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BIOGAS PRODUCTION

FROM ORGANIC WASTE, A SUSTAINABLE ALTERNATIVE FOR THE ENVIRONMENTAL DEVELOPMENT

PRODUCCIÓN DE BIOGÁS A PARTIR DE RESIDUOS ORGÁNICOS, UNA ALTERNATIVA SUSTENTABLE PARA EL DESARROLLO AMBIENTAL

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ABSTRACT

Vinasses are the main organic waste from alcohol obtention with numerous toxic compounds for the environment. The discharge of untreated vinasses in water bodies or soils has significant environmental and social impacts. Anaerobic digestion is a method used for the treatment of organic wastes such as vinasses, and the simultaneous production of bioenergy and biomaterials. The use of biofilms in bioreactors favors biogas production, reducing the number of toxic components. In the present study, the use of a low-cost biofilm made of PET or polyethylene terephthalate was evaluated in a 6 L bioreactor digesting mezcal vinasses and cow manure. The production of biogas and methane was analyzed. The biofilm bioreactor generated 40 and 70 % more biogas and methane and lower hydrogen sulfide, in comparison to the control bioreactor. Organic acids did not accumulate in the bioreactor with biofilm, while the control bioreactor showed an accumulation of these acids. This work presents an alternative use of reusable and low-cost biofilms, improving the effectiveness of anaerobic digestion.

Keywords: Biogas, Methane, Biofilm, PET, Contamination.

RESUMEN

Las vinazas son el principal residuo orgánico de la obtención de alcohol, residuo que contiene numerosos compuestos tóxicos para el medio ambiente. La descarga de vinazas sin tratamiento previo en cuerpos de agua o suelos tiene importantes impactos ambientales y sociales. La digestión anaeróbica es un método utilizado para el tratamiento de residuos orgánicos como vinazas y la producción simultánea de bioenergía y biomateriales. El uso de biopelículas en biorreactores favorece la producción de biogás, reduciendo la cantidad de componentes tóxicos. En el presente estudio se evaluó el uso de una biopelícula de bajo costo hecha de PET o tereftalato de polietileno en un biorreactor de 6 L que digiere vinazas de mezcal y estiércol de vaca. Se analizó la producción de biogás y metano. El biorreactor con biopelícula generó entre un 40 y un 70 % más de biogás y metano, así como menos sulfuro de hidrógeno, en comparación con el biorreactor de control. Los ácidos orgánicos no se acumularon en el biorreactor con biopelícula, mientras que el biorreactor control mostró una acumulación de estos ácidos. Este trabajo presenta un uso alternativo de biopelículas reutilizables y de bajo costo, mejorando la efectividad de la digestión anaeróbica.

Palabras clave: Biogas, Metano, Biopelícula, PET.

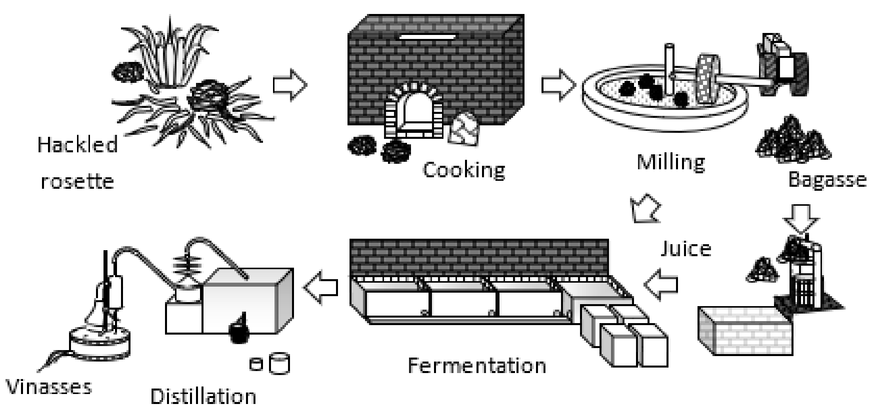
INTRODUCTION

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.

Tequila and Mezcal are very important products for the Mexican economy and are produced from different kinds of Agave. 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). 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. The denomination of origin of Tequila includes the states of Jalisco, Nayarit, Guanajuato, Tamaulipas, and Michoacán. 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, 2024; CRM, 2024).

