measurements

The daily amount of biogas contained in the tedlar bags was measured according to the displacement principle using an Erlenmeyer flask and a digital scale from Media Data PS-5. The content of methane carbon dioxide and hydrogen sulphide was measured with a biogas analyzer Multitec 540 from the German company SEWERIN GmbH. At least three times a week, the effluent was measured regarding pH, REDOX (mV), TDS (ppm) and conductivity (µS/cm). FOS/TAC was measured before and after each feeding took place. REDOX and pH were measured with a pH-meter VWR-110. TDS and conductivity were measured with a waterproof tester HI-98311 from HANNA Instruments. FOS/TAC, the quotient of the volatile organic acids and the total inorganic carbon, was measured throughout the titration of sulphuric acid 0.05 M ($H_2SO_4$) in the bioreactor effluent. FOS indicates the amount of volatile organic acids or VFA, mostly acetic acid (mgHAc/L), and TAC indicates the total inorganic carbon or buffer capacity (mg$CaCO_3$/L) (Mézes *et al.* 2011, Moerschner 2015). At the beginning and end of the experiments, COD, total solids (TS) and volatile solids (VS) were measured according to the norms DIN 38414-9:1986-09 (DIN 1986) and VDI 4630 (VDI 2016). At the end of the experiments, biofilm was analyzed with an optical microscope LEICA DMS 1000.

## 5.5 Kinetic modelling

A mathematical sigmoidal bacterial growth curve was used in order to understand the kinetics of biogas and methane generation. Transference, logistic and Gompertz models were evaluated (Ware and Power 2017, Li *et al.* 2018). The biogas and methane production curves were fitted to the curve generated from the transference model of Eq. 5.1, so that the specific growth rate and lag phase λ of the microbial population could be evaluated. The lag phase λ is the time at which the maximum microbial growth rate µm is achieved. At λ, the hydrolitic bacteria convert the fat, protein and carbohydrates in fatty acids, aminoacids and sugar (Ware and Power 2017).

$$y = N * (1 - \exp(-\mu m * (\lambda - t)/No)) \qquad \text{(Eq. 5.1)},$$

y: cumulative gas yield ($L_{CH4}$/kgVS)

N: maximum production potential ($L_{CH4}$/kgVS)

No: start gas production ($L_{CH4}$/kgVS)

µm: maximum specific yield growth rate ($L_{CH4}$/kgVS*d)

λ: lag phase (days)

t: incubation time (days)

The correlation between experimental and theoretical data was analyzed by means of the correlation coefficient r (Rolfe *et al.* 2012, Ware and Power 2017). By means of the software Statistica 13, a regression analysis of non-linear least squares was performed with a 95 % confidence interval.

## 5.6 Results and discussion

### 5.6.1 Biogas, methane and hydrogen sulphide production

The cumulative biogas and methane production is shown in **figure 5.1**. After 80 days of experiments, bioreactor without biofilm produced 2827 $L_{biogas}$/kgVS and 1284 $L_{CH4}$/kgVS, whereas bioreactor with biofilm produced 45 % more biogas and 70 % more methane, 4123 and 2279 L/kgVS, correspondingly. By day 30, the biogas and methane production of both bioreactors were similar, but in both cases, after day 60, when the feeding began to be done every two days, instead of seven days, a significant increase in both, biogas and methane, was given. Martí-Herrero *et al.* (2014) reported a 40 % biogas enhancement when using PET bottles as biofilm carrier in a reactor digesting cattle manure for 300 days. Liu *et al.* (2017a) reported a biogas and methane enhancement of 40 % and 49 %, correspondingly, when using a polypropilene fiber as biofilm carrier. Gong *et al.* (2017) achieved also 40 % enhancement for both, biogas and methane production, when using activated carbon fiber. Other fibers used such as polyvinyl alcohol fiber and glass fiber caused AD inhibition. The results of the present assays demostrate that PET is an accurate alternative as biofilm carrier, besides the fact that the overproduction of PET worldwide has becomme a serios environmental problem. Reusing PET bottles for AD could hinder their disposal in landfills and water.

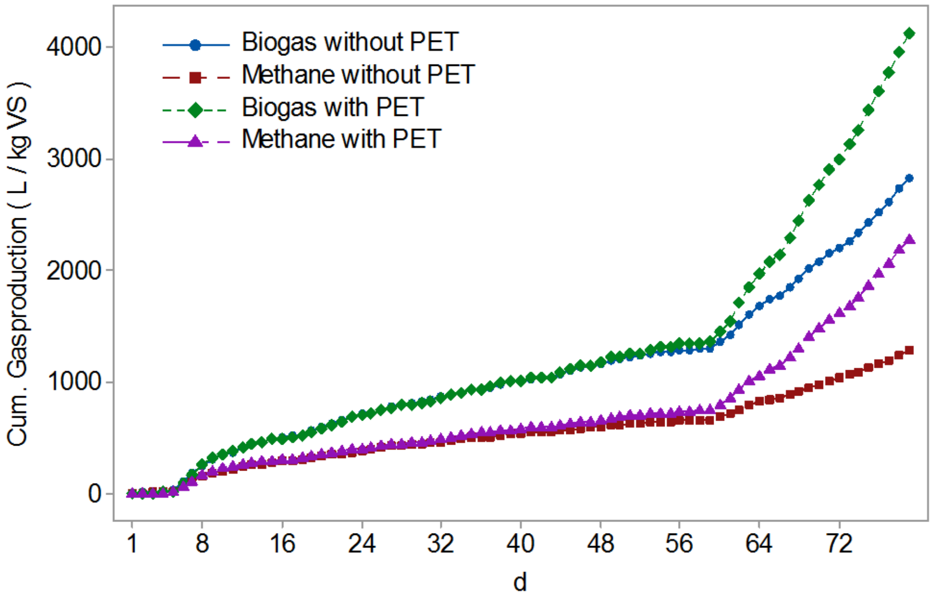
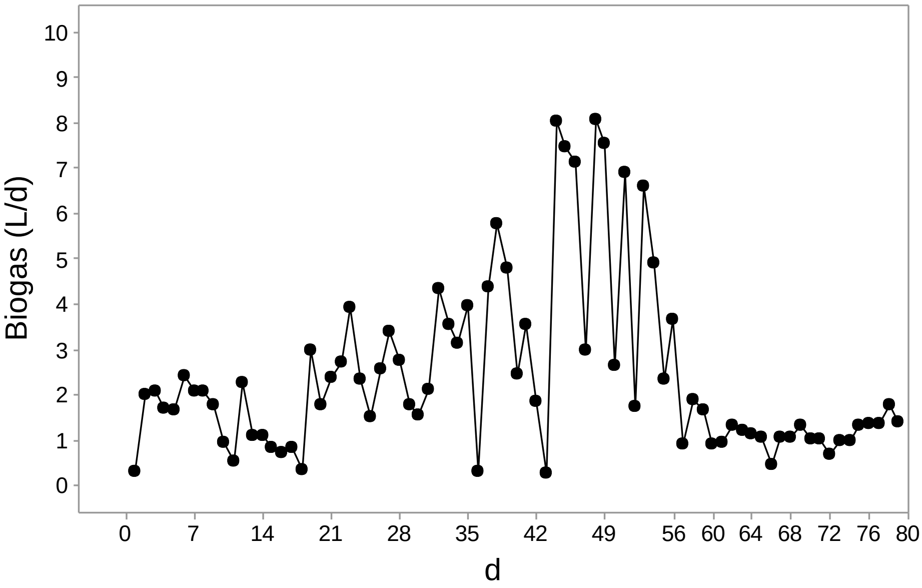


**FIGURE 5.1** Cumulative biogas and methane production in L/kgVS (volatile solids) vinasses

The daily biogas produced in L and its methane content in % are shown in **figures 5.2** and **5.3**. In general terms, the highest biogas production took place around day 50. The methane production achieved a higher value in BF producing 68 % methane by day 35. In comparison, B0 achieved 62 % by the third day of experiments, and afterwards achieved the highest value of 60 % by day 27. During the first days of experiments, BF produced a little amount of biogas (0.04 L/d), whereas B0 began to produce 1.8 L/d, with 25 % methane content. These facts suggests, that the biofilm formation took place during several days. Similar results were reported by Langer *et al.* (2014). When detecting the microcolones formed in the biofilm during AD, microorganisms were appreciated after three days of incubation.



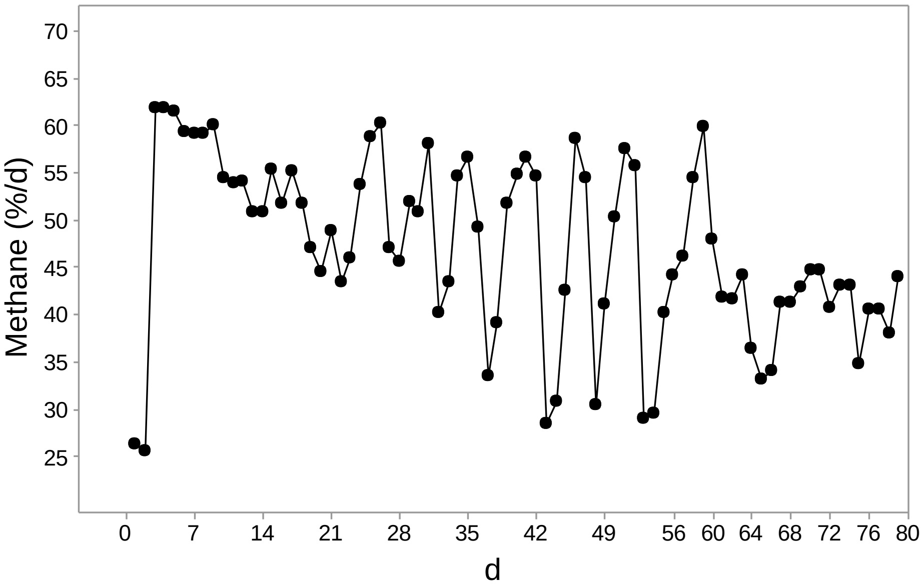
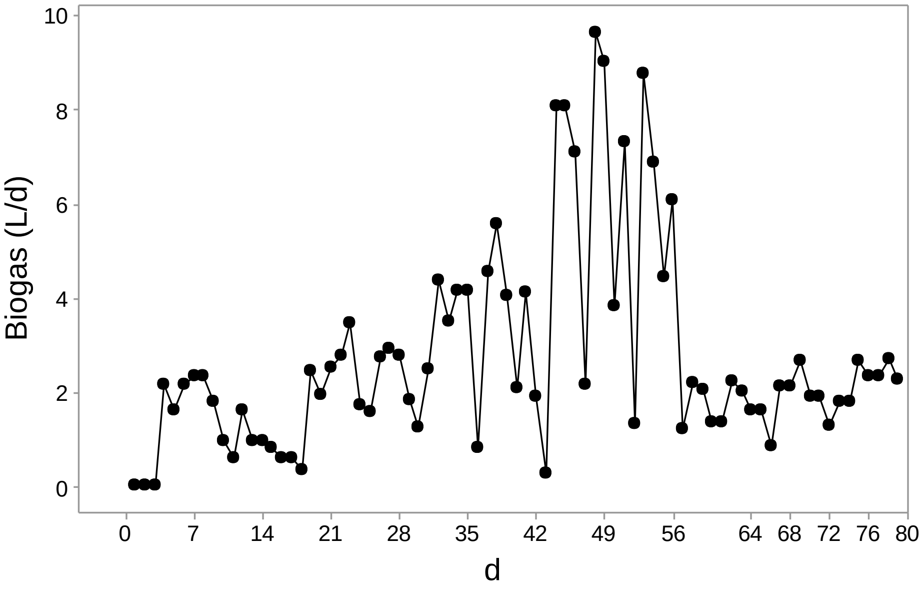


**FIGURE 5.2** Up: daily biogas quantity in L/d, down: daily methane content in %/d, in bioreactor without biofilm (B0)



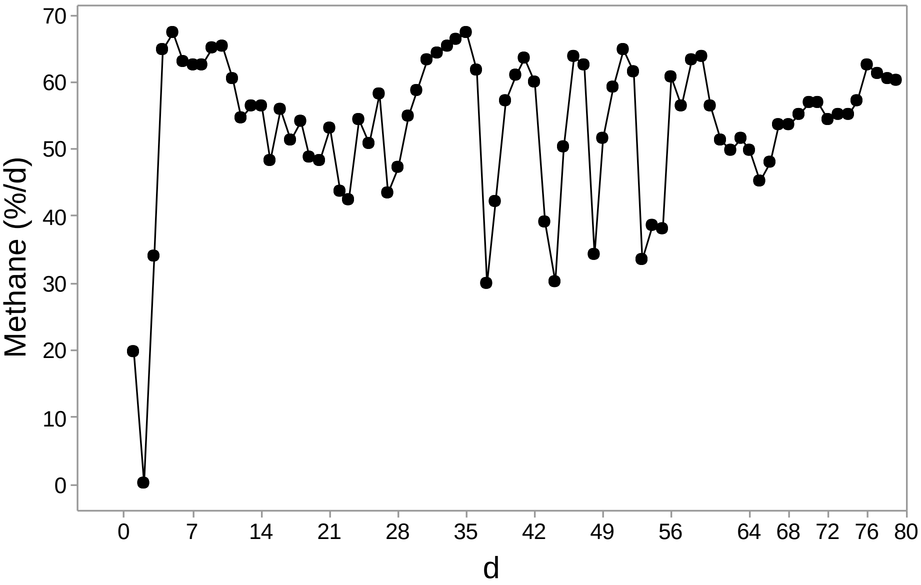


**FIGURE 5.3** Up: daily biogas quantity in L/d, down: daily methane content in %/d, in biofilm bioreactor (BF)

For BF and B0, after a high methane value was achieved, methane content decreased the next day up to 30 %, this fact suggests that the feeding steps could had been done more frequently than every seven days. In the case of BF, after day 70 an stable methane content around 55 and 60 % was achieved. Regarding B0, a stable methane content could not be obtained, because the last ten days of experiments, methane content varied between 34 and 44 %.

The $H_2S$ content in biogas was also affected when the biofilm was placed in the bioreactor. B0 produced 12.58 g/L $H_2S$, whereas BF produced 20 % less $H_2S$ (10.28 g/L). $H_2S$ is considered as biogas impurity and is not desired. This trace element is found in biogas in the ranges of 50 – 10000 ppm (or mg/L). It can cause corrosion in the engine and metal parts, where biogas is converted to energy. $H_2S$ emmits $SO_2$ when biogas is being combusted. In practical applications, the content of $H_2S$ in biogas has been a limiting factor of the use of biogas for power generation (Friehe *et al.* 2013, Rashed *et al.* 2015). Several technologies for $H_2S$ removal has been developed. As a biological treatment, sulphide oxidizing microorganisms convert biogas sulphur compounds in elementary sulphur. The posibility to reduce the amount of $H_2S$ in biogas, through the use of biofilm carriers in the bioreactor should be deeply studied in further experiments.

### 5.6.2 Biofilm comparison

**Figure 5.4** shows the photographs of the biofilm taken at different sides of the sanded PET bottles after finishing the experiments.

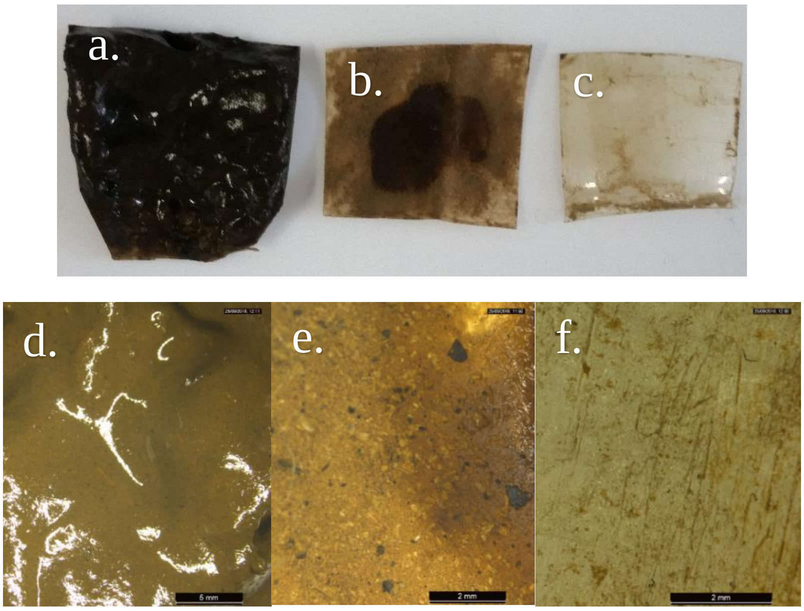


**FIGURE 5.4** Biofilm by the end of the experiments, comparison of **a.** and **d.** stacked internal side, **b.** and **e.** internal side, and **c.** and **f.** external side

**Figures 5.4d**, **5.4e** and **5.4f** were taken with the optical microscop. **Figures 5.4a**, **5.4b** and **5.4c** were taken with a conventional camera. The bottles were stacked so that the internal side of one bottle made direct contact with the external side of the other one, contributing the biofilm formation in the space between. At the end of the experiments the stacked internal side of the bottles showed the highest amount of adhered microbial population, like in **figures 5.4a** and **5.4d**. The internal side of the bottles, which could not be stacked, accumulated less microorganisms, like in **figure 5.4b** and **5.4e**. The external side of the bottles is shown in **figure 5.4c** and **5.4f**, which showed the fewest amount of microorganisms adhered. The sanded surfaced can be seen in **figure 5.4c** and **5.4f**. It can be noticed that sanding the surface did not led to microorganisms adhesion, like in the case of stacked bottles.

### 5.6.3 Kinetic modelling

Regarding the kinetic modelling, Gompertz, logistic and transference models were used to compare μm or the maximum specific growth rate (L/kgVS*d), and the lag phase λ (d), in which μm is achieved. Gompertz and logistic predicted curves did not show good fits to the real biogas and methane production curves. Transference function curves showed the best correlation and similarity to the real curves. **Table 5.2** shows the comparison of the values μm and λ calculated with the transference function.