For tequila and mezcal production, agave is harvested from arid regions. When the stem begins to grow, many sugars are synthetized, so that it can continue growing. Afterwards the plant reproduces 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. At the end of the process, vinasses remain as an agricultural residue. In figure 1 a general schema of the mezcal production is presented.

Fig 1: Scheme of Tequila and Mezcal production from Agave



Source: Godínez (2017)

Great volumes of vinasses with high pollutant charge remains in Mexico every year. From each liter of tequila produced, around 10- and 12-liters vinasses are being generated and from mezcal production around 8 and 15 liters. The number of produced vinasses and characteristics depends on the Agave species and schnapps production processes. 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 due to their low pH in the range between 3 and 5, high organic matter content up to 50 000 mg O2/L as BOD (Biological Oxygen Demand) and 150 000 mg O2/L as COD (Chemical Oxygen Demand). Approximately 80 % of vinasses are discharged into water bodies and soils, what causes severe environmental problems. The high contents of salts in vinasses can lead to sodicity and salinity, what deteriorate soil porosity, structure, and fertility. Besides, accumulation of high suspended solid loads can lead to phytotoxicity and might inhibit seed germination. If vinasses are discharged at temperatures around 50 and 80 °C, they may rise water temperature up, so that dissolved oxygen lowers under levels where fish survival is no longer possible. The high contents of phosphor and nitrogen could also lead to eutrophication in water bodies, a significant contamination issue (López-López et al. 2010).

Nevertheless, anaerobic digestion (AD) is one of the most used methods for wastewater and organic wastes treatment due to the low operational costs and the possible generation of bioenergy and biomaterials. Much research has been done in regard to this technology (Méndez-Acosta et al. 2010; Nunes et al. 2022). Through AD, not only bioenergy in form of methane, and biomaterials such as biofertilizers could be produced, but also the removal of organic matter and other toxic agents can be achieved.

Depending on the biomass source a special management may be needed. For example, residues such as vinasses contain high levels of salt and organic contents. When used in a bioreactor for biogas production, they could lead to high OLRs and thus poor AD efficiencies (Arif et al. 2018). Active bacteria can continuously flow out of the bioreactor when extracting the treated substrate, which implies higher retention times, microorganism's washout effect and decrease of active microbial population. An alternative to solve this problem is the bacteria immobilization on a solid support, accumulating the number of microorganisms in bioreactor, and thus increasing the capacity to degrade organic matter (Nguyen et al. 2021). With the use of biofilms, the hydraulic retention time can be separated from solid retention time, diminishing the bacteria washout effect in the bioreactor, and incrementing 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 anaerobic digestion process (Cayetano et al. 2022). Support materials such as carbon fiber, resin, concrete, polyurethane foam, seashell, charcoal, break, gravel, ceramic, sintered glass, fire bricks, natural stones including limestone, gravel, pumice, clay, rocky aggregates, sand granual activated carbon, saponite, and synthetic plastic materials have been tested (Arif et al. 2018; Liu et al. 2017). Martí-Herrero et al. (2014) used strips of polyethylene terephthalate (PET) bottles as biofilm carrier to test the anaerobic digestion of cow manure at psychrophile temperature. Biogas production was enhanced at 6.1°C.

PET is a thermoplastic polymer widely used in the modern world for manufacturing bottles, food packages and even textiles. PET is lightweight and has high tensile strength and chemical resistance. It can resist up to 80 °C without losing mechanical properties. Besides, it is recyclable for its further use in manufacturing textile, carpets or clothing (Dhaka et al. 2022). However, the demand on PET has increased worldwide. In 2019 around 650 billion PET plastic bottles were produced, ending as plastic waste and even worst, contaminating marine environment. It was estimated that more than eight million tons of PET land into the ocean every day (Pudack et al. 2020). For this reason, it is important to develop strategies for PET reuse and recycling. The most common PET recycling techniques are mechanical recycling, chemical recycling, and bio recycling (Joseph et al. 2024). Mechanical recycling involves used PET collecting and shredding, for its further washing and melting to form new PET products. Mechanical recycling is cost-effective, but implies limitations such as quantity of collected PET and quality of the further output products manufactured. Therefore, other PET recycling technologies should also be implemented.

This paper pursues different goals, considering technical, academic, and scientific aspects. First goal was to analyze the effect of using a biofilm carrier by the start-up and operation of a bioreactor. A low-cost PET biofilm was placed inside a bioreactor where mezcal vinasses and cattle manure were anaerobic digested. A similar bioreactor without biofilm was also prepared for anaerobic digestion. The comparison regarding efficiency of biogas and methane production was performed between both bioreactors. Biogas was daily measured with regard to quantity and quality.

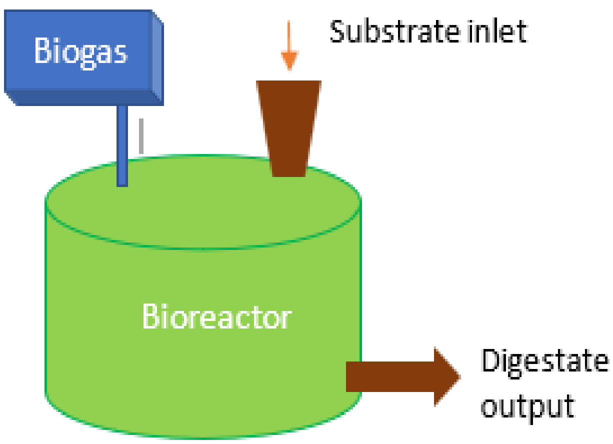
A further goal pursued through the present study is to bring together not only theory, but also empirical evidence and statistical data regarding the use of PET biofilms for anaerobic digestion. Much research has been done regarding the anaerobic digestion of vinasses, but few focusses on the use of PET material as biofilm carriers. Publishing the present research paper aims to be an important instrument for passing on knowledge to scientists working in the field of anerobic digestion and efficiency through biofilm carriers. Furthermore, a deep analysis of bacteria consortia can be proposed, where bacteria capable to produce biogas and degrade PET is implemented for waste mitigation, as well as biomaterials and bioenergy production.

MATERIALS AND METHODS

Bioreactor configuration

Two bioreactors made of PVC (polyvinyl chloride) with a volumetric capacity of 6 L were filled with 4 L cattle manure and 1.1 L mezcal vinasses. At the top of the bioreactor, a tedlar bag was connected in order to collect the biogas produced daily (see figure 2).

Fig 2: Bioreactors scheme.



Source: Own creation

Inside the bioreactor with biofilm, called BF, a biofilm made of six sanded and overlapped PET bottles was placed. The contact surface of the complete biofilm placed in the bioreactor was 0.212 m2. The bottles were sanded in order to achieve a porous surface, which could help enhancing the adsorption capacity of the material, and so its capacity to retain the microorganisms forming the biofilm. In bioreactor called B0 no biofilm was placed. Vinasses pH was adjusted to 7 with NaOH (sodium hydroxide) prior to start-up and each feeding (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 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).

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 1 shows the characteristics measured in the cattle manure and mezcal vinasses.

Table 1: Characteristics of cattle manure and mezcal vinasses.

Parameter	Cattle manure	Mezcal vinasses
pH @ 27°C	8.29	4.41
Chemical oxygen demand COD (g/L)	31.10	63.73
Total solids (TS)	3.07	5.26
Volatile solids (VS)	1.80	2.88 2.88
Total dissolved solids TDS (g/L)	14.14	5.87
Total nitrogen (g/L)	1.80	0.13
Conductivity (µS/cm)	28240	11750
REDOX (mV)	-352	-142
Volatile organic acids (gHAc/L)	1585	N/A
Total inorganic carbonate (gCaCO3/L)	9525	N/A
FOS/TAC (volatile organic acids / total inorganic carbonate)	0.17	N/A

Source: Own creation

Bioreactor start-up

The start-up consisted of filling BF and B0 at a SI-ratio (substrate to inoculum 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 by the 60th day of experiments. OLRs increased weekly from 0.4 to 6.17 gVS/Ld. After the stepwise increase, a stable methane content above 60 % could be appreciated in BF. After this point only 0.05 L of substrate, which means 1 % v/v of mezcal vinasses were added every two days.