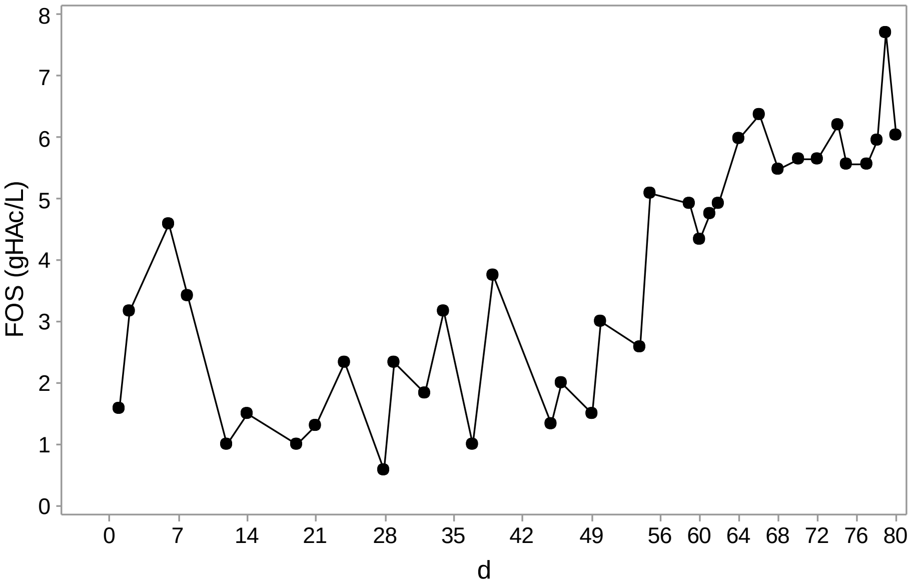
**TABLE 5.2** Kinetic parameters calculated with the transference function for bioreactor without biofilms B0 and bioreactor with biofilms BF

| | *B0* | *BF* |
|---|---|---|
| Biogas yield | | |
| Maximum specific growth rate μm (L/kgVS*d) | 425 | 564 |
| Lag phase λ (d) | 0 | 7 |
| Methane yield | | |
| Maximum specific growth rate μm (L/kgVS*d) | 194 | 302 |
| Lag phase λ (d) | 2 | 8 |

As expected, the lag phase of BF was longer than B0. In the case of biogas, the maximum specific gas production growth rate was achieved in two days in the case of B0, and in eight days in the case of BF. For methane, the maximum specific gas production growth rate was reached at the beginning of the experiments for B0, and by day seven for BF. It can be confirmed, that AD start-up was displaced when using biofilm, due to the time of biofilm formation. Langer *et al.* (2014) published that the life cycle of a biofilm begins when the single cells are adhered to the carrier, afterwards an accumulation of microcolonies in the surface takes place, and at the end microorganisms dissipation occurs. A deeper analysis of the dynamic of biofilm formation should be carried out in future experimentations.

### 5.6.4 FOS/TAC

**Figures 5.5** and **5.6** show the content of FOS (gHAc/L) and TAC ($gCaCO_3/L$) in BF and B0. For both bioreactors, when each feeding took place, the amount of FOS increased and TAC decreased. In B0 the amount of accumulated FOS or VFA increased with the time. In comparison, BF did not show an accumulation of acids, even though they were fed simultaneously.



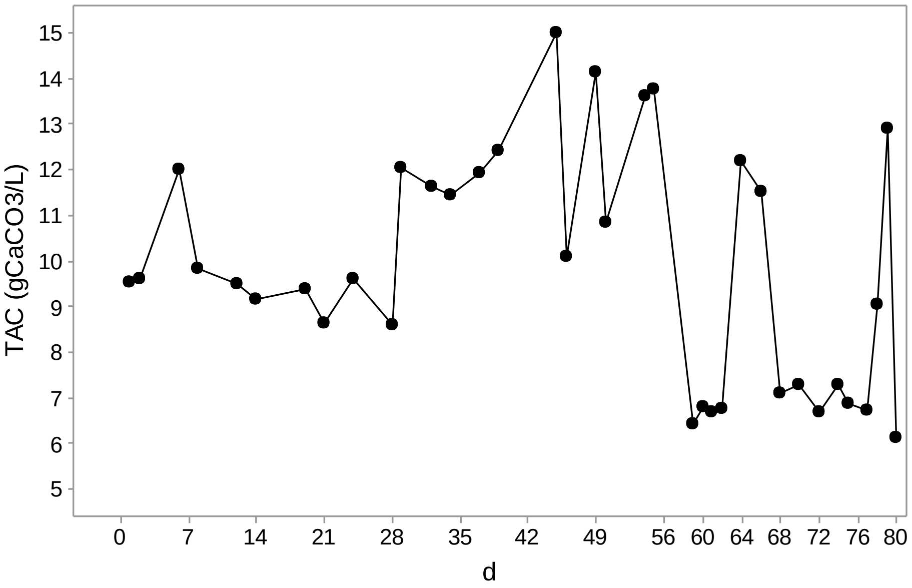


**FIGURE 5.5** Volatile organic acids in gHAc/L (FOS), and total inorganic carbon in $gCaCO_3/L$ (TAC), in bioreactor without biofilm B0

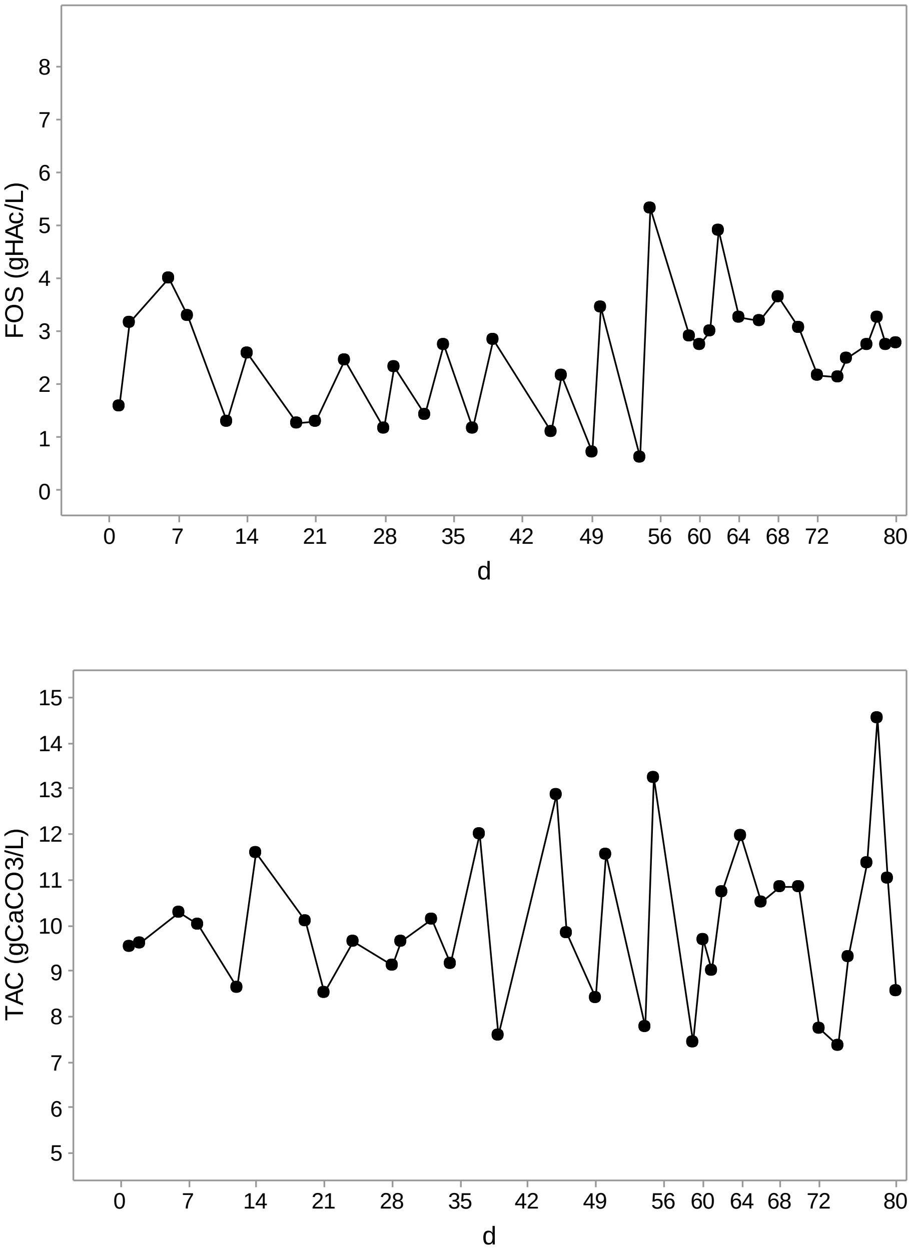


**FIGURE 5.6** Volatile organic acids in gHAc/L (FOS) and total inorganic carbon in gCaCO$_3$/L (TAC), in bioreactor with biofilm BF

According to Mézes *et al*. (2011), if FOS exceeds 10 g/L, an incomplete metabolism given by a high organic acid content might inhibit AD. In this experiments no inhibition took place, nevertheless the methane content was lower in B0, whereas the FOS was higher.

**Figure 5.7** shows the measured FOS/TAC during the 80 days of experiments.

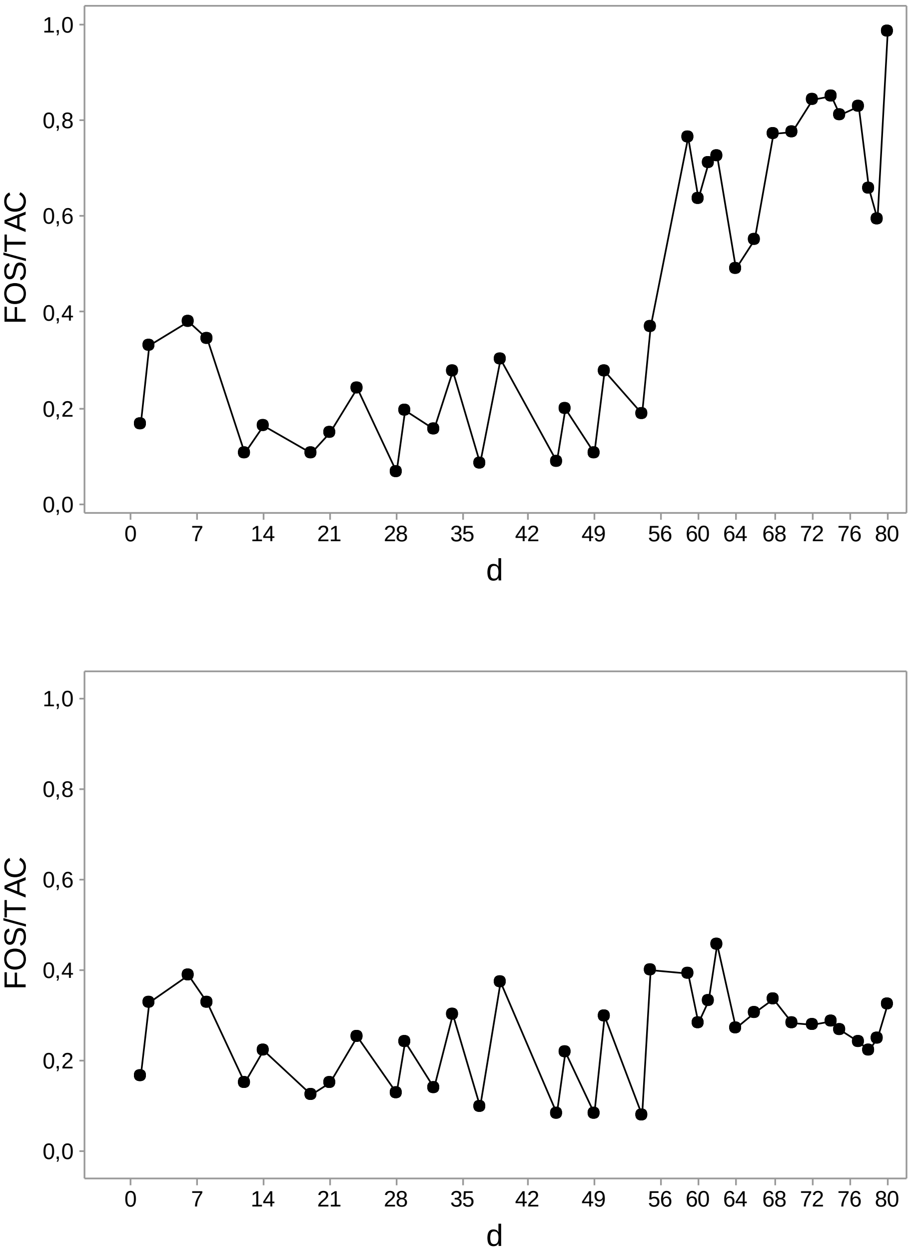


**FIGURE 5.7** FOS/TAC (ratio of volatile organic acids and total inorganic carbon), up: bioreactor without biofilm B0, down: bioreactor with biofilm BF

According to Lossie and Pütz (2008), for an optimal AD and methane production, FOS/TAC value should oscilate between 0.3 and 0.4. A FOS/TAC value under 0.3 indicate that the biomass input is too low, values above 0.4 mean that the system is heavily loaded, and the biomass input should be lowered. For B0, the optimal FOS/TAC value of 0.3 - 0.4 was not often achieved. When the feeding steps began to be done every two days, the system was heavily loaded. In comparison, the use of a biofilm could guarantee much stable FOS/TAC values between 0.2 and 0.4 when the feeding was done more frequently. A frequent feeding should result in a FOS accumulation, this was not the case due to the biofilm (BF).

According to Moerschner (2015), if TAC oscilate between 8.5 and 13 g/L, the buffer capacity of the system is suitable, a low TAC indicates a higher amount of organic acids in the system. This can be confirmed in **figure 5.5,** related to B0, whereas FOS/TAC was mostly out of the recommended limits. B0 showed more TAC measurements below the recommended limit, especially when feeding took place more frequently. This means that the buffer capacity of the system without biofilm was low. An adequate TAC indicates a good balance between carbon and $NH_4^+/ NH_3$ in bioreactor. BF showed mostly values between 8.5 and 13 g/L. The use of biofilm could guarantee a higher buffer capacity of the system, and thus a more aqurate FOS/TAC. These findings suggests, that the microorganisms adhered on the biofilm could degrade the biomass so that the VFAs could be succesfully converted into biogas and methane.

## 5.7 Total dissolved solids, conductivity and organic matter removal

**Figure 5.8** and **5.9** show the results of the conductivity and TDS measurements.

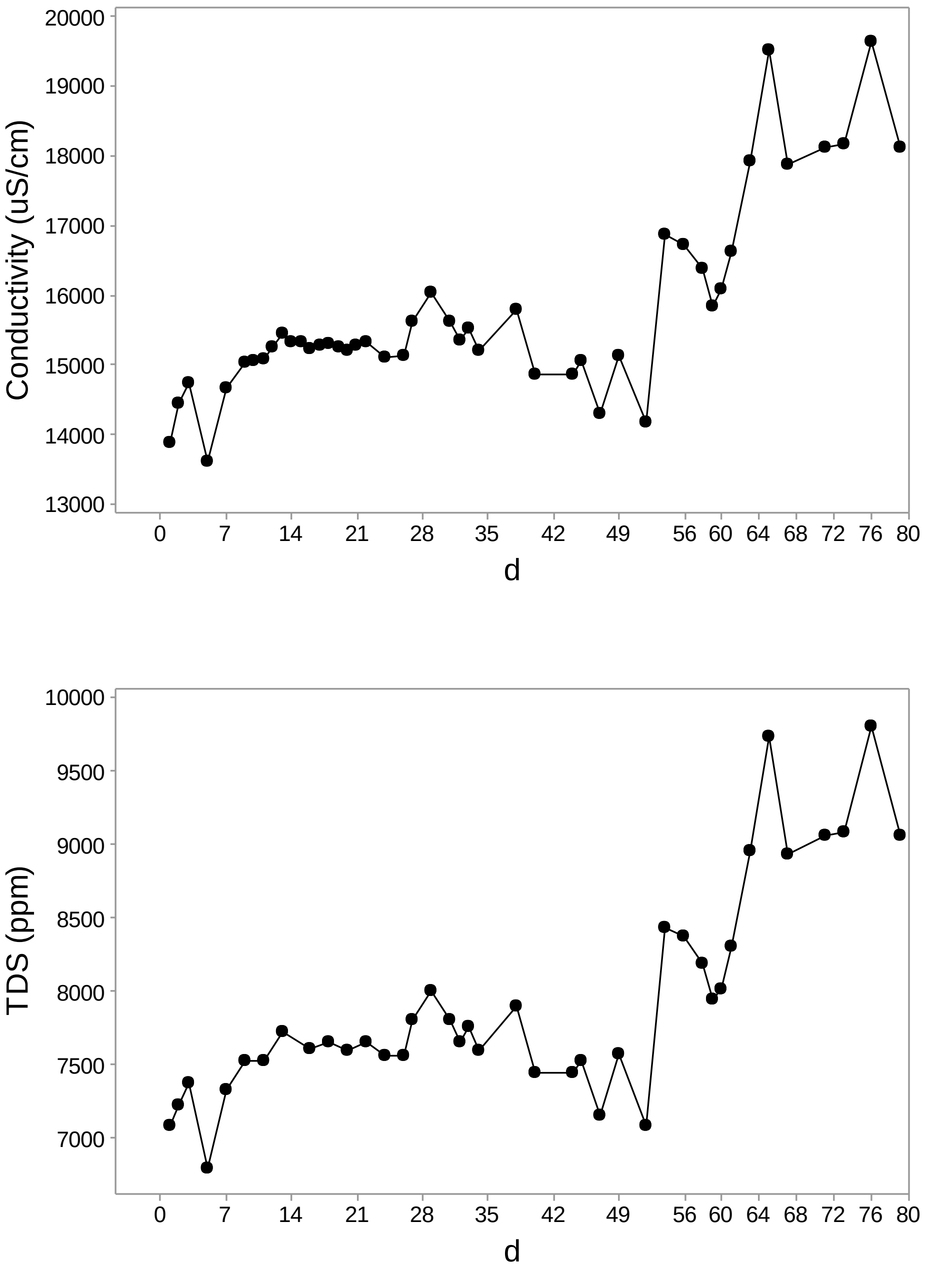


**FIGURE 5.8** Conductivity in μS/cm and total dissolved solids (TDS) in ppm, for bioreactor without biofilm B0

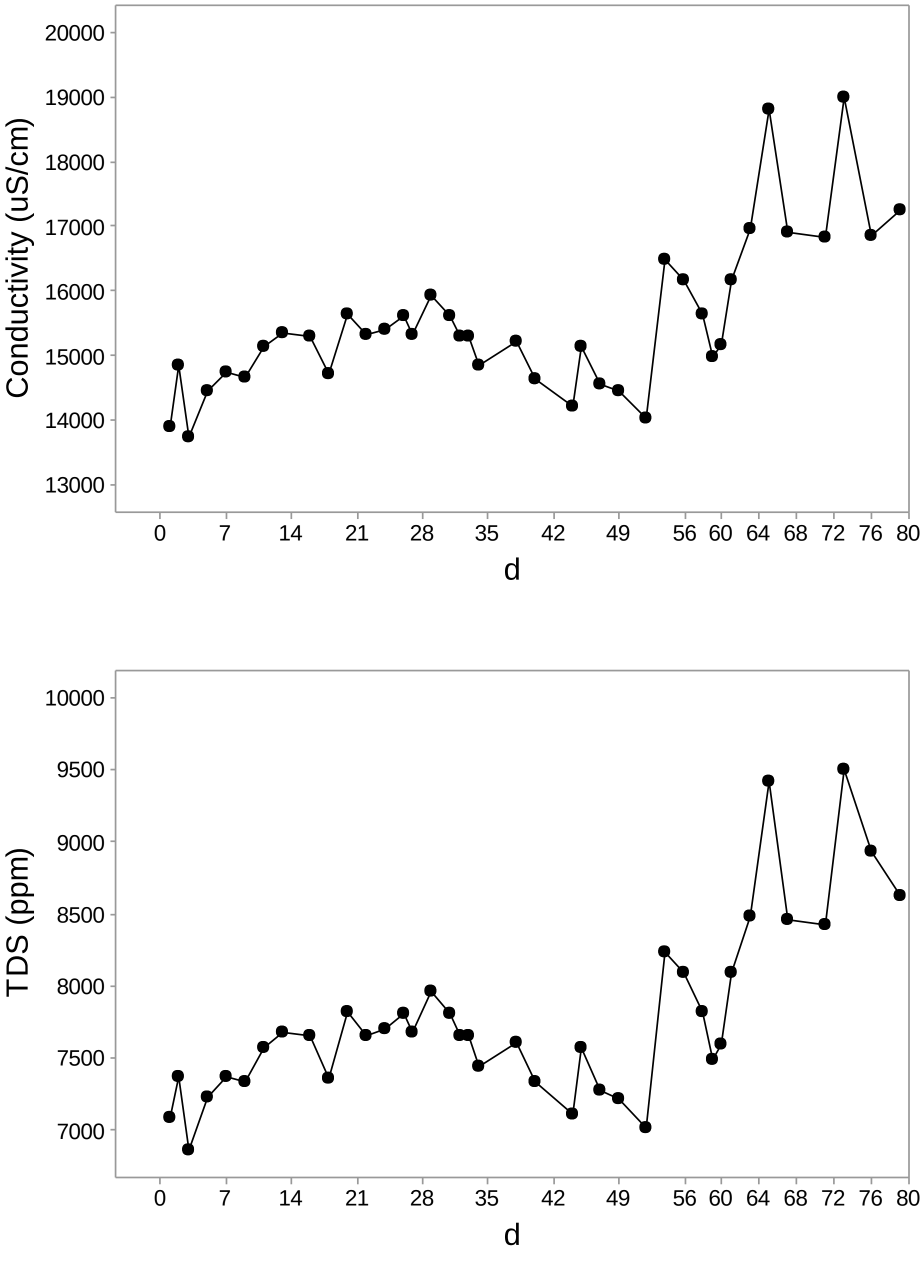


**FIGURE 5.9** Conductivity in µS/cm and total dissolved solids (TDS) in ppm, for bioreactor without biofilm BF

For both bioreactors, conductivity and TDS content increased when increasing the biomass input. Salts indicate indirectly the amount of ammonium ($NH_4^+$-N). According to Moerschner (2015) the conductivity increase with the increase of salt content, such as ammonium content. It can be said that 10 mS conductivity corresponds to 1 g/L $NH_4^+$-N. Both, conductivity and TDS were much lower in BF. This fact suggests that the microorganisms adhered on the biofilm could hinder the accumulation of salts, whereas the dissolved solids could be better degraded.