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 sulfide were measured with a biogas analyzer Multitec 540 from the German company SEWERIN GmbH. Vinasses and cattle manure were characterized regarding pH, REDOX, TDS (ppm) and conductivity (µS/cm). FOS/TAC was measured with a manual titration device. REDOX and pH were measured with a pH-meter VWR-110. TDS and conductivity were measured with a waterproof tester from HANNA Instruments HI-98311. COD, TS (total solids), and VS (volatile solids) were measured according to the norms DIN 38414-9:1986-09 (1986) and VDI 4630 (2016). At the end of the experiments, biofilm was analyzed with a conventional SONY W800/B 20 MP digital camera and an optical microscope LEICA DMS 1000.

Statistical analysis

An ANOVA (analysis of variance) was performed in order to confirm if biogas and methane produced in BF were statistically significantly different to B0. Data were statistically analyzed by one-way ANOVA and the Tukey´s post hoc test in Minitab® 17.1.0 Statistical Software was used to determine the statistically difference between both bioreactors at the 0.05 level.

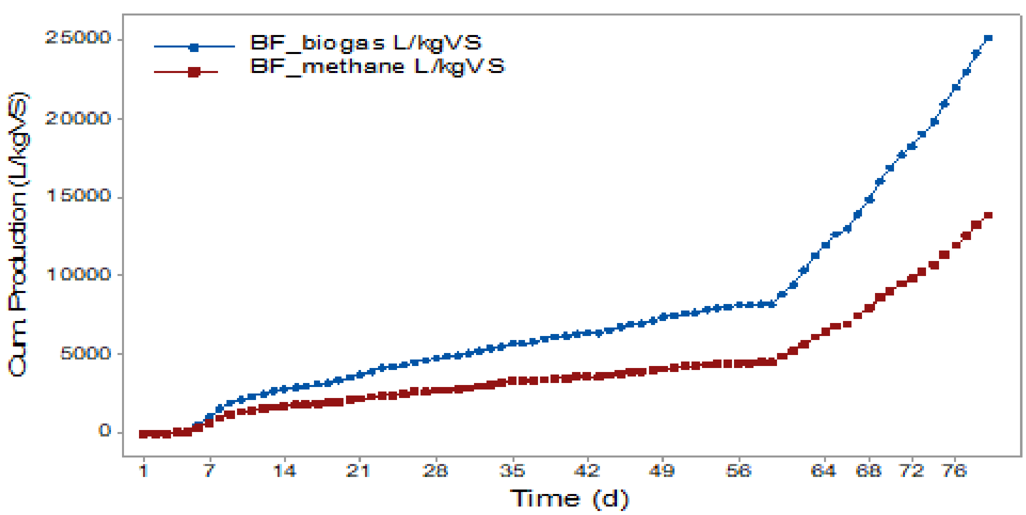
RESULTS AND DISCUSSION

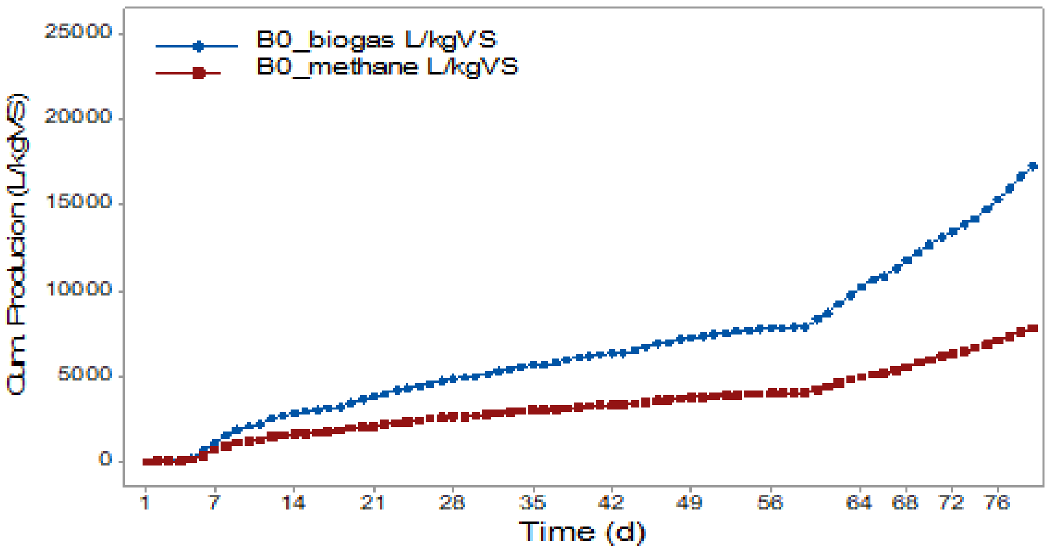
Biogas, methane and hydrogen sulfide production

Biogas and methane production were reported according to the norm VDI 4630 (2016). The cumulative biogas and methane production curves are shown in figure 3. After 80 days of experiments, B0 produced 17244 Lbiogas/kgVS and 7833 LCH4/kgVS, whereas BF produced 40 % more biogas and 70 % more methane, 25151 and 13899 L/kgVS correspondingly. When looking at the solely daily methane produced, BF and B0 produced during the first 60 days of experiments between 50 and 100 LCH_4d/kgVS. By the end of the assays, methane production achieved nearly 250 LCH_4d/kgVS, whereas BF production reached 700 LCH_4d/kgVS. 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. (2017) reported a biogas and methane enhancement of 40 and 49 %, correspondingly, when using a polypropylene fiber as biofilm carrier. Gong et al. (2011) 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. Juárez-García et al. (2022) obtained a high organic matter and fatty acids removal of more than 80 %, digesting municipal solid wastes at high organic loading rates, using a silica sand biofilm. The results of the present assays demonstrate that PET is an accurate alternative as biofilm carrier, besides the fact that the overproduction of PET worldwide has become a serios environmental problem. Reusing PET bottles for AD could hinder their disposal in landfills and water.

Fig 3: Cumulative biogas and methane production in L/kgVS (volatile solids) vinasses, B0 bioreactor without biofilm, BF bioreactor with biofilm.