The amount of TS and VS decreased after AD. For B0, TS and VS diminished correspondingly 44 % and 51 %. BF resulted in a TS decrease of 50 % and VS decrease of 60 %. Martí-Herrero *et al.* (2014) achieved 35 % TS and 36 % VS removals when digesting cattle manure without PET biofilm. When using PET biofilm TS and VS removal increased to 57 % and 60 %, correspondingly.

Contrary to the TDS content, organic matter content, in terms of COD decreased after the 80 days of experiments. COD at the beginning of the experiments was 38.8 g/L in BF and B0. By the end of the assays COD removal was 4 % in B0 and 21 % in BF. CODs at the end of the experiments were 37.55 and 30.65 g/L, correspondingly. It can be inferred, that the microorganisms adhered to the biofilm could also enhance the organic matter removal. Gong *et al.* (2011) reported an increase around 40 % in the biogas and methane production, as well as TS and VS removals, when using an activated carbon fiber, digesting sludge and cattle manure. COD removal increased up to 80 %. When using polyvinyl alcohol fiber and glass fiber, an inhibition in the biogas and methane production took place, and no significant VS, TS and COD removals could be achieved. Liu *et al.* (2017a) compared different fiber sources during AD. When using polypropylene fiber, COD removal was 40 % higher, TS removal 57 % and VS removal 30 %. Both findings from Gong *et al.* (2011) and Liu *et al.* (2017a) showed much higher values than reported in these experiments using the polyethylene terephthalate PET bottles as biofilm carriers. Nevertheless, the low cost related to the PET bottles in addition to the contamination risks avoided when reusing PET, makes this alternative competitive for AD enhancement.

## 5.8 Conclusions

This paper shows the advantages of using biofilm bioreactors for Mezcal vinasses AD. A better performance on the biogas and methane yield could be achieved and the organic matter removal could be enhanced. In this study, when using biofilms, biogas and methane production increased 40 % and 60 %, correspondingly. Besides, the accumulation of organic acids was inhibited and the buffer capacity of the system increased. These results suggests more efficient conversion of organic acids into biogas and methane, when using PET bottles as a biofilm carrier. PET bottles were first sanded in order to achieve a porous surface, in which a higher amount of microorganisms could be adhered. The results of the optical analysis showed that sanding the PET bottles did not led to a higher amount of adhered microorganisms. Results regarding methane production and FOS/TAC values indicate that feeding BF could had taken place more frequently than 7 days, increasing the biogas and methane production. COD removal using biofilm was 20 %. This value is certainly low in comparison to literature, but if considering that PET bottles are low-cost biofilm carriers, and their reutilization can avoid environmental problems, PET can be consider as a good alternative to other expensive materials. The amount of undesirable hydrogen sulphide was 20 % reduced in the biogas produced by the biofilm bioreactor. Through this work, new alternatives are opened for a deeper analysis on the dynamics of the biofilm formation, as well as the reduction of $H_2S$ in biogas through biofilm bioreactors.

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# 6 Performance of a microbial fuel cell operated with vinasses using different chemical oxygen demand concentrations

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## 6.1 Abstract

Vinasses are one of the main wastes generated from the Mezcal industry in Mexico. Due to their high organic matter content and low pH, vinasses have negative environmental impacts if discharged without any treatment. An alternative treatment of vinasses is their use in microbial fuel cells (MFC) for organic matter removal and electricity production. In this paper, the performance of a MFC using vinasses is analyzed. Different organic matter concentrations in terms of chemical oxygen demand (COD) were tested and compared regarding power density, internal resistance, and voltage production. The results demonstrated that the highest COD tested resulted in a poor MFC performance. When using vinasses in MFC for 10 days, COD of 6 g/L generated 80.64 W/m$^3$ and when using vinasses with a COD of 17.1 g/L, the power density dropped to 5.13 W/m$^3$. Further tests of COD performance in MFC were made with 10.6 and 6.7 g/L for 68 days. It was demonstrated that a COD of 10.6 g/L only produced 0.61 V, while COD of 6.7 g/L reached 0.81 V. Regarding the organic matter removal, the highest COD removed (92 %) was obtained when using vinasses with a COD of 10.6 g/L. By operating the cell with 6 g/L, COD removal was 83 % and with 17.1 g/L, 49 %. To achieve a better MFC performance, the organic matter content in the electrolyte should not exceed 6 g/L so that the MFC does not achieve a saturated state that hinders the oxidation mechanisms and thus electricity production and COD removal.

**Keywords**: *Agave*, chemical oxygen demand, organic matter removal, electricity production

## 6.2 Introduction

### 6.2.1 Vinasses from Mezcal and Tequila production

In Mexico, *Agave* is a natural resource of great economical, sociological, and agro-ecological importance. More than 200 different *Agave* species, 75 % of the total, are found in the country. *Agave* is used to produce two important commercial Mexican products; Tequila and Mezcal. About 8 million liters Mezcal are produced yearly in Mexico, from which 90 million liters of vinasses remain as organic waste from this industry. Depending on the feedstock and distillation parameters, Mezcal vinasses consist of organic substances such as acetic and lactic acids, glycerol, polyphenols, phenols, melanoidins, and inorganic substances such as sulphates or phosphates. The general characteristics for vinasses are low pH (3-5) and high organic matter content (biological oxygen demand of 35 - 50 g/L and chemical oxygen demand of 70 – 150 g/L). Due to their acidic composition, high concentration of mineral salts, and high recalcitrant organic matter, vinasses can contaminate the environment and their discharge into soils and water can have a negative impact on the ecosystem. When used on fertile soils, vinasses may affect the soil structure, nutrient uptake and crop yield (López-López *et al.* 2010, Robles-González *et al.* 2012, Moraes *et al.* 2014). For this reason, biological and physicochemical treatments of vinasses have been researched recently. The principal target is to reduce the biodegradable organic matter, convert major toxic organic substances to compounds that can be easily biodegraded, and reach the permissible levels of contaminants in wastewater discharges into national waters according to the NOM-001-SEMARNAT-1996 (SEMARNAT 1996).

### 6.2.2 Microbial fuel cells

MFCs are electro-chemical reactors, which use microorganisms from an electrolyte solution as catalysts to generate current by the oxidization of organic matter such as acetate, glucose and volatile fatty acids, or inorganic matter such as sulphides. This system generates clean electricity directly from chemical energy in one step, treating wastewater simultaneously (Higgins *et al.* 2013, Xiao *et al.* 2014, Baicha *et al.* 2016). The production of electricity is not the only objective of MFC operation. Also the removal of pollutants such as nitrites, sulphides, or sulphates, and especially of organic matter and thus wastewater treatment are targets of the MFC. During the recent years, the interest in lab-scale and large-scale applications has increased tremendously, as well as the power output generated from MFCs. The efficiency and performance of MFCs, including the power density and coulombic efficiency, depend on the chemical and biological composition of the substrate used in the cell (Pant *et al.* 2010).

MFC performance has been tested with different substrates as electrolyte solution, such as swine wastewater, domestic wastewater, distillery wastewater, alkaline substrates, glucose, acetate and microalgae, among others (Martin *et al.* 2010, Mohanakrishna *et al.* 2010, Liao *et al.* 2014, Baicha *et al.* 2016, Kim *et al.* 2016).

### 6.2.3 Vinasses in microbial fuel cells

The results found in the literature in regards to power density, coulombic efficiency, organic matter, and pollutant removal in MFCs differ according to the substrates utilized, cell design, operating conditions, and electrode materials. Vinasses from *Agave* have a high content of organic matter as well as sulphides or phosphates, which could be used as electron donor in

MFCs. Few research has been done in regards to vinasses in MFCs, even though this substrate is very promising. MFC technology is a promising alternative to treat the recalcitrant compounds of vinasses before they are being discharged into soils and water, in addition to the potential for electricity production. Few papers report results regarding the electricity production from MFCs using substrates with high organic matter in terms of chemical oxygen demand (COD). The aim of this study was to analyze different vinasses COD concentrations tested in MFCs fuel cells for the purpose of electricity generation and organic matter degradation.

## 6.3 Materials and methods

### 6.3.1 Microbial fuel cell configuration

MFC-900 consisted of two plexiglas chambers separated by a Nafion 117 Proton Exchange Membrane with a surface of 7 $cm^2$. The total volume of the MFC was 900 ml, with 450 ml in each chamber. Temperature was kept at 34 °C using a water bath. The cathodic chamber was aerated by means of an Elite 801. Anode was made of graphite from supplier, Rooe Group, with a volume of 4.8 $cm^3$. The cathode consisted of a AISI 304 stainless steel plate with the dimensions of 4 x 2 x 0.1 cm. Electrodes were connected with a stainless steel wire with a diameter of 0.7 mm and the distance between electrodes was 15 cm. External resistance was 5000 Ω. In the anodic chamber, wastewater was mixed with different concentrations of vinasses from *Agave salmiana* Mezcal production, while in the cathodic chamber deionized water was used.

Vinasses samples were stored at 4 °C until they were used. The pH and the conductivity of concentrated vinasses were 4.22 and 7.35 mS/cm. The wastewater had a pH of 8 and a conductivity of 2.25 mS/cm. The pH of the vinasses diluted with wastewater varied from 7.5 to 7.9. Every time a new concentration of a specific amount of vinasses diluted with wastewater was tested, new wastewater was used and the biofilm was kept in deionized water injected with nitrogen in order to achieve the absence of oxygen. COD of the wastewater ranged between 1.1 and 1.5 g/L. COD of concentrated vinasses was 140 g/L.

### 6.3.2 Start-up and operation

As an inoculum, sludge was provided by a treatment plant, in which the wastewater produced was being aerobically treated for removal of organic matter and recalcitrant compounds. Sludge was used in the MFC-900 without vinasses for 30 days at 34 °C for inoculation. To test the COD effect on the electricity production, different vinasses concentrations were used as electrolyte solution in the MFC by diluting them with wastewater. The different vinasses concentrations were chosen according to values in the literature regarding the use of other substrates used in MFCs with high CODs tested. No values for *Agave* vinasses were found for comparison. Wastewater was used to facilitate the formation of the biofilm, to buffer the low pH of vinasses, and to provide a varied microbial community for organic matter degradation. Three experiments were carried out. Due to lack of capacity, no blank test with only wastewater was performed. First, vinasses were diluted resulting in CODs of 1.2, 4.1, 6 and 17.1 g/L correspondingly. These tests were run in the MFC-900 until a constant voltage production was achieved or until the voltage decreased. The second experiment consisted of testing a high COD (10.6 g/L) in the MFC-900 over a longer period. This was done to check if the microbial community would begin to degrade the vinasses once hydrolyzed substrate was available for the exoelectrogenic microorganisms and to test if a thicker biofilm could be formed using a longer time period. The MFC was run out over two months. In the third experiment lower COD (6.7 g/L) was tested over two months in MFC-900 in order to corroborate if a lower COD would result in a better MFC performance.

### 6.3.3 Measurement and calculations

Voltage data was recorded continuously by the data acquisition system, Labview 2011 from National Instruments, and measured daily with a multimeter Steren Mult-010. Power density P was calculated according to Eq. 6.1, and current I according to Eq. 6.2, where R means resistance (Ω), V means voltage and Vanode means the volume of the anodic chamber.

$$P = \frac{V\ x\ I}{V\ anode} \quad \text{(Eq. 6.1)}$$

$$I = \frac{V}{R} \quad \text{(Eq. 6.2)}$$

Polarization curves were calculated only in the first experiment, when a constant voltage was shown for more than 48 hours. In the second and third experiment no polarization curves were calculated, in order not to interrupt voltage production. Polarization curves were calculated with a potentiostat BASi Epsilon-EC, Bioanalytical Systems, Inc. Internal resistance (Ω) was calculated according to polarization curves data. COD was measured at the start and the end of each experiment according to the German Standard Method DIN 38409-41:1980-12 (DIN 1980). Three samples were analyzed for COD measurement. Prior to these measurements, a COD calibration curve was done with controlled samples using wavelength of 620 nm. COD removal was calculated as the ratio between the removed COD and initial COD. The pH and the conductivity were measured with an Orion 4 Star pH-meter from Thermo Scientific. Power density and internal resistance were calculated using the different CODs by means of polarization tests, measuring anode and cathode potentials against an Ag/AgCl in NaCl reference electrode.

## 6.4 Results and discussion

### 6.4.1 Effect of chemical oxygen demand on power output and internal resistance

**Figure 6.1** shows the polarization curves of the first experiment, where vinasses at CODs of a) 1.2, b) 4.1, c) 6 and d) 17.1 g/L were tested.

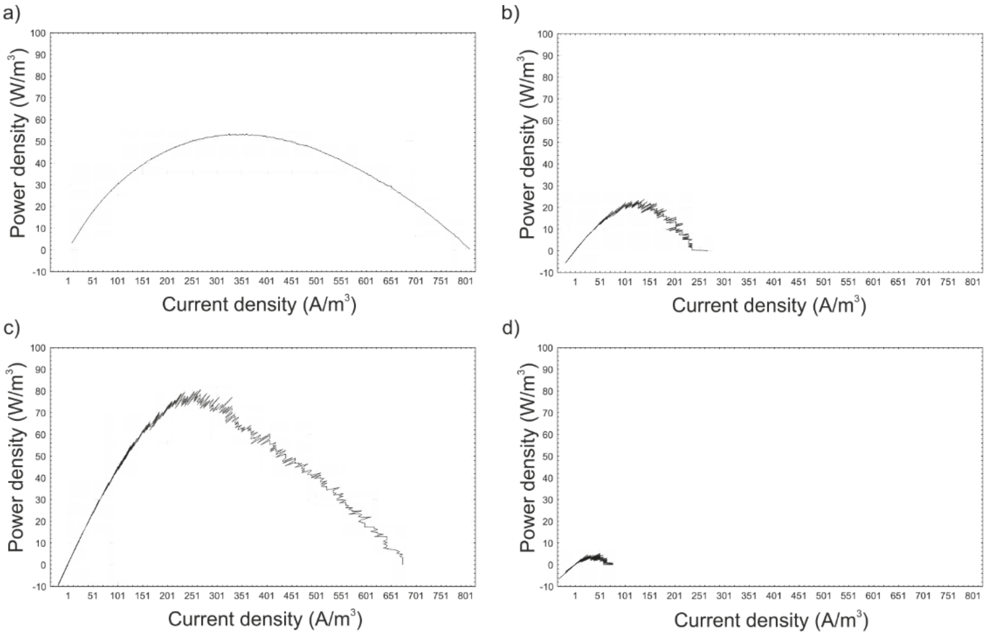

**FIGURE 6.1** Polarization curves using different organic matter concentrations in electrolyte composed of vinasses and wastewater: a) Chemical oxygen demand of 1.2 g/L, b) Chemical oxygen demand of 4.1 g/L, c) Chemical oxygen demand of 6 g/L and d) Chemical oxygen demand of 17.1 g/L

The highest power density was achieved when using the COD concentration of 6 g/L resulting in 80.64 W/m$^3$ (c). When COD increased to 17.1 g/L, the power density was the lowest recorded with 5.138 W/m$^3$ (d). COD of 1.2 g/L and 4.1 g/L achieved 53.39 (a) and 23.78 W/m$^3$ (b) correspondingly. The fact that the highest COD concentration showed the lowest power output occurred because the anodic reactions in the anodic chamber of the MFC depend on the substrate characteristics and carbon availability. If the substrate has colloidal particles, as the case for vinasses, these particles can act as limiting factors and increase the internal resistance, so that power density is diminished. Power density rises when the organic loading rate increases up to a certain concentration. When the organic load exceeds the specific concentration, power density decreases, although organic load removal increases. High CODs could produce a saturated state in the MFC generating a power density decrease (Nam *et al.* 2010, Liu *et al.* 2011, Oliveira *et al.* 2013).

Similar results were obtained by Belafi-Bako *et al.* (2014), who tested the power output of an MFC inoculated with anaerobic sludge from an AD (anaerobic digestion) plant using wastewater from a sugar beet factory as substrate. The highest power density of 8652 mW/m$^2$

was achieved with COD of 7.1 g/L and the lowest power density of 3380 mW/m$^2$ with a higher COD of 19.800 g/L. COD of 4.1 g/L yielded 4500 mW/m$^2$. Nam *et al.* (2010) found the highest power density of 2981 mW/m$^2$ by testing fermented wastewater produced from hydrogen fermentation of coffee processing wastewater with an organic loading rate of 3.8 g/L/d. However, an increase of the organic loading rate up to 4.8 g/L/d generated less power; 2959 mW/m$^3$. The influent characteristics and consortium's metabolism affected the power generation. Reddy *et al.* (2010) tested four different organic loading rates (OLR) of an anaerobic mixed consortia of UASB treating wastewater with CODs of 0.195, 0.458, 0.911 and 1.589 g/Ld, where power generation increased with increasing OLR but only up to 0.911 g/Ld generating 76.17 mW/m$^2$. With the highest OLR tested, 1.589 g/Ld, the power generation decreased to 49.86 mW/m$^2$. Martin *et al.* (2010) tested different concentrations of glucose and acetate. Using glucose as a substrate, a power output of 8.2W/m$^3$ was achieved using an organic load with a COD of 3.72 g/Ld. With the further increase of organic load of 7.44 g/Ld, the power output decreased to 6.6 W/m$^3$. Similar results occurred when testing acetate in the MFC. OLR of 4 g/L/d produced 53.3W/m$^3$ and 8 g/L/d produced only 50.6 W/m$^3$. When increasing the glucose load, substrate availability for the methanogenic population also increased. Thus, at a higher load, 34 % of substrate was used to produce $CH_4$ and 2 % to produce electricity.

**Figure 6.2** shows the results of the polarization tests regarding internal resistance and maximal power density of the first experiments, in which vinasses with COD of 1.2, 4.1, 6 and 17.1 g/L were tested for power generation.

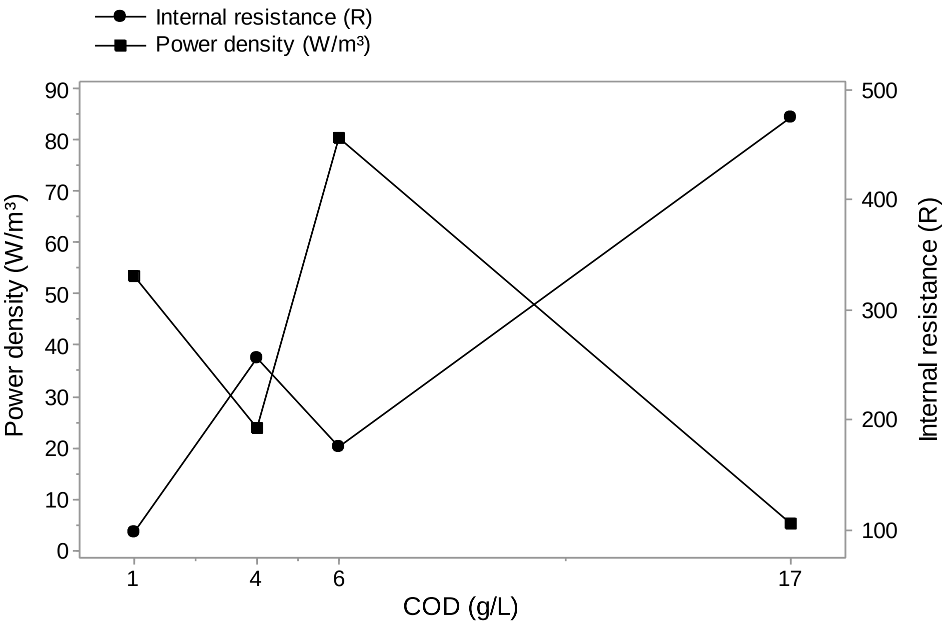

**FIGURE 6.2** Power density in W/m3 and internal resistance in ohms (Ω) of the microbial fuel cell using different chemical oxygen demand (COD) concentrations of vinasses

The internal resistance was correlated with the organic matter availability. With the lowest COD tested, 1.2 g/L, the internal resistance was the lowest achieved, 97.10 Ω. The highest internal resistance of 474.58 Ω was achieved with the highest COD of 17.1 g/L. The internal resistance increases if the substrate has colloidal particles, as is the case for the vinasses (Nam *et al.* 2010). Contrary to what was expected, Martin *et al.* (2010) found out that using glucose in a MFC, the lowest internal resistance was achieved using the highest glucose load, suggesting that a high volatile fatty acid concentration resulted not only in the increase of ionic strength, but also the catalytic activity and density of anodophilic microorganisms increased, which resulted in the internal resistance drop.

### 6.4.2 Effect of chemical oxygen demand on voltage output

**Figure 6.3** shows the voltage measured with multimeter during the first experiment, where CODs of 1.2, 4.1, 6 and 17.1 g/L were tested. The highest voltage was produced when using vinasses with COD of 4.1 g/L and the lowest when using vinasses with a COD of 17.1 g/L.

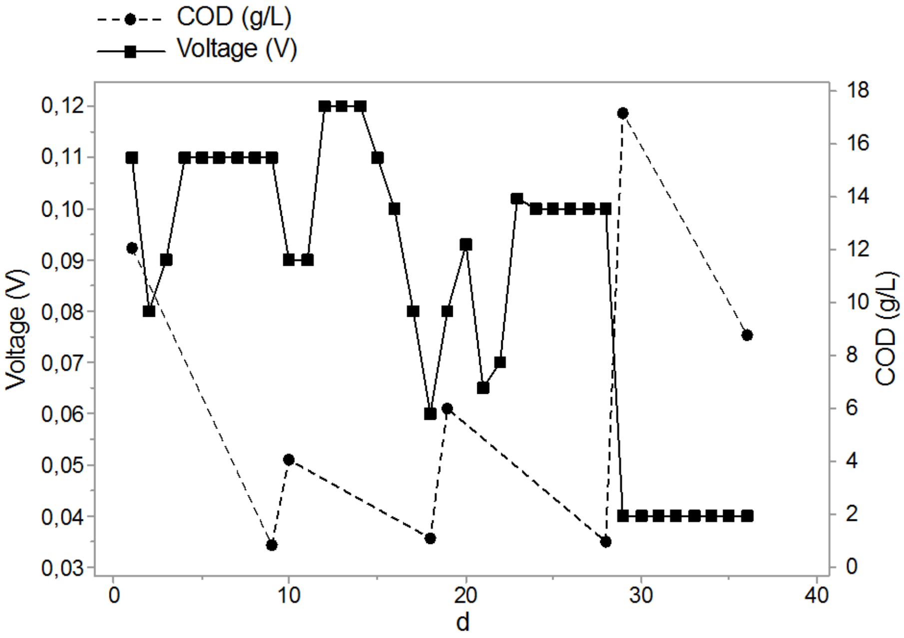

**FIGURE 6.3** Voltage output using different chemical oxygen demand concentrations (COD) in the electrolyte composed of vinasses and wastewater

As already described by the effect of COD on power density and in internal resistance, if COD exceeds a specific concentration, the electricity production could be hindered through the saturated state of the substrate. Vinasses from alcohol production, distillery slops, contain a high percentage of organic matter. The contained colloidal particles increase the vinasses density, depending on the distillation parameters, up to 1.72 g/cm$^3$. The potential of electricity production in a MFC is given by the bacterial metabolic activity or in other words by the reduction - oxidation reaction generating electrons and protons, as well as by the electron acceptor conditions. This is influenced by the anode potential and substrate used as electrolyte. The electrolyte solution has a significant amount of colloidal particles, the electron and proton transfer could be hindered.

Regarding the last two experiments, voltage production tested over a longer period (68 days) was compared using two different CODs; 10.6 and 6.7 g/L. Results are shown in **figure 6.4** and f**igure 6.5** respectively.

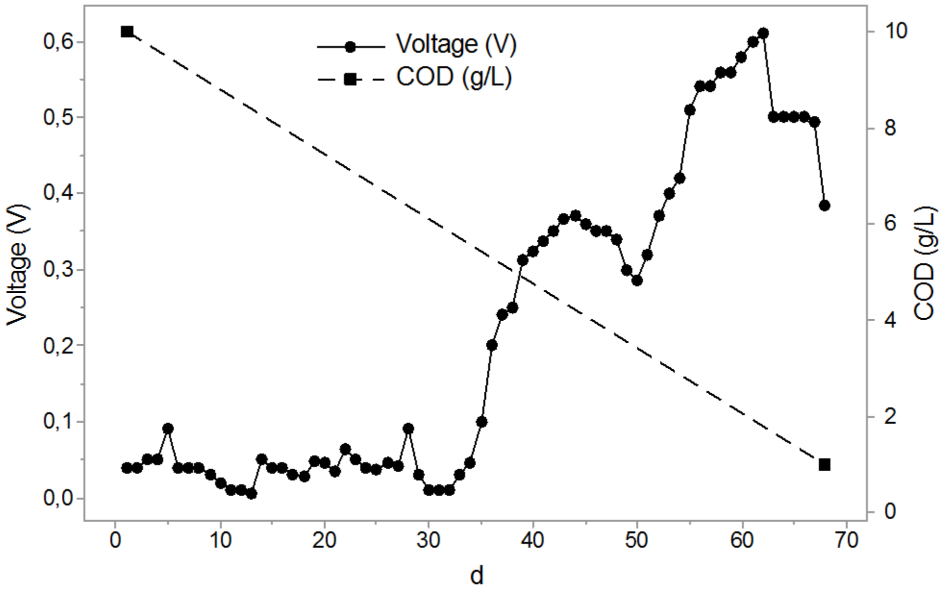

**FIGURE 6.4** Voltage output of batch microbial fuel cell MFC-900 using an electrolyte chemical oxygen demand (COD; vinasses and wastewater) of 10.6 g/L

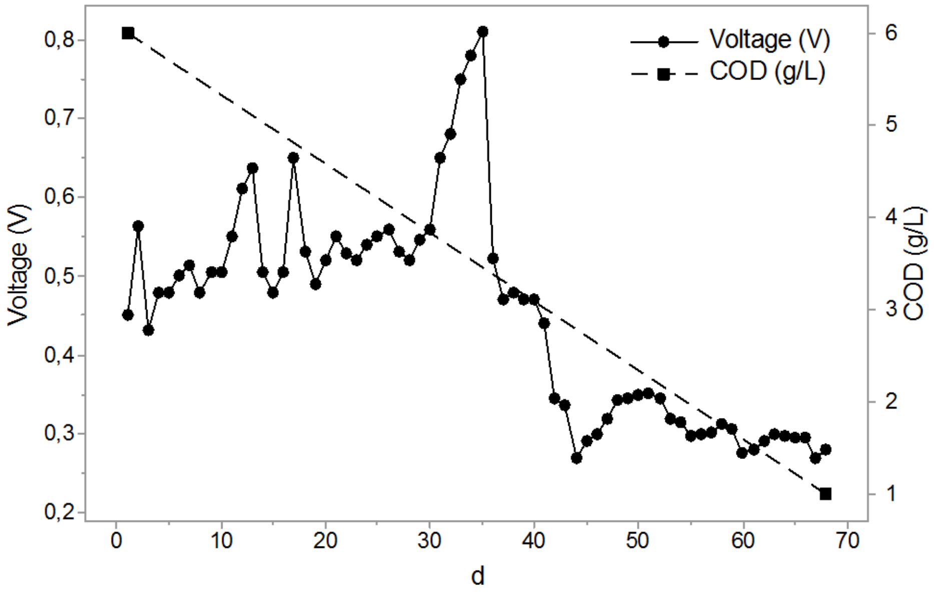

**FIGURE 6.5** Voltage output of batch microbial fuel cell MFC-900 using an electrolyte chemical oxygen demand (COD; vinasses and wastewater) of 6.7 g/L

Regarding the test using vinasses with a COD of 10.6 g/L, during the first 34 days, a low voltage, between 0.006 and 0.1 V was recorded. Between day 35 and 44 voltage rose to 0.37 V and then decreased over the next 6 days. From day 51 to 62 voltage increased up to the highest value of 0.61 V. The last six days the voltage dropped down to 0.38 V, when the experiment was suspended. Voltage production of MFC using vinasses with a COD of 6 g/L showed higher voltage production. The first 31 days voltage varied between 0.42 and 0.65 V. On the day 35, voltage reached the highest value of 0.81 V. Between day 36 and 42 voltage dropped to 0.35 V and oscillated around 0.27 and 0.35 V until the experiment was suspended on day 68. With a higher COD of 10.6 g/L, 0.61 V were obtained after 62 days and with a lower COD of 6.7 g/L, 0.62 V were obtained only after 13 days. Because of the high COD, the reactants availability was not very high during the initial phase, until the hydrolysis of vinasses took place (Nam *et al.* 2010). When using vinasses with a higher COD concentration, more organic matter needs to be degraded before a significant electron and proton exchange starts and higher voltages can be produced. Molecules need to be first broken into smaller ones in order to be more suitable as MFC fuels.

With COD of 6.7 g/L the voltage increased further to 0.81 V after 35 days. Voltage production with a COD of 10.6 g/L did not increase higher than 0.61 V. According to Vogl *et al.* (2016) substrates, which are easy to degrade lead to higher power densities in comparison to substrates with a high amount of organic pollutants. A portion of the substrate is used for biomass synthesis. Complex substrates, as in the case of vinasses with high COD, lead to high internal resistance and low power output (Nam *et al.* 2010). The anodic reactions thus increase electricity production when carbon sources are available with a low COD. This is not the case of wastewater with a high vinasses concentration, because vinasses are complex substrates that contain high concentrations of dissolved solids (such as reducing sugars), nonvolatile compounds, and high concentrations of mineral salts.

### 6.4.3 Chemical oxygen demand removal

**Table 6.1** shows the results of COD degradation of all the experiments performed. From a COD of 1.2 to 10.6 g/L, the COD removal rate increased to 92 %. Nevertheless, at the highest COD of 17.1 g/L, COD removal diminished by 49 %.

**TABLE 6.1** Percentage of removed chemical oxygen demand (COD) in the electrolyte solution before and after microbial fuel cell operation

| *Initial COD (g/L)* | *Final COD (g/L)* | *COD removal (%)* |
|---|---|---|
| 1.2 | 8.6 | 29 |
| 4.1 | 1.1 | 73 |
| 6.0 | 9.9 | 83 |
| 6.7 (68 days) | 13.7 | 80 |
| 10.6 (68 days) | 0.8 | 92 |
| 17.1 | 8.7 | 49 |

The most efficient COD removal was shown at a COD of 10.6 g/L and the lowest at a COD of 1.2 g/L. The increase in substrate removal at high load rates (in this study up to 10.6 g/L) could have occurred because of the direct anodic oxidation mechanism in the anodic chamber. There was an increase in microorganism consumption of organic matter. If the organic load in the substrate is higher, in this case 17.1 g/L, the saturated state of the electrolyte hindered the oxidation mechanism.

Similar results were obtained by Belafi-Bako *et al.* (2014), when higher CODs were tested, COD removal diminished considerably. For example, when using a COD of 19.8 g/L, only 25 % COD was removed. When using 5 g/L, COD removal was 61 %.

## 6.5 Conclusions

The analysis of testing different vinasses concentrations in MFC demonstrate that an increase in COD from 1.2 up to 10.6 g/L results in an efficient MFC performance in terms of electricity production and COD removal achieving values higher than 80 W/m$^3$ produced with a COD of 6 g/L and 92 % COD removed from a COD of 10.6 g/L. The anodic reactions were able to occur more efficiently when carbon sources within the electrolyte were better available. However, when using a higher COD of 17.1 g/L, power output dropped to 5 W/m$^3$ and COD removal reduced to 49 %. Internal resistance increased with the organic load due to a higher percentage of colloidal particles in the electrolyte. Over a longer operation period of MFC operation, substrate with lower COD (6.7 g/L) produced 0.6 V only after 13 days, while substrate with a higher COD (10.6 g/L) needed 62 days to reach the 0.6 V. This happened due to the high organic content of vinasses, which needed to be hydrolyzed before an important amount of electrons can be released for electron and proton exchange and thus voltage production. On the other hand, the voltage from the MFC operating with 6.7 g/L, continued increasing up to 0.8 V after 35 days, whereas the MFC operating with 10.6 g/L did not produced more than 0.6 V. This confirmed the results of the first experiments, where a higher COD showed a lower MFC performance, due to the saturated state of the electrolyte solution and lack of availability of carbon sources in the anodic chamber. When COD in the electrolyte solution increased up to 10.6 g/L, substrate removal occurred because of the direct anodic oxidation mechanism in the anodic chamber. Due to the high amount of colloidal particles in vinasses, a higher COD in the electrolyte solution inhibits the oxidation reactions and thus substrate degradation.

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# 7 Simultaneous electricity and biogas generation of vinasses and cattle manure

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## 7.1 Abstract

A new design for the simultaneous generation of electricity through microbial fuel cells (MFCs) and biogas production through anaerobic digestion (AD) was drafted. A computational fluid dynamics (CFD) simulation was carried out in order to analyze the best inlet diameter and flow rate, at which no sediments are built in the reactor. Voltage production and biogas generation were daily recorded at different Mezcal vinasses and cattle manure ratios, as well as chemical oxygen demands (CODs). A control test with only cattle manure was carried out. When comparting the control test with the concentrations with vinasses, vinasses resulted in inhibition of voltage output. On the contrary, if no vinasses were used, no biogas production took place, revealing that the inoculum did not have activity itself. The concentration with the lowest vinasses content and COD showed the poorest AD efficiency and lowest voltage output. Power density increased with increased organic matter content (from 8.95 to 14.85 g/L), until a certain limit. The concentration showing a COD of 17.80 g/L resulted in a voltage drop and showed the lowest COD removal rate. Contrary to the effect of COD for the voltage production, biogas yield and methane content increased with increased organic matter content. These results show that the combination of these technologies is not suitable for the simultaneous voltage production and biogas generation. If a high COD is used, a low power density will be generated. When diluting the substrate to achieve higher power outputs, a low biogas production will be given.

**Keywords**: anaerobic digestion, chemical oxygen demand, microbial fuel cells, FOS/TAC, voltage

## 7.2 Introduction

There is a broad variety of technologies either on use or on development for the utilization of organic wastes to generate bioenergy. Conversion routes for bioenergy production are mostly thermal and biochemical technologies. Anaerobic digesters and microbial fuel cells (MFC) are both suited technologies for biomass treatment and bioenergy production. AD is one of the most common technologies for bioenergy production at industrial scales, while MFCs has not yet found significant practical applications to bioenergy production.

Through the MFC technology, electrogenic bacteria is used to oxidize a great amount of substrates such as glucose, acetate, organic acids, or also inorganic substances like sulphates or phosphates. MFC transforms chemical energy contained in the substrate, into electricity by means of reduction and oxidation (REDOX) reactions. Therefore, through bacterial respiration, the reduction and oxidation of organic molecules take place (Higgins *et al.* 2013). Through AD, almost all organic wastes can be anaerobically degraded for biogas and methane production. The nutrient-rich digestate remaining after AD is normally used as fertilizer. Nevertheless it has been demonstrated, that an important amount of gases, such as methane $NH_3$ and $N_20$ remain in the digestate. These gases are released to the environment when using digestate as fertilizer. This is still an important and challenging topic, since the use of biomass should be climate neutral (Lukehurst *et al.* 2010, Menardo *et al.* 2011, Rico *et al.* 2011). If the digestate is used before released to the environment, gas emissions could be diminished.

Not only the efficient bioenergy generation but also the removal of organic matter is pursued in laboratory and industrial scales. A plenty amount of substrates, such as vinasses, consist of a high concentration of mineral salts and high recalcitrant organic matter in terms of chemical and biological oxygen demand (COD and BOD). Besides bioenergy generation, the target is to reduce the degradable organic matter, convert major toxic organic substances to compounds that can be easily biodegraded, and reach the permissible levels of contaminants in waste discharges.