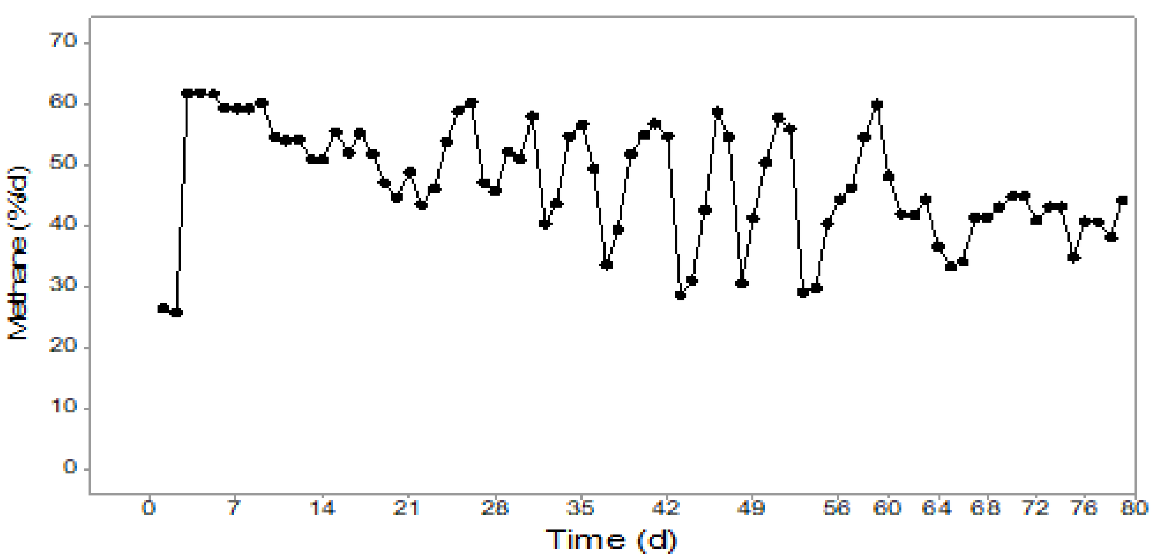


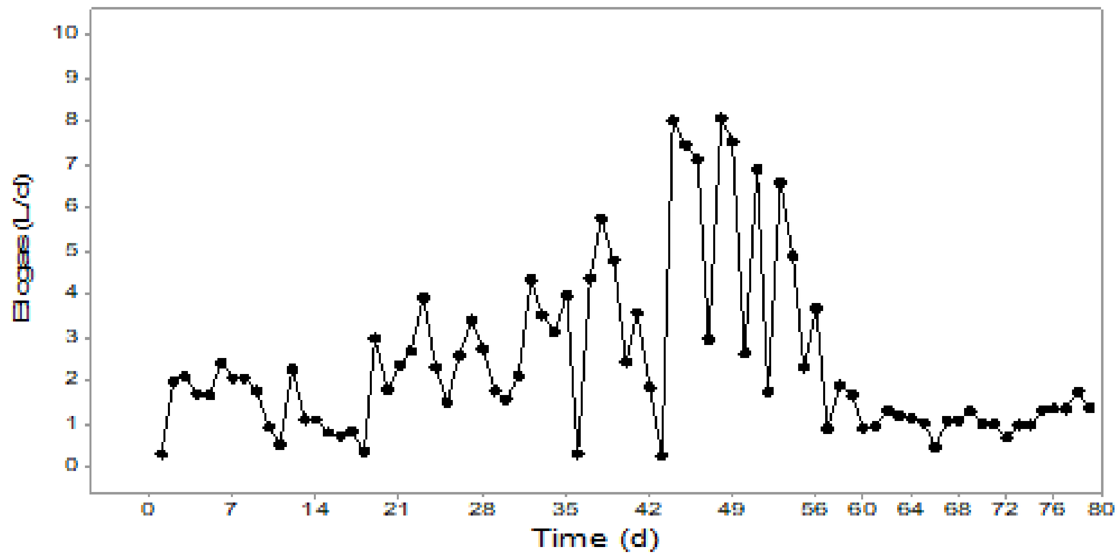
Source: Own creation.

The daily biogas produced in L and its methane content in % are shown in figures 4 and 5. In both bioreactors, vinasses addition took place every seven days, increasing weekly the amount of influent starting with 0.1, 0.15, 0.25, 0.35, 0.45, 0.55, 0.75, 0.85, 1.05, 1.15 and 1.5 L by the 60th day of experiments. Afterwards, when the methane content achieved stable values for BF, feeding took place every two days. The addition of vinasses is directly reflected in figures 4 and 5 just directly after every feeding, when an increased biogas and methane production can be noticed. Five or six days after feeding took place, biogas and methane generation decreased, and augmented again after feeding once more time. BF shows a constant increasingly methane production the last three weeks

of experiments.

Fig 4: Biogas quantity in L/d and methane content in %/d, in bioreactor without biofilm (B0).