Until now, the power generated by MFC is low for large-scale wastewater treatment. The only MFC design used in a large scale application produces power from sediment by embedding an anode in sediment, and connecting it to the cathode, which is placed in the overlying aerobic seawater, through an electrical circuit (Pant *et al.* 2009). The development of this technology is challenging, especially because of the cost of membranes and electrodes, potential of substrate-biofouling, and high internal resistance that limits the power generation. Some improvements on MFC design point out, that the use of open air bio-cathodes and replacement of platinized with non-platinized cathodes, as well as the use of stainless steel and nickel or manganese dioxide cathodes are alternatives to be used (Pant *et al.* 2009). The research in regards to new alternative substrates for the efficient use of MFCs at large scales is necessary, especially regarding substrates with high organic loads which are produced in high amounts, like vinasses.

The concept of a biorefinery comprehends an integrative overall concept, where the biomass will be used as far as possible in the sustainable generation of energy or materials (FNR 2012). Coupling AD and MFC could be one step of a biorefinery concept, where the digestate produced after AD is used in MFCs. Few research regarding coupling AD and MFC technologies has been recently carried out. Typically, AD has been used for COD reduction, especially when processing high strength wastewaters. MFC was proposed for AD effluents treatment and for enhancement of organic matter removal. In the practice, total ammonia nitrogen hinders COD removal during AD. If AD digestate could be used in a MFC, ammonia nitrogen could be removed (Higgins *et al.* 2013, Kim *et al.* 2015). Also the diminution of toxic gases released to the environment could be goaled, when using digestate in MFC. No studies have described the approach of AD and MFC technologies operated with vinasses, for the simultaneous biogas and electricity production. This substrate is, due to the low pH-value and high organic content, very promising not only to produce electricity, but also to treat the vinasses before they are being discharged into soils and water. On this account, aim of this work is to test a new design developed as a small biorefinery, where the digestate generated from vinasses AD with cattle manure as inoculum, can be used as input material for MFC operation. The configurations of open air cathode and membrane-less MFCs will be studied to make this technology a low-cost alternative for green energy production at large scale.

## 7.3 Materials and methods

### 7.3.1 Reactor design

The reactor consisted of three chambers placed side by side. The first chamber (under anaerobic conditions) was a bioreactor, in which 400 $cm^3$ vinasses and cattle manure were digested. The second chamber, anodic chamber, was designed also to guarantee anaerobic conditions, and had a fluid volumetric capacity of 400 $cm^3$. The last chamber, cathodic chamber, was designed so that one side of the cathode had direct air contact, to guarantee aerobic conditions. Thus, the open air cathode would make the costs of MFC construction and operation much cheaper. The volumetric capacity of the cathodic chamber was 700 $cm^3$. The total fluid volume contained in the reactor was 1500 $cm^3$. Anode and cathode were connected through an external resistance of 1000 Ω and a stainless steel wire of 0.7 mm diameter. The distance between anode and cathode was approximately 7 cm. Anode and cathode were made of activated carbon felt, with volumes of 115 $cm^3$ and 255 $cm^3$, correspondingly. Anode and cathode were inoculated with cattle manure, one month before experiments started-up. The reactor was kept under mesophilic temperatures around 32 – 33 °C, with a ceramic hotplate SP88857100 from Thermo Scientific. A 250 ml Erlenmeyer flask was connected to the first chamber (bioreactor), in order to collect the daily biogas produced.

An external feeding tank with the mixture Mezcal vinasses and cattle manure was placed next to the reactor and was connected to it through a peristaltic pump TS7892K07 from Thomas Scientific. The effluent from the reactor was recirculated to the feeding tank.

The reactor was designed with the solid modeling computer-aided design (CAD) Solidsworks 23 and the simulation of the substrate flow was done with the CFD software ANSYS Fluent 14.5. The CFD simulation was done in order to analyze the dynamics and fluid displacement in the reactor, according to the fluid density, anode-cathode porous mediums related to a loss of pressure, and the minimal inlet velocity reached with the available peristaltic pump. With the CFD simulation, the correct dimension regarding the inlet diameter, could be found. **Figure 7.1** shows the designed bioreactor. **Figure 7.2** shows the CFD analysis performed in ANSYS

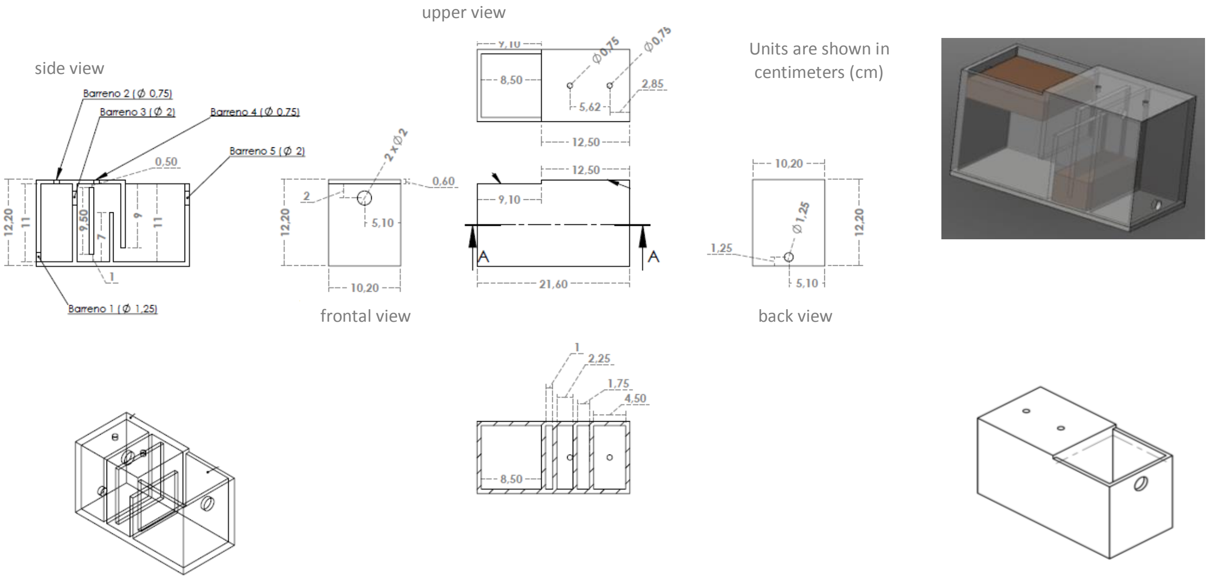

**FIGURE 7.1** Scheme of the designed bioreactor in cm

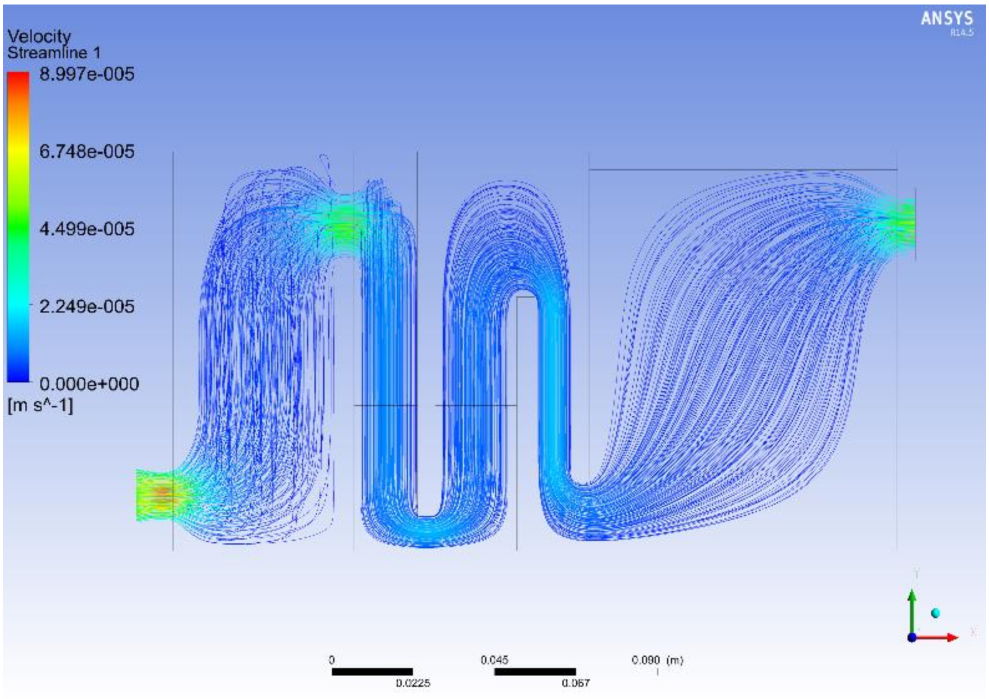

**FIGURE 7.2** Streamlines velocity analysis in reactor in m/s

For the CFD simulation, the porous media model was used. This model can be used in a wide variety of simulations, including flow distribution through filter cloth. A cell zone was defined, in which this model is applied to determine the pressure loss in the flow. Different parameters were considered such as the fluid density and viscosity (1 g/cm$^3$ and 1.007x10$^{-2}$ Poise), as well as the density of the anode and cathode (0.097 g/cm$^3$). Isotropic porosity of the anode and cathode was stablished, to simulate velocity loss during the streamline. Default values of inlet turbulence were retained (10 %), and the velocity and diameter were stablished according to the available peristaltic pump and according to **figure 7.1**. Outlet turbulence of 5 % was set as default, outlet diameter of 2 cm was chosen (according to **figure 7.1**).

The streamlines show the path that a mass particle (substrate) would take through the current flow. The lowest speed achieved with the peristaltic pump was 0.347 $cm^3$/min. According to Eq. 7.1 for flow rate estimation, the calculated flow speed, with the optimal inlet diameter of 1.25 cm, was $4.172 \times 10^{-5}$ m/s, which corresponds to the velocity streamline in the green area of **figure 7.2.** With the parameters of inflow velocity and inlet diameter, no accumulation of sediments could be seen through the CFD simulation. After simulation, reactor was manufactured from an external supplier, according to **figure 7.1**.

$$V = \frac{V\,\pi\phi^2}{4} \qquad \text{(Eq. 7.1)}$$

### 7.3.2 Reactor operation

Experiments were carried out for 92 days. Four different concentrations of Mezcal vinasses digested with cattle manure, diluted with deionized water, were tested. Concentrations were tested one after the other. Each concentration test was interrupted, when whether voltage nor biogas were produced. CA was tested for nine days, CB for 24, CC for 26 and CD for 23. A control test with only cattle manure was performed for 12 days at the beginning of the assays. **Table 7.1** shows the tested concentrations CA, CB, CC and CD, chemical oxygen demand (COD) in g/L related to each concentration, as well as substrate to inoculum ratios used (S:I-ratio).

**TABLE 7.1** Tested concentrations CA, CB, CC and CD, chemical oxygen demand COD (g/L) and substrate to inoculum ratio (S:I-ratio)

| | *Control* | *CA* | *CB* | *CC* | *CD* |
|---|---|---|---|---|---|
| Mezcal vinasses (L) | 0 | 0.4 | 0.6 | 0.8 | 1 |
| Cattle manure (L) | 3.5 | 1 | 1 | 1 | 1 |
| COD (g/L) | 12.20 | 8.95 | 11.90 | 14.85 | 17.80 |
| S:I-ratio | N/A | 0.26 | 0.40 | 0.53 | 0.66 |

### 7.3.3 Inoculum and substrate

Vinasses generated from the Mezcal production from *Agave Salmiana* were collected from the Mezcal factory Laguna Seca in San Luis Potosi in Mexico, and were stored in the refrigerator at 4°C prior to use. Cattle manure was collected from a local pasture-raised dairy and was left at room temperature, in order to eliminate the microbial activity of the inoculum itself (VDI 2016). **Table 7.2** shows the characteristics measured in both, Mezcal vinasses and cattle manure, before the experiment started.

**TABLE 7.2** Characteristics of cattle manure and Mezcal vinasses

| *Parameters* | *Cattle manure* | *Mezcal vinasses* |
|---|---|---|
| pH @ 27°C | 7.95 | 4.41 |
| Chemical oxygen demand COD (g/L) | 12.20 | 63.73 |
| Total solids TS (% FM) | 3.70 | 5.26 |
| Volatile solids VS (% FM) | 1.80 | 2.88 |
| Total dissolved solids TDS (g/L) | 8.11 | 5.87 |
| Conductivity mS/cm | 12.24 | 11.75 |
| REDOX mV | -211 | -142 |
| Volatile organic acids mgHAc/L | 1585 | N/A |
| Total inorganic carbon mgCaCO3/L | 9525 | N/A |
| FOS/TAC (volatile organic acids / total inorganic carbon) | 0.17 | N/A |

### 7.3.4 Measurement and calculations

The amount of biogas was determined according to the water displacement principle. The water displaced from the Erlenmeyer flask connected to the first chamber, was weighed with a digital scale from Media Data PS-5 and converted to volume biogas, according to the biogas density 1.2 $m^3$/kg (Uni Bremen 2009). The biogas quality regarding $CH_4$, $CO_2$, $O_2$, $H_2S$ and CO contents, was measured with a biogas analyzer Multitec 540 from Sewerin GmbH.

The voltage produced between the anode and cathode was daily recorded with a Fluke 115/EFSP Digital Multimeter. Power density P was estimated according to Eq. 7.2 and current I according to Eq. 7.3, where R means resistance (**Ω**), V means voltage and Vanode means the volume of the anodic chamber. Polarization curves were calculated with Excel 2013 and plotted with the software Minitab 17.

$$P = \frac{V\ x\ I}{V\ anode} \qquad \text{(Eq. 7.2)}$$

$$I = \frac{V}{R} \qquad \text{(Eq. 7.3)}$$

Each concentration was characterized at the beginning and end of every test, regarding pH, REDOX (mV), FOS/TAC, TDS (ppm) and conductivity (µS/cm) with a waterproof tester from HANNA Instruments HI-98311 and a pH-meter VWR-110. FOS/TAC, the ratio of volatile organic acids and total inorganic carbon, was measured throughout the titration of sulphuric acid 0.05 M ($H_2SO_4$) to pH 5 and 4.4 (Lossie and Pütz 2008). FOS indicates the amount of volatile organic acids, mostly acetic acid (mgHAc/L) and TAC indicates the total inorganic carbon or buffer capacity (mgCaCO$_3$/L) (Mézes *et al.* 2011, Moerschner 2015). COD, total solids (TS) and volatile solids (VS) were measured according to the norms DIN 38414-9:1986-09 (DIN 1986) and VDI 4630 (VDI 2016).

## 7.4 Results and discussion

### 7.4.1 Voltage output

The results of the voltage produced are shown in **figure 7.3**. It can be said, that vinasses content in substrate inhibit the voltage production.

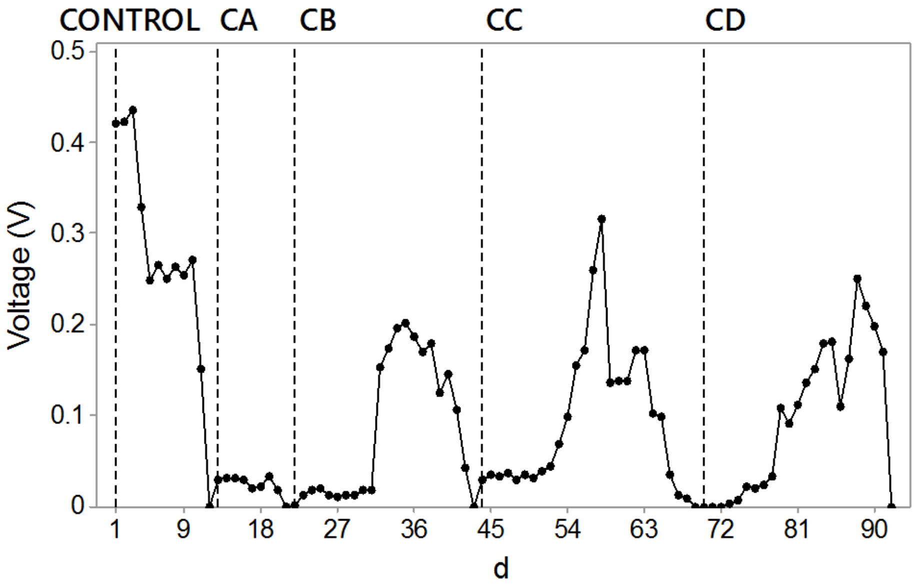

**FIGURE 7.3** Voltage produced by control test, CA concentration, CB concentration, CC concentration, and CD concentration

Control test was tested for 12 days and achieved the highest voltage of 0.436 V already by third day. CA, with the lowest COD and vinasses content, showed the worst results, producing only 0.032 V by the second day of tests and was carried out only nine days. CB produced 0.202 V by day 14 and was carried out for 22 days, while CC generated the highest voltage output of 0.317 V by day 15 and was carried out for 26 days. CD was carried out for 23 days and achieved 0.25 V by day 19. Few values for *Agave* vinasses were found for comparison.

López Velarde S.M. *et al.* (2017) used vinasses diluted with water for the electricity production in aerated-cathode with proton exchange membrane (PEM) at batch conditions. For short term operation (10 days), using a COD of 4.1 g/L, the highest voltage output of 0.12 V was achieved. The highest vinasses content with a COD of 17.1 g/L produced the lowest voltage output of 0.04 V. The highest power output was shown using a COD of 6.7 g/L. When comparing the results of the present work with the results of the long term operation (68 days) reported by López Velarde S.M. *et al.* (2017), this assays showed lower values.

Another finding in this study was that the highest the vinasses content, the longest the time to achieve the highest voltage output. This suggests that the microorganisms in the anode took more time to oxidize substrate with high organic matter content. This fact can be confirmed by Vogl *et al.*(2016), who reported that easily degradable substrates produce higher power densities. In this study the voltage output and power density increased with increased vinasses content (CODs of 8.95, 11.90 and 14.85 g/L), until a certain point (COD of 17.80 g/L). Afterwards a voltage drop took place, when the saturated state of the electrolyte inhibit the oxidation mechanisms. **Figure 7.4** shows the power/current density curves of the concentrations tested.

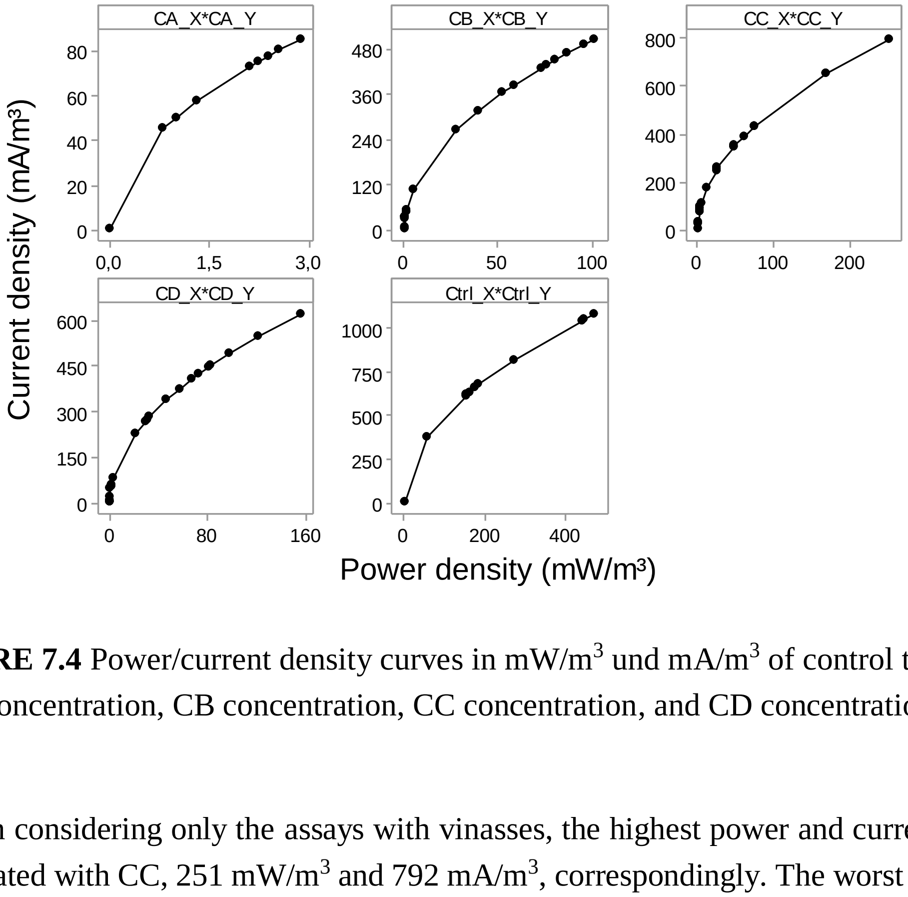

**FIGURE 7.4** Power/current density curves in mW/m$^3$ und mA/m$^3$ of control test, CA concentration, CB concentration, CC concentration, and CD concentration

When considering only the assays with vinasses, the highest power and current densities were generated with CC, 251 mW/m$^3$ and 792 mA/m$^3$, correspondingly. The worst results were obtained with CA, which contained also the lowest vinasses content and COD.

Results of these assays are comparable to Belafi-Bako *et al.* (2014). AD effluent from a sugar factor wastewater plant was used in a MFC for electricity production. With a COD of

7.15 g/L, the highest power density of 8652 $mW/m^2$ was achieved. The lowest power density was generated with the highest COD of 19.8 g/L. Nam *et al.* (2010) found similar results. The highest power density of almost 3 $W/m^2$ was obtained with an organic loading rate (OLR) of 3.84 g/Ld, whilst an increase on OLR to 4.80 g/Ld resulted in a decrease of the power density. The reduction and oxidation reactions of the microorganisms adhered in the anode determines the power output of a MFC. If the electrolyte solution has a high organic matter content, a difficult electron and proton transfer takes place (Nam *et al.* 2010). Schievano *et al.* (2016) reported that MFC performance decreased at higher COD concentrations, which were tested to avoid high substrate dilutions.

### 7.4.2 Biogas and methane production

The bar charts regarding the cumulative biogas and methane production generated by CA, CB, CC and CD are shown in **figure 7.5**.

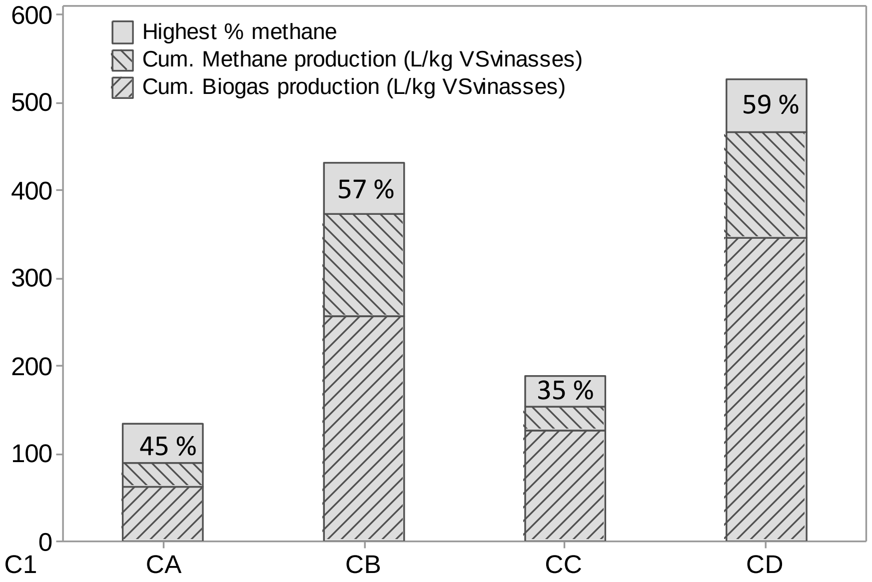

**FIGURE 7.5** Cumulative biogas and methane production, as well as methane content generated by CA, CB, CC and CD

CA produced 63 $L_{biogas}$/kgVSvinasses with a highest methane content of 45 %. CB produced 257 $L_{biogas}$/kgVSvinasses with a highest methane content of 57 %. CC generated 127 $L_{biogas}$/kgVSvinasses and achieved a methane production of 35 %, and CD produced 347 $L_{biogas}$/kgVSvinasses with the highest methane content of all concentrations tested (59 %). The highest biogas production was generated by CD. CB showed the highest methane production of 117 $L_{CH4}$/kgVSvinasses, one of the highest methane content (57 %) and showed also the highest

COD removal of 93 %. CC, which produced the highest voltage output, as well as power and current densities, did not show significant biogas and methane yields, and showed the lowest methane content of 35 %. CA showed the worst results regarding not only electricity production, but also AD efficiency. This indicates that the lack of organic matter available was insufficient for both, electricity and biogas production.

An important finding was that CC produced less biogas and methane in comparison to CB and CA, but generated the highest power density and voltage output recorded. The conversion of organic acids into biogas was not successfully, but the microorganisms in anode could oxidize the organic acids to generate more voltage. Zhao *et al.* (2012) carried out experiments using the anode chamber as anaerobic digester. The results suggested that fermentation, more precisely the methanogenesis, compete with the electricity generation, what resulted in a low power output and biogas yield. The coulombic efficiency was 2.79 % and the biogas yield persisted only 8 days with a maximum production of 0.285 L/d on a 15 L biodigester.

### 7.4.3 Chemical oxygen demand removal, FOS/TAC, total dissolved solids and conductivity

**Table 7.3** shows the results regarding COD removal in CA, CB, CC and CD. CC showed the best removal of 93 % and CD the lowest. Results are comparable to López Velarde S.M. *et al.* (2017), who obtained the worst COD removal with the highest COD tested. The high amount of organic matter results in a saturated state of the electrolyte inhibiting the oxidation mechanisms for COD removal.

**TABLE 7.3** Characteristics of cattle manure and Mezcal vinasses

| | *CA* | *CB* | *CC* | *CD* |
|---|---|---|---|---|
| Initial COD (g/L) | 8.95 | 11.90 | 14.85 | 17.80 |
| Final COD (g/L) | 1.34 | 0.83 | 2.07 | 9.43 |
| COD removal (%) | 85 | 93 | 86 | 47 |

The amount of TDS and conductivity were higher with a higher vinasses content and COD. **Table 7.4** indicates the amount of volatile organic acids (FOS) and total inorganic carbon (TAC), as well as the quotient FOS/TAC.

**TABLE 7.4** FOS/TAC values measured at the beginning and end of each assay

| | *FOS mgHAc/L* | *TAC mgCaCO$_3$/L* | *FOS/TAC* |
|---|---|---|---|
| CA_I | 713.5 | 1750 | 0.41 |
| CA_F | 174 | 2400 | 0.07 |
| CB_I | 838 | 1950 | 0.43 |
| CB_F | 49.5 | 2312.5 | 0.02 |
| CC_I | 2166 | 1725 | 1.26 |
| CC_F | 49.5 | 2625 | 0.02 |
| CD_I | 1253 | 1425 | 0.88 |
| CD_M | 215.5 | 2687.5 | 0.08 |

With the increase of vinasses content, the volatile organic acids, as well as the FOS/TAC increased. The buffer capacity of the system decreased with a high COD. When comparing the beginning and end of each concentration test, the amount of volatile organic acids decreased dramatically, what indicates that they were quickly adhered to the anode. When comparing beginning and end of every concentration tested, the buffer capacity of the system achieved always higher values than at the beginning. A lowest REDOX was shown, when the vinasses content, COD and S:I-ratio diminished.

Although FOS/TAC values where optimal for CA and CB according to values proposed by Lossie und Pütz (2008) of 0.3 - 0.4, the separated FOS and TAC values where much lower than recommended. FOS values should be higher than 10 g/L and TAC values should lie between 8.5 and 13 g/L (Mézes *et al.* 2011, Moerschner 2015). This was the reason of the low AD efficiency of the system. For concentration CC, the FOS/TAC value of 1.26 was higher than recommended by Moerschner (2015) and Lossie and Putz (2008). This resulted in a drop of the biogas and methane yields.

According to Kretzschmar *et al.* (2016), volatile fatty acids are correlated to the currrent production. Inhibitions in MFC were found out when the amount of organic acids increased above 4 g/L. In the present study, the best voltage output was obtained when the amount of FOS was 2.16 g/L, which showed also the highest amount of organic acid concentration.

## 7.5 Conclusions

The combination of bioenergy technologies offers a wide range of both, sustainable energy generation and byproducts further utilization. Substrates consisting of high amounts of organic and inorganic matter should stay in focus for treatment before discharge in soil and water. Through this study, the consecutive implementation of AD and MFC technologies was tested with Mezcal vinasses and cattle manure as inoculum source for AD. It was found out that the use of vinasses in MFC inhibits the process of voltage generation, in comparison to the solely use of cattle manure. Besides, MFCs does not tolerate substrates with high amounts of COD. In the present study, voltage output increased with increasing vinasses content, until a certain limit of 14.85 g/L. The highest COD tested was 17.80 g/L, at which the voltage dropped. In regards to biogas, the higher the COD content, the higher the biogas yield and methane content. It can be concluded that both technologies cannot be sequentially implemented without COD adjustment. This study presents an alternative for the further and deeper investigation of the use of both technologies consecutively, so that the organic acids content in substrate could result in a successfully AD and MFC operation.

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# 8 General results and discussion

Through these studies, the hypotheses proposed at the beginning of this work could be cleared. It could be found out, that the agricultural residues of the *Agave* processing to Tequila and Mezcal can be successfully used for bioenergy production, through both anaerobic digestion (AD) and microbial fuel cells (MFC). Enhancement of AD efficiency could be achieved when using a low-cost biofilm (PET bottles) in the bioreactor, when using a more suitable inoculum source (cattle manure), and when adjusting S:I-ratio to 0.3 in batch fermentation. The simultaneous energy generation of biogas and voltage was not efficient in the AD-MFC configuration, so that these technologies can be coupled, only when the chemical oxygen demand (COD) in digestate is adjusted, prior to MFC operation.

Few research has been done regarding important topics investigated in this work. Findings of this study are relevant, because gaps in knowledge were closed, in relation of the use of the agricultural residues of *Agave* processing for bioenergy production, and organic matter removal. Such knowledge gaps included the effect of the inoculum source on vinasses AD. In general, inocula effect has been subject of research, but only since recent years. Besides, inocula effect when digesting vinasses was not studied before. Another knowledge gap was the effect of using low-cost PET biofilms in vinasses AD. Biofilms has been widely researched, nevertheless only one study shows the effect of using low-cost PET biofilms. A further knowledge gap, which could be closed in this work, was the importance of the S:I-ratio in batch fermentation. It is common to consider a 0.5 S:I-ratio as optimal, regardless of the inoculum source and substrate to be digested. Batch fermentation is very common in developing countries like Mexico. There was also a knowledge gap in relation to the use of vinasses in MFCs. Few literature is found in this regard. Coupling AD digestate as MFC influent has not been widely researched. Finally, the use of mathematical modelling has been used for describing bacterial growth in AD, but only since the past years. Vinasses AD was mathematically modelled in this work, probably for the first time. Therefore, new alternatives to perform and describe bioenergy production from agricultural residues of *Agave* processing are proposed. Results can be transferred in large scale applications, which will guarantee an enhancement in biogas and methane production.

## 8.1 Effect of inoculum source

AD efficiency using manure was higher than sludge, for both, batch (BMP) and semi-continuous tests. Biochemical methane potential (BMP) tests using cattle manure as inoculum, showed a cumulative biogas production of almost 600 L/kgVSvinasse with a maximal methane content of 81 %. In the contrary, sludge produced 200 L/kgVSvinasses, with maximal 50 % methane. Semi-continuous tests using manure showed after 30 days of experiments, a cumulative biogas production of 1500 L/kgVSvinasse, with 55 – 60 % methane. Sludge showed only 217 L/kgVSvinasses with 50 – 55 % methane. The difference between AD efficiency in both inocula could be explained comparing the content of organic matter, organic acids, total inorganic carbon, as well as COD:N-ratio.

Sludge had a volatile organic acid content (FOS) aroud 20 g/L and total inorganic carbon contents (TAC) between 14 and 20 g/L, while manure showed lower values around 4 and 9 g/L, for FOS and TAC correspondingly. Mézes *et al.* (2011) found out that a high organic acid content (FOS > 10 g/L) could result in an incomplete bacterial metabolism, which might lead to process inhibition. If at the same time the buffer capacity of the system is adequate, the inhibition will not be evidenced. Moerschner (2015) suggested optimal TAC values between 8.5 and 13 g/L. In this study, assays with sludge showed FOS and TAC values out of the recommended limits. Regarding the assays with manure, some days the TAC values were slightly out of the limits, but direct afterwards, these values were again within the limits. For this reason, manure showed a higher buffer capacity than sludge, and so a higher balance between ammonium and ammoniac $NH_4^+/NH_3$ formation. Manure indicated also a higher conductivity than sludge and thus higher $NH_4^+$-N content. Manure and sludge conductivities were 28.24 mS/cm and 2.98 mS/cm, correspondingly. Moerschner (2015) reported that 10 mS conductivity corresponds to 1 g/L $NH_4^+$-N content in substrate. The total nitrogen content was higher in manure (1.5 g/L) than in sludge (0.042 g/L). These facts explain the high buffer capacity of manure. With pH increase by organic acids consumption, the concentration of $H^+$-ions increases too, and the $NH_4^+/NH_3$–balance could had moved to ammoniac $NH_4^+$. Regarding the semi-continuous tests, when feeding every seven days, TAC value in manure assays kept within the recommended limits, while sludge continued to increase with the time, up to 31 g/L. In general, manure assays showed a better buffer capacity than sludge assays. Besides the nitrogen content, the high carbon availability or COD content could had been responsible for the high buffer capacity. Sludge showed a COD of 5.7 g/L, while manure 31.1 g/L.

Regarding the semi-continuous AD tests, when a more frequently feeding in the bioreactor digesting with cattle manure took place (every two days), biogas production increased significantly up to 21561 L/kgVSvinasses by day 80, and methane content achieved stable values between 55 – 60 %. Feeding frequency in sludge semi-continuous assays was done only every seven days, showing a lower buffer capacity and conversion efficiency to biogas and methane, After 40 days of experiments, only 426 $L_{biogas}$/kgVS were produced. Methane content remained around 50 %.

The bacterial growth rate could be defined by means of mathematical modelling (Ware and Power 2017, Li *et al.* 2018). Gompertz curves showed best fits and correlation to the assay with sludge, whereas transference function described better the assay with manure. The lag phase, or initial biomass break down, was 2 days for both, sludge and manure, and the maximal growth rates µm were 328 and 425 L/kgVS•d, correspondingly.

Anaerobic sludge had a low amount of oxidizable matter, because COD and TS (total solids) values were very similar, 5.7 and 5.39 g/L, correspondingly. This was the same case of cattle manure, where COD was 31.1 g/L and TS 30.7 g/L. In both cases, inocula showed a low amount of oxidizable matter, suggesting that the highest oxidizable matter was provided by vinasses. Vinasses showed an unbalanced COD:N-ratio. According to the literature (Moletta 2005), for optimal AD, COD:N-ratio should lie around 800:5. COD:N-ratio of sludge was 679:5, while vinasses 2341:5, three times lower than it should be. It is suggested either adding nitrogen to the bioreactor (e.g. urea addition) or using a protein-rich sustrate or inoculum, such as cattle manure. Total nitrogen in sludge was 0.04 g/L, whilst in manure 1.5 g/L. Due to the high COD, a complete carbon metabolism could not had taken place. In addition, if the nitrogen content is high, the amount of ammoniac ($NH_3$) could also be high, and pH drops (Friehe *et al.* 2013). The high COD amonut in vinasses, causing an unbalanced COD:N in the bioreactor, could be balanced through the low COD:N ratio in cattle manure. COD:N-ratio of manure was 104:5, much lower than recommended. The use of manure as inoculum source has also been compared in the literature. Similar results were obtained by Gu *et al.* (2014), who reported manure as the most efficient of the inoculum sources tested, because it showed a higher amount of micronutrients content. In this study, nitrogen content was higher in manure. When comparing the biodegradability index, it was demonstrated, that a better degradation was given by the microbial population in manure, in comparison to sludge. Córdoba *et al.* (2015) showed contrary results when comparing sludge to manure as inoculum. Bacteria in manure was not able to consume the available volatiles fatty acids and showed a lower methane generation. Sludge was reported to have more volatile fatty acids or VFAs (comparable to FOS) than manure, 1.5 $gCaCO_3$/L against 1.4 $gCaCO_3$/L. The adequate inoculum and S:I-ratio promote VFAs consumption and methane production, otherwise there is an accumulation that could inhibit the methanogenic activity. The efficient AD process requires a large diversity of methane-forming population and active microbial communities (Gerardi 2003).

Although AD did not show high COD removal rates, BMP manure assays achieved 20 % more organic matter removal in terms of VS (volatile solids), in comparison to sludge. This suggests that cattle manure contains microorganisms that produce enzymes, which could hydrolyze the vinasses for the efficient AD. A deeper analysis of the existing microorganisms should be further carried out.

## 8.2 Effect of S:I-ratio

S:I-ratios comparison indicate that the highest AD efficiency was achieved with S:I-ratio 0.3 for both inocula. It is difficult for the microbial population to degrade high contents of organic material, especially because the methanogenic bacteria does not reproduce at the same rate as the hydrolytic or acidogenic bacteria, creating a bottleneck for material degradation (Budiyono *et al.* 2013). This was the case of the highest S:I-ratios tested, 0.4 for sludge and 0.7 for manure, which showed the lowest biogas and methane yields. A high organic matter content, or high concentration of vinasses, might lead to accumulation of organic acids, exceeding the microorganism's degradation capacity, reducing the methanogenic activity (Fagbohungbe *et al.*

2015). By S:I-ratio 0.3, the organic load was slightly lower than the microorganism's degradation capacity, preventing organic acids accumulation. A low S:I-ratio was also not efficient, because of the lack of substrate to be converted in biogas and methane. Manure showed that the amount of FOS, TAC, and FOS/TAC values incremented proportionally to the vinasses content or high S:I-ratios. Sludge showed a decreasing buffer capacity, with increasing S:I-ratio and FOS content. Similar results happened in the semi-continuous tests when comparing inocula. In agreement, Zhou *et al.* (2011) reported higher methanogenic activity by AD of bean curd when using S:I-ratios between 0.3 and 0.6, rather than S:I-ratios between 0.7 and 3. Methane production decreased when substrate load increased. Liu and Sung (2002) reported a significant decrease in the methane conversion efficiency using algal residue as a substrate, when S:I-ratio was higher than 1. S:I-ratio 0.3 tend to be more promising than S:I-ratio 0.5, which is recommended by VDI 4630 (VDI 2016).

Regarding kinetic modelling, three mathematical models of bacterial growth were compared. For both inocula, S:I-ratio 0.3 showed the highest specific methane growth rate µm. The lowest µm was shown by the lowest S:I-ratio digested. In comparison to sludge, manure indicated a steeper slope, meaning a high µm. The lag phase was longer for the assays with manure, although the AD efficiency was better. These long lag phase could be explained by the high amount of organic matter to be degraded in manure, in terms of VS and TS, in comparison to sludge. This is also confirmed when comparing the error (%) between experimental and theoretical methane yields. Good fits within the theoretical and experimental methane production curves implies an uncomplicated digestion of the substrate (Ware and Power 2017). This was not the case of the use of manure with higher S:I-ratios (0.5 and 0.7), which had a high vinasses content and showed inhibition. For both, sludge and manure, the best visual fits to the mathematical models are shown by assays resulting in the highest AD. S:I-ratio 0.5 and 0.7 with manure showed elevated errors, long lag phase (more than 25 days) and lower µm than S:I-ratio 0.3. On the contrary, S:I-ratios 0.1 and 0.4 with sludge showed a much lower µm between 12 and 2 L/kgVS, in spite of the short lag phases near to cero. This indicate the complicated vinasses digestion, resulting in a low methane production.

## 8.3 Effect of biofilm

According to the suggestions resulting from the inoculum source comparison, a biofilm carrier was used in the bioreactor, to enhance the availability of microorganisms to metabolize the high organic matter content, despite an unbalanced COD:N ratio, especially due to vinasses. The effect of biofilms was carried out in assays digesting with cattle manure as inoculum, due to the better results obtained before. Start-up was carried out with a S:I-ratio of 0.3, as pointed out in previous assays. After 80 days of assays, the cumulative biogas production of the biofilm bioreactor generated 4123 L/kgVSvinasses, while the control bioreactor produced only 2827 L/kgVSvinasses. Until 60th day, when feeding was done every 7 days, cumulative biogas production had achieved similar values for both reactors, around 1300 L/kgVSvinasses. Nevertheless, methane content had achieved higher values in biofilm bioreactor. When the feeding took place more frequently, biofilm bioreactor showed quickly a much more efficient AD, with higher methane production. Biofilm bioreactor achieved stable methane values around 55 - 60 %, while control bioreactor achieved more unstable values around 34 - 44 %. In general, biofilm bioreactor produced 40 % more biogas and 60 % more methane, than control bioreactor. The amount of produced $H_2S$ was 20 % lower for bioreactor biofilm, suggesting that biofilm carrier in bioreactor could be considered as a biological treatment for $H_2S$. This findings suggested that sulphide oxidizing microorganisms were adhered to the biofilm, so that a better amino acids conversion, responsible of the $H_2S$ production, could take place.

In the bioreactor without biofilm, the amount of accumulated organic acids increased with the time. In comparison, when using the biofilm, no accumulation of acids took place, even though they were fed simultaneously. FOS/TAC values stayed within recommended range (0.3 - 0.4) for biofilm bioreactor, while in the bioreactor without biofilms it increased up to 1.6, when a frequent feeding took place. Regarding TAC values, biofilm bioreactor showed more values within the recommended limits of 8.5 – 13 g/L, suggesting a higher buffer capacity. An accurate TAC value indicates a good balance between carbon and $NH_4^+/\ NH_3$. These findings suggests, that the microbial population adhered on the biofilm convert the organic acids into biogas and methane succesfully.

Conductivity and TS showed lower values in the biofilm bioreactor, while COD, TS and VS removals increased. These results suggest that the microorganisms adhered to the biofilm could hinder the accumulation of salts, which have direct relation with the conductivity, whereas the COD and dissolved solids could be better degraded. The kinetic modelling showed in biofilm bioreactor lag phases of 7 and 8 days for biogas and methane, correspondingly. Control bioreactor showed lag phases of 0 and 2, for biogas and methane curves. This study suggests that biofilm formation took 7-8 days long, what could be overcome with the time. For optimal AD performance, biofilm should be inoculated for at least seven days prior to AD assays. The µm of cumulative and methane curves was also higher for biofilm bioreactor, than control bioreactor. Control bioreactor showed 425 L/kgVS*d for biogas curve and 194 L/kgVS*d for methane curve, while biofilm bioreactor showed 564 L/kgVS*d for biogas and 302 L/kgVS*d for methane.

When analyzing the biofilm with the optical microscope, it was found out that sanding the PET surface did not lead to a higher microorganism’s accumulation, like the case of stacked PET bottles, which showed a much thicker biofilm formation.

In agreement with the results obtained through this study, Liu *et al.* (2011) reported a biogas and methane enhancement of 40 % and 49 %, correspondingly, when using a polypropylene fiber as biofilm carrier. Gong *et al.* (2017) achieved also 40 % enhancement for both, biogas and methane production, when using activated carbon fiber. Other fibers used such as polyvinyl alcohol fiber and glass fiber caused AD inhibition. Martí-Herrero *et al.* (2014) reported a 40 % biogas enhancement when using PET bottles as biofilm carrier in a reactor digesting cattle manure for 300 days. The results of the biofilm assays demostrate that polyethylene terephthalate (PET) is a low-cost and efficient alternative as biofilm carrier. It is important to consider that the overproduction of PET worldwide has becomme a serios environmental problem, and reusing PET bottles for AD could hinder their disposal in landfills and water.

## 8.4 Microbial fuel cell and anaerobic digestion

Regarding the use of vinasses in a conventional MFC, the highest power density was achieved when the COD content was 6 g/L resulting in 80.64 W/m$^3$. COD of 1.2 g/L and 4.06 g/L produced 53.39 and 23.78 W/m$^3$, correspondingly. The highest COD tested resulted in the lowest power density generated (17.14 g/L, 5.13 W/m$^3$). This finding suggests that a low power output occurs because the anodic reactions in the anodic chamber depend on the substrate characteristics and carbon availability. Power density rises when the organic load increases up to a certain concentration. If the electrolyte solution has a significant amount of colloidal particles, like vinasses, the electron and proton transfer could be hindered. These colloidal particles can act as limiting factors and increase the internal resistance, so that power density decreases, although COD removal increases (Nam *et al.* 2010, Liu *et al.* 2011, Oliveira *et al.* 2013). When using substrates with a high COD content, like vinasses, more organic matter needs to be degraded before a significant electron and proton exchange starts, and before higher voltage can be produced. Molecules need to be first broken into smaller ones in order to be more suitable as MFC fuels. The potential of electricity production in a MFC is given by the bacterial metabolic activity or in other words by the reduction - oxidation reactions, generating electrons and protons, as well as by the electron acceptor conditions. This is influenced by the anode potential and substrate used as anolyte. This can be confirmed when measuring the internal resistance. With the lowest COD tested, the lowest internal resistance of 97.10 Ω was achieved. The highest internal resistance of 474.58 Ω was achieved with the highest COD tested.

From the CODs tested, the most efficient COD removal of 92 % was shown with a COD of 10.60 g/L and the lowest with a COD of 1.2 g/L. The increase in substrate removal at high load rates (in this study up to 10.60 g/L) could have occurred because of the direct anodic oxidation mechanism in the anodic chamber. At higher organic matter contents, the oxidation mechanisms were hindered through the saturated state of the anode and electrolyte (Belafi-Bako *et al.* 2014).

Similar results were obtained by Belafi-Bako *et al.* (2014), who tested the power output of a batch MFC inoculated with anaerobic sludge from an AD plant using wastewater from a sugar beet factory. The highest power density of 8652 mW/m$^2$ was achieved through a COD of

7.15 g/L and the lowest power density of 3380 mW/m$^2$ with a higher COD of 19.80 g/L. COD of 4.1 g/L yielded 4500 mW/m$^2$. Nam *et al.* (2010) found the highest power density of 2981 mW/m$^2$ by testing fermented wastewater produced from hydrogen fermentation of coffee processing wastewater, with an organic loading rate of 3.84 g/Ld. However, an increase in the organic loading rate up to 4.80 g/L/d generated less power (2959 mW/m$^3$). The influent characteristics and consortium's metabolism affected the power generation. Reddy *et al.* (2010) tested four different organic loading rates (OLR) of the anaerobic mixed consortia from the UASB treating wastewater, with OLRs of 0.195, 0.458, 0.911 and 1.589 g/Ld. Power generation increased with increasing OLR but only up to 0.911 g/Ld, generating 76.17 mW/m$^2$. With the highest OLR tested, 1.589 g/Ld, the power generation decreased to 49.86 mW/m$^2$. Martin *et al.* (2010) tested different concentrations of glucose and acetate. Using glucose as substrate, a power output of 8.2 W/m$^3$ was achieved using an organic load with a COD content of 3.72 g/Ld. With the further increase of organic load to a COD of 7.44 g/Ld, the power output decreased to 6.6 W/m$^3$. Similar results occurred when testing acetate in the MFC. OLR of 4 g/Ld produced 53.3W/m$^3$ and 8 g/L/d produced only 50.6 W/m$^3$. When increasing the glucose load, substrate availability for the methanogenic population also increased. Thus, at a higher load, 34 % of substrate was used to produce $CH_4$ and 2 % to produce electricity.

When designing the reactor to treat the AD digestate as influent for MFC, the optimal inlet diameter of 0.0125 cm was found to prevent sediment accumulation. A computer fluid dynamics (CFD) simulation was performed. Inflow velocities were tested, especially the lowest one of 0.347 cm$^3$/min, achieved from the available peristaltic pump. Vinasses were mixed with inoculum for AD. Results regarding power outputs showed much lower values for the designed MFC. It was found out, that the anolyte with only manure (no vinasses) achieved the highest power output of 475 mW/m$^3$. Cattle manure was diluted so that a COD of 12.2 g/L could be guaranteed in MFC. When comparing only the concentrations containing vinasses, the concentration with S:I-ratio 0.5 and COD of 14.85 g/L, achieved the highest power output of 251 mW/m$^3$. CODs of 8.95, 11.9 and 17.8 g/L showed lower power outputs, suggesting that optimal COD concentrations for this MFC configuration should lie around 12.2 and 14.85 g/L. Results are comparable with the findings of the conventional batch MFC assays. If a very high organic matter content is used for electricity production, power outputs could be limited through the saturation state of the anolyte. More COD or molecules need to be broken into smaller ones, before a significant electron and proton exchange can start (Nam *et al.* 2010, Schievano *et al.* 2016). According to Kretzschmar *et al.* (2016), volatile fatty acids are correlated to the currrent production. Inhibitions in MFC were found out when the amount of organic acids increased above 4 g/L. In the present study, the best voltage output was obtained with a FOS content of 2.16 g/L.

Regarding AD, the overall results were not efficient. The highest biogas production was achieved with the highest vinasses content (S:I-ratio 0.66 and COD of 17 g/L). Nevertheless, the highest methane content was achieved with S:I-ratio 0.4 (14 g/L), which is an optimal S:I-ratio for AD efficiency. Even when the FOS/TAC values were within the recommended limits (Lossie and Pütz 2008), the separated FOS and TAC values where much lower than recommended. FOS values should be lower than 10 g/L and TAC values should lie between 8.5 and 13 g/L (Mézes *et al.* 2011, Moerschner 2015). This was the reason of the low AD efficiency of the system. When comparing power output and biogas production, the concentration with the highest voltage output, as well as power and current densities, did not show significant biogas and methane yields, and showed the lowest methane content of 35 %. The conversion of organic acids into biogas was not successfully, but the microorganisms in anode could oxidize the organic acids to generate more voltage. Zhao *et al.* (2012) demonstrated that methanogenesis compete with the electricity generation, what result in a low power output and biogas yield.

COD removal in both MFCs tested was much higher, in comparison to the solely AD. Batch MFC achieved the highest COD removal of 92 % at COD 10.6 g/L, while continuous AD – MFC achieved a similar value of 93 % at COD of 11.9 g/L. A very low COD of 1.2 g/L achieved only a 29 % removal (for batch MFC), while the highest COD around 17 g/L for batch and continuous assays, resulted in 49 and 47 % COD removals. Similar results were obtained by Belafi-Bako *et al.* (2014). The increase in substrate removal occurred because of the direct anodic oxidation mechanism in the anodic chamber. If the organic load in the substrate is very high (around 17 g/L for vinasses), the saturated state of the electrolyte hindered the oxidation mechanism. Comparing COD removal efficiencies of MFC and AD, MFC achieved much higher values of 93 % against 34 and 20 % in AD. Anode works like a biofilm, where the microorganisms oxidize the available biomass. In continuous MFC, anode was an activated carbon felt, which guaranties a much higher surface contact for microbial adhesion. This could be a reason why continuous MFC achieved higher power outputs at higher CODs (14 g/L), in comparison to batch MFC, where highest power output was achieved by a lower COD content of 6 g/L. Microbial fuel cells were at first developed for wastewater treatment, what makes this technology more suitable for organic matter removal. MFC has been pointed out for bio-electrochemical treatment of phenols, sulphur, chromium, and other heavy metals (Mohanakrishna *et al.* 2010, Pant *et al.* 2010). The direct oxidation of organic matter in the anodic chamber results in a better COD removal, in comparison to AD, where the oxidation processes depend on a variety of parameters of bioreactor operation.

MFC showed a lower tolerance to high organic matter contents, than AD. By BMP assays, COD of 38.3 g/L was successfully used for AD, while COD content of 17 g/L resulted in a drastic power output decrease in MFC. Coupling AD and MFC showed a much lower voltage production and biogas generation, than separated AD and MFC. These results show that the configuration designed in this work is not suitable for the simultaneous biogas and voltage generation, without COD adjustment. High CODs result in low power densities, so that if substrate is diluted to achieve higher power outputs, a low biogas production takes place.

## 8.5 Transferability in large scale applications

This study is important, because the results obtained regarding the use of agricultural residues of *Agave* processing for bioenergy production, can be transferred to large scale applications. The research began in laboratory scale, in order to find out, in small scale, the best parameters resulting in an AD enhancement. The investigation was based on the comparison regarding use of biofilms in a bioreactor, the use of a more effective inoculum source, as well as the use of different S:I-ratios. The results obtained through this work regarding vinasses AD, and its enhancement, can be transferred to industrial biogas plants, working at optimal temperature conditions, continuous stirring and feeding, as well as continuous bioreactor control. If at first, the parameters intending a higher AD efficiency are tested in small scales, investigation costs drop and the risk to achieve no favorable results, does not have important negative implications.

In this study, favorable results for biogas and methane production enhancement were found out when using stacked biofilms made of PET bottles. Active microorganisms were adhered between the PET surfaces, so that more amount of volatile organic acids could be successfully converted to biogas and methane. In addition, the unbalanced COD:N-ratio of vinasses and manure, could be overcome. The microorganisms adhered to the biofilm could metabolize the high amount of organic matter, preventing accumulation of acids, resulting also in a high buffer capacity in bioreactor. When considering that PET biofilms could be used to digest substrates with high amounts of organic matter (in the case of vinasses), the construction of a biogas plant with PET biofilms can be more attractive. Also, $H_2S$ production was lower when using the PET biofilm, suggesting that the microbial population adhered to biofilm carrier, could had been sulphide oxidizing. In practical applications, the fact that biogas contains small amount of $H_2S$, is an obstacle for the construction of biogas plants. A deeper research regarding the effect of biofilms in $H_2S$ could be meaningful.

Regarding S:I-ratios, the correct S:I-ratio found out (0.3), can be used for more accurate BMP assays, for an optimal bioreactor start-up, or for batch fermentation design, obtaining a higher AD efficiency.

Especially in the state of Jalisco, where the highest amount of Tequila and vinasses are produced (CRT 2018), anaerobic sludge of wastewater treatment plants is used as inoculum, according to the literature research. When comparing the vinasses AD using anaerobic sludge and cattle manure, results suggest that it is worthwhile to consider manure as inoculum, despite transportation costs. Cattle manure showed a higher amount of nitrogen, and according to the literature also other nutrients, as well as a higher buffer capacity, than sludge. This could prevent acidification of the bioreactor, anticipating AD inhibition, which otherwise could result in high operational costs.

With the vinasses characteristics and biogas and methane yields determined in this study, the dimensioning of a large scale biogas plant was carried out (Rieker 2010, Kilic 2016). Cattle manure was considered as inoculum source. In the Mezcal factory Laguna Seca in San Luis

Potosi, from which the vinasses for this study were obtained, around 4000 L/week vinasses are produced from a batch of *Agave* shredding, cooking, fermentation and distillation. If 163 cattle heads are considered, approximately 3100 T/a manure and 208 T/a vinasses could be gathered. According to Fischer (1998), cattle manure production could achieve 52 kg/d, by animals 450 kg heavy. If the amount of 3100 T/a manure and 208 T/a vinasses were anaerobically digested, a reactor volume of 110 $m^3$ would be needed to generate 18 kW electricity with a micro CHP. Optimal operation parameters of the bioreactor should be organic loading rate of 3.5 gVS/Ld, 40 % CHP electrical efficiency, and 8000 h/a operating hours. Methane content in the mixture would lie around 62 %. If considering the 163 cattle heads lonely, without vinasses, electricity produced would lie by 15 kW. If the Mezcal factory facilities were bigger, and one batch could be produced in one day, 1460 T/a vinasses would be available. The power output of the micro CHP would be 33 $kW_{el}$. In Mexico, the energy consumption in a household with 4 people lies around 225 kWh/month (Sánchez Peña 2012). When producing 18 $kW_{el}$, the electricity consumption of 57 households could be covered. Electricity costs of one household consuming 225 kWh/month ascend to $ 360 Mexican pesos, considering the tariffs of the Federal Electricity Supplier (CFE 2018). The 57 households would pay monthly the amount of $ 20 508 Mexican pesos, or $ 246 096 pesos/a (yearly). After 20 years, and considering an increment in electricity price of 1 %, as reported by SENER (2013) for year 2012, households would had paid almost $ 5 418 789 million pesos of electricity. According to Kayser (2016), the investment costs of a small biogas plant with an electric capacity of 40 $kW_{el}$ could ascend to 470 000 € (in Germany) or $ 10 258 000 Mexican pesos. There is no information regarding the cost of a biogas plant built in Mexico. From this brief analysis, it can be said, that for a small amount of vinasses, like in the case of this small Mezcal factory Laguna Seca, the investment of a biogas plant for electricity production would not be profitably. Instead, an anaerobic digester can be implemented operated in batch fermentation, with an S:I-ratio 0.3, for the biogas production. $H_2S$ content in biogas could be removed, and biogas could be combusted for the cooking and distillation of shredded *Agave*. Hence, the problem of vinasses disposal would be solved. If a higher amount of vinasses could be available, as the case of large scale Mezcal or Tequila factories, biogas technology could be successfully implemented for electricity generation. The state of Jalisco concentrates 99 % of Tequila production. According to CRT (2018), the yearly Tequila production ascends to 271 000 T/a. If from every liter Tequila produced, 10 L vinasses remain as residue, the yearly vinasses production in Jalisco ascends to 2 710 000 T/a. Under optimal bioreactor conditions, a total power output of 33 $MW_{el}$ could be produced from the biogas, what means electricity for more than 105 000 average households. Heat produced from the CHP could be used for *Agave* cooking or *Agave* juice distillation. When considering that 90 000 T/a Mezcal vinasses are produced in Mexico (Robles-González et al. 2012), electricity generation in the state of Zacatecas would lie around 498 $kW_{el}$ from the whole regional Mezcal vinasses production. In Oaxaca, the Mezcal vinasses generation could contribute with 595 $kW_{el}$. A 500 $kW_{el}$ CHP could supply electricity for more than 1600 average Mexican households, without considering transportation losses.

With the amount of electricity briefly calculated in this study, the construction of biogas plants for vinasses AD could be attractive, if a high vinasses amount is available. Batch fermentation of vinasses could be successfully implemented for small factories. Much more aspects need to be considered, like the location of the distillation factories, their proximity with each other, the possibilities to grid injection, financing and invertors, as well as energy Mexican policies, and boundary conditions.

Either way, the potential use of digestate as fertilizer should be analyzed regarding organic matter content, salts and other inorganic fraction. The results of this study suggest the use of low-cost MFCs to minimize the organic load and other contaminants in digestate, to reach the permissible levels in wastewater discharges. A small amount of electricity would be produced.

This work open alternatives to consider the agricultural residues of *Agave* processing in middle and large scale applications for the generation of bioenergy. At the same time, the vinasses disposal problem could be overcome, as well as PET disposal, if PET bottles were used as biofilms for AD enhancement. Digestate could be used as fertilizer, although the long term use of PET bottles in bioreactors should be analyzed according to the respective norms and permissible contaminant levels, before being used in soils.

From this work, some areas of opportunity could be detected. Not only the COD should had been analyzed after AD, but also the content of phenols, or inorganic compounds like sulphates and phosphates salts. More inoculum sources could had been also analyzed. The microorganisms adhered in the biofilm could had been characterized. Vinasses AD in a middle scale bioreactor could had reinforced the findings of these assays. Through these findings obtained, further alternatives for the analysis of vinasses AD can be proposed.

## 8.6 References

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# 9 Summary

The present work analyses alternatives to use agricultural residues of *Agave* processing (vinasses) for bioenergy production, and organic matter removal. The hypotheses proposed at the beginning of this work, are cleared and a deeper understanding of vinasses anaerobic digestion (AD) is achieved. Anaerobic digestion of vinasses results to be a suitable alternative for bioenergy production, thus the chemical oxygen demand (COD) removal rates are higher when using microbial fuel cells (MFC). The enhancement of biogas production is achieved when using cattle manure, instead of the conventional anaerobic sludge. The volatile organic acid (FOS) content is much higher in manure, and so the total inorganic carbon (TAC). In manure, FOS and TAC show values within the recommended in the literature, while in sludge both values are mostly out of the limits. This indicates an incomplete metabolism of the substrate, when using sludge as inoculum. Manure shows a higher buffer capacity than sludge, due to the high conductivity and nitrogen content (N), suggesting a good balance between carbon and ammonium/ammoniac ($NH_4^+/NH_3$). In the literature it is reported that manure contained a higher nutrient content (such as nitrogen and other trace elements) for the microorganisms, so that AD is more efficient.

The use of biofilms can also goal a better AD performance, in terms of biogas and methane production. When comparing, biofilm bioreactor shows 45 % more cumulative biogas production and 70 % cumulative methane production, than control bioreactor. Methane content in biofilm bioreactor achieve stable values between 55 % and 60 %, while control bioreactor only 34 % – 44 %. Due to the high COD content in vinasses, related to the high volatile organic acids, the optimal COD:N-ratio to be used in AD is out of the limits. When an inoculum source, with low nitrogen content is used (sludge), the high amount of carbon sources in vinasses hinders the metabolic activities of the microbial population, lowering methanogenesis. The use of the biofilm hinders the bioreactor wash out effect, separating the hydraulic retention time, from the solid retention time. The microbial population accumulation is guaranteed, so that COD:N adjustment is not necessary. The accumulation of volatile organic acids is not given, so that their successfully conversion to methane takes place. The buffer capacity in the bioreactor is also higher when using biofilms, indicating a good balance between carbon and $NH_4^+/NH_3$. Another advantage of using biofilms is that the amount of hydrogen sulphide ($H_2S$) in biogas is lower, indicating that sulphide oxidizing microorganisms were adhered in the biofilm, so that the successfully conversion of amino acids in methane, instead of $H_2S$ is achieved.

Biochemical methane potential (BMP) assays shows that the optimal substrate to inoculum ratio (S:I-ratio) to be used in batch fermentation of vinasses with cattle manure or anaerobic sludge, should lie by 0.3. High amounts of organic matter could difficult substrate degradation, especially because the methanogenic bacteria reproduces at slower rates than hydrolytic, acetogenic or acidogenic bacteria, creating a bottleneck for material degradation. An accumulation of acids, by high organic matter content, might exceed the microorganism's degradation capacity, reducing the methanogenic activity. Results regarding the optimal S:I-ratio are confirmed with the mathematical kinetic modelling. For both inocula, S:I-ratio 0.3 shows the highest specific methane growth rate µm. The lag phase λ is longer for the assays with manure, which is explained by the higher organic matter content, in terms of COD, volatile solids (VS) and total solids (TS), in comparison to sludge. Modelling curves of assays with manure show good fits to the real curves. Good fits within the theoretical and experimental methane production curves imply an uncomplicated digestion of the substrate. Higher S:I-ratios of 0.5 and 0.7 shows elevated errors or bad fits and long lag phase, indicating the complicated vinasses digestion, due to the high amount of organic matter, resulting in a low methane production potential.

Regarding MFC, for both, batch and continuous tests, the highest power outputs increase with increased COD content in anolyte, up to a COD of 6 g/L for batch and 14 g/L for continuous tests. Higher COD contents (17 g/L) has negative effects in the voltage generation. The electricity production in a MFC is given by the bacterial metabolic activity or REDOX reactions (electrons and protons generation) in the anodic chamber, as well as by the electron acceptor conditions. These conditions are influenced by the anode potential and substrate used as electrolyte. If the electrolyte solution has a significant amount of colloidal particles, internal resistance increase and the electron and proton transfer is hindered. The internal resistance at high COD content (17 g/L) is 474.58 Ω, while internal resistance of the lowest COD tested is 97.1 Ω. The highest COD removal of 93 % is achieved with CODs around 10 – 12 g/L. COD removal occurs because of the direct anodic oxidation mechanism in the anodic chamber. A higher organic matter content hinders the oxidation mechanisms through the saturated state of the anolyte and anode.

COD removal efficiencies between MFC and AD show very different rates. COD removal in MFC is 93 %, while AD reaches 34 and 20 %. Due to the nature of their operation, microbial fuel cells were at first developed for wastewater treatment. Organic or inorganic matter is adhered to the anode for electron transfer. The direct substrate oxidation in the anodic chamber results in a better COD removal, in comparison to AD, where the oxidation processes depend on a variety of parameters of bioreactor operation.

The CFD simulation of the designed reactor shows the optimal inlet diameter to be used for avoiding substrate sedimentation. Also the inflow velocity is tested. Results of this designed reactor, where AD digestate is used as input material for MFC, are not suitable for simultaneous biogas and voltage generation, without COD adjustment. High CODs results in low power densities, so that if substrate is diluted to achieve higher power outputs, a low biogas production takes place.

The present work shows a deeper understanding of the processes related to vinasses AD, and their direct conversion in electricity through MFCs. Comparison of organic load is studied, and new approaches for AD enhancement are researched. The potential use of digestate as fertilizer should be analyzed, regarding a possible high organic and inorganic fraction. Coupling AD – MFC could be used to minimize the organic load and other contaminants in digestate, thus a small amount of electricity can be generated.

According to the present results, it can be concluded that the agricultural wastes of *Agave* processing (vinasses) are suitable for AD. Biogas and methane production can be enhanced, using low-cost alternatives. In large scale distilleries, electricity and heat can be produced from vinasses AD. At middle scales, biogas can directly be used for combustion for the *Agave* cooking and juice distillation. Vinasses AD could be combined with cattle manure, and biogas can be used also for cooking in rural areas. In this way, the problem of vinasses disposal will be overcome, accelerating the development of markets for renewable energy technologies and promoting access to cleaner energy.

# 10 Zusammenfassung

Durch die vorliegende Arbeit wird die Verwendung von organischen Abfällen der *Agave* Verarbeitung (Schlempe) für die Bioenergieerzeugung sowie der Abbau deren organischer Substanz analysiert und bewertet. Schlempe als Reststoff der Alkoholherstellung ist aufgrund des hohen Gehalts an organischen Anteilen, seines Salzgehalts und weiterer Inhaltstoffe umweltschädlich und sollte nicht ohne eine Vorbehandlung Gewässern oder Böden zugeführt werden.

Die zu Beginn aufgestellte Arbeitshypothese wird mittels Laborversuchen im Detail überprüft und das Verständnis bezüglich der anaeroben Vergärung der Schlempe vertieft. Es stellt sich heraus, dass die anaerobe Vergärung eine sinnvolle Möglichkeit der Bioenergieerzeugung ist, obwohl der Abbau organischer Substanz viel höher ist, wenn die Schlempe in mikrobiellen Brennstoffzellen (MBZ) verwendet wird. Die Verwendung von Rindergülle als Impfschlamm in der anaeroben Vergärung liefert höhere Biogas- und Methanerträge als „konventioneller“ anaerober Schlamm aus Kläranlagen. Die chemischen Analysen zeigen, dass, im Vergleich zu Schlamm, Rindergülle ein höheres Puffervermögen in Form von TAC (gesamter anorganischer Kohlenstoff / Carbonat) sowie einen höheren Gehalt an flüchtigen organischen Säuren (FOS) aufweist. Der FOS- sowie der TAC-Gehalt in Rindergülle liegen immer innerhalb der in der Literatur empfohlenen Grenzwerte, während sich die jeweiligen Werte im konventionellen Schlamm meist außerhalb der Grenzwerte befinden. Dies weist auf einen unvollständigen bakteriellen Stoffwechsel beim anaeroben Schlamm hin. Das Puffervermögen in Gülle ist aufgrund der höheren Werte an Leitfähigkeit und Stickstoff (anorg. N) besser. Es besteht ein Gleichgewicht zwischen den kohlenstoffhaltigen Verbindungen und Ammonium/Ammoniak ($NH_4^+/NH_3$). In der Literatur wird Rindergülle mit einem höheren Nährstoffgehalt angegeben. In der vorliegenden Studie wird der Stickstoffgehalt in beiden Inokula gemessen. Rindergülle zeigt einen Wert von 1,5 g/L auf, im Vergleich zu Schlamm mit 0,04 g/L. Die anaerobe Vergärung ist somit viel effizienter mit Rindergülle.

Die Erzeugung von Biofilmen mittels Aufwuchskörpern im Bioreaktor weist auf eine erhöhte Biogas- und Methanproduktion hin. Durch die Erzeugung von Biofilmen werden etwa 40 % mehr Biogas und ein 60 % höherer Methangehalt produziert. Der Methangehalt im Bioreaktor mit einer Nutzung von Biofilmen beträgt 55 – 60 %, während der Reaktor ohne eine Nutzung von Biofilmen nur 34 – 44 % Methangehalt aufweist. Mezcal-Schlempe weist einen sehr hohen chemischen Sauerstoffgehalt (CSB) auf im Vergleich zu anderen Substraten. Das CSB:N-Verhältnis liegt deshalb außerhalb der empfohlenen Grenzen. Bei der Verwendung von anaerobem Schlamm werden die metabolischen Aktivitäten im Bioreaktor bezüglich eines Abbaus der Kohlenstoffquellen verhindert, dadurch dass der Stickstoffgehalt in Schlamm zu niedrig ist. Das CSB:N-Verhältnis befindet sich nicht im Gleichgewicht. Durch Erzeugung von Biofilmen kann der Auswascheffekt der Mikroorganismen im Bioreaktor verhindert werden. Die hydraulische Verweilzeit wird von der Feststoffverweilzeit getrennt. Die Mikroorganismendichte kann konstant hoch gehalten werden und das unausgewogene CSB:N-

Verhältnis kann die Vergärung nicht beeinträchtigen. Die Anhäufung flüchtiger organischer Säuren im Bioreaktor stellt ein Problem für viele Bakteriengruppen dar und behindert deren Stoffwechselaktivitäten. Der Biofilm-Bioreaktor zeigt keine Anhäufung von FOS und erreicht eine unkomplizierte Umwandlung von FOS in Methan. Das Puffervermögen eines derartigen Bioreaktors weist auch höhere Werte auf, es liegt ein Gleichgewicht zwischen den Kohlenstoffverbindungen und $NH_4^+/NH_3$ vor. Zusätzlich liegt der Schwefelwasserstoffgehalt ($H_2S$) im Biogas aus dem Bioreaktor mit Biofilmen um bis zu 20 % tiefer. Sulfid-oxidierende Bakterien sammeln sich offensichtlich im Biofilm an, so dass eine hohe Umwandlung von Aminosäuren in Methan möglich ist.

Versuche mit einer absatzweisen Vergärung zeigen ein optimales Verhältnis von Substrat zu Inokulum (S:I-Verhältnis) von 0,3 bezüglich der organischen Trockensubstanz auf. Wenn die Beladung an organischen Substanzen im Substrat im Verhältnis zur aktiven Bakterienmasse zu hoch ist, wird der Substratabbau behindert. Die Vermehrungsrate methanbildender Bakterien ist überwiegend langsamer als die der hydrolytischen, säurebildenden oder essigsäurebildenden Bakterien. Das wirkt sich auf die Methanbildungsrate aus. Das optimale S:I-Verhältnis wird anhand einer mathematischen Modellierung beschrieben. Die Modellierung deutet ein S:I-Verhältnis von 0,3 als optimal an, sowohl bei anaerobem Schlamm als auch bei Rindergülle. Die Verzögerungsphase $\lambda$ bei der Wachstumskurve von Mikroorganismen ist bei Rindergülle länger als beim Schlamm. Rindergülle zeigt höhere Kohlenstoffgehalte gemessen als organische Trockensubstanz und CSB. Die Kurven der Methanerzeugung aus Schlempe mittels Rindergülle zeigen eine bessere Übereinstimmung zwischen Modell und gemessenen Daten als mit Schlamm als Impfmaterial. Ähnlichkeiten zwischen den experimentellen und modellierten Kurven weisen auf eine unkomplizierte anaerobe Vergärung hin. Liegt das S:I-Verhältnis deutlich höher, z.B. bei 0,5 oder 0,7, dann ist der Kurvenverlauf stark abweichend und die Verzögerungsphase ist länger. Dies deutet auf einen komplizierten Abbau aufgrund des überhöhten Gehaltes an organischer Substanz hin.

Die absatzweisen und kontinuierlichen Versuche mittels mikrobiellen Brennstoffzellen (MBZ) zeigen eine Verbesserung der Leistungsausbeute bei steigendem CSB-Gehalt im Substrat mit maximalen Werten bei 6 g/L im Batchansatz und 14 g/L bei den kontinuierlichen Versuchen. Höhere CSB-Gehalte (10 oder 17 g/L) verursachen eine Hemmung der Spannungserzeugung. Die Spannungserzeugung einer MBZ wird durch die Stoffwechselaktivitäten der Bakterien im Elektrolyt, dem Elektronenakzeptor für die Oxidation in der Anode und die Redoxreaktionen bestimmt (Elektronen- und Protonenerzeugung). Der Innenwiderstand in der MBZ steigt, wenn der Elektrolyt viele kolloidale Partikel aufweist (im Fall der Mezcal-Schlempe). Gleichzeitig sinken die Elektronen- und Protonentransferprozesse. Der Innenwiderstand beim höchsten CSB-Gehalt von 17 g/L beträgt 474,5 Ω, während der Innenwiderstand beim niedrigsten CSB-Gehalt nur 97,1 Ω beträgt. Der Abbau organischer Substanz wird analysiert. Der höchste Abbau von 93 % wird anhand der CSB-Gehalte gemessen. Der Abbau von CSB geschieht aufgrund der anodischen Oxidation im Anodenkompartiment. Aufgrund des hohen CSB-Gehalts erreichen der Elektrolyt und die Anode den gesättigten Zustand, wodurch der Innenwiederstand steigt und die Oxidation verhindert wird. Folglich sinkt die Leistungsdichte.

Anaerobe Vergärung und MBZ zeigen eine sehr unterschiedliche Abbaurate des CSB. Der Abbau in der MBZ erreicht 93 %, während bei der anaeroben Vergärung nur ein Abbau von 20 % bis 34 % erreicht wird. MBZs werden grundsätzlich in der Praxis für den Abbau organischer und anorganischer Substanzen eingesetzt, wobei diese Substanzen an der Anode zugegeben werden. Die Redoxreaktionen, die unmittelbar im Anodenkompartiment stattfinden, ergeben einen höheren CSB-Abbau im Vergleich zur anaeroben Vergärung, wobei Oxidation und Umwandlungsprozesse von vielen anderen Bioreaktorparametern abhängen. Mittels CFD-Simulation (computergestützte Fluiddynamik) können der Zulaufdurchmesser und der minimale Durchfluss überprüft werden, um eine Sedimentation im Reaktor zu verhindern. Der Gärrest aus der anaeroben Vergärung (Mezcal-Schlempe mit Rindergülle) dient als Einsatzstoff für eine MBZ. Dabei werden sowohl Biogas und als auch Spannung in einem geringen Maße erzeugt. Eine höhere Biogasausbeute wird durch einen höheren CSB-Gehalt erzielt, gleichzeitig sinkt jedoch die Spannungserzeugung. Ein niederer CSB-Gehalt ergibt höhere Spannungswerte, die Biogasausbeute nimmt allerdings ab.

Diese Arbeitsergebnisse vertiefen das Verständnis der anaeroben Vergärung von Mezcal-Schlempe, sowie dessen Umwandlung in Elektrizität durch eine MBZ. Das Betreiben von MBZ mit Mezcal-Schlempe sowie deren Koppelung mit einer anaerober Vergärung zeigen neue Perspektiven für weitere Recherchen zur energetischen Nutzung von Mezcal-Schlempe.

Die Ergebnisse dieser Arbeit zeigen, dass landwirtschaftliche Reststoffe der Alkoholproduktion aus *Agave* für die Weiterverwendung in der anaeroben Vergärung geeignet sind. Diese Technologie kann erfolgreich für die Erzeugung von Elektrizität und Wärme in großen Destillerien verwendet werden. In kleineren Destillerien kann das gewonnene Biogas gereinigt und direkt für die Erhitzung von *Agave* und die Destillation des fermentierten Safts benutzt werden.

Die Verwendung des verbleibenden Gärrests aus der Schlempe-Vergärung soll tiefer recherchiert werden, besonders wegen des hohen Gehalts an organischer Substanz, anorganischen Salzen und anderer Inhaltstoffe. Die Koppelung einer MBZ mit der anaeroben Vergärung kann die unerwünschten Inhaltsstoffe aus dem Gärrest entfernen, so dass dieser als Dünger verwendet werden kann. Zusätzlich kann eine geringe Spannung erzeugt werden.

Durch die Nutzung der Schlempe zur Bioenergieerzeugung wird eine Alternative zur Entsorgung geschaffen, die sich zudem positiv auf die Entwicklung des Marktes für erneuerbare Energien auswirkt.