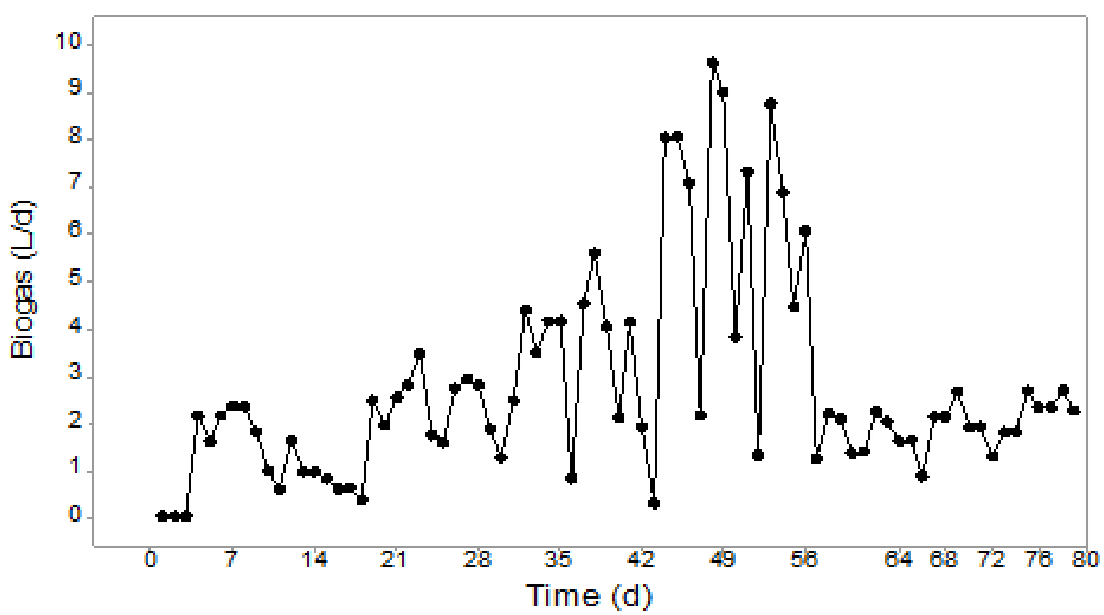


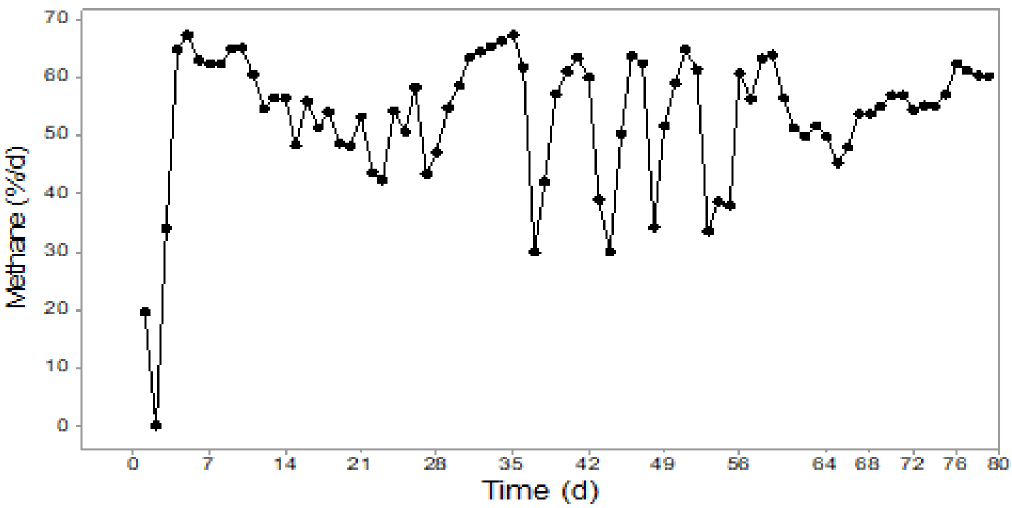


Source: Own creation

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 biogas with 25 % methane by the second day of experiments. These facts suggest, that the biofilm formation took place during three days. Similar results were reported by Langer et al. (2014). When detecting the microcolons formed in the biofilm during AD, microorganisms were appreciated after three days of incubation.

Fig 5: Biogas quantity in L/d and methane content in %/d, in bioreactor with biofilm (BF).





Source: Own creation

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 a 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 (hydrogen sulfide) content in biogas was also affected when the biofilm was placed in the bioreactor. B0 produced 12588 mg/L $H_2$2S, whereas BF produced 20 % less H_2S (10283 mg/L). H_2S is considered as biogas impurity and is not desired in biogas. This trace element is found in biogas in ranges the ranges 50 – 10000 ppm (or mg/L). It can cause corrosion in the engine and metal parts, where biogas is converted to energy. H_2S emits SO_2 (sulfur dioxide) when biogas is being combusted. In practical applications, the content of H_2S in biogas has been a limiting factor for power generation from biogas (Friehe et al. 2016). Several technologies for H_2S removal have been developed. As a biological treatment, sulfide oxidizing microorganisms convert biogas sulfur compounds in elementary sulfur. The possibility 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.

Statistical analysis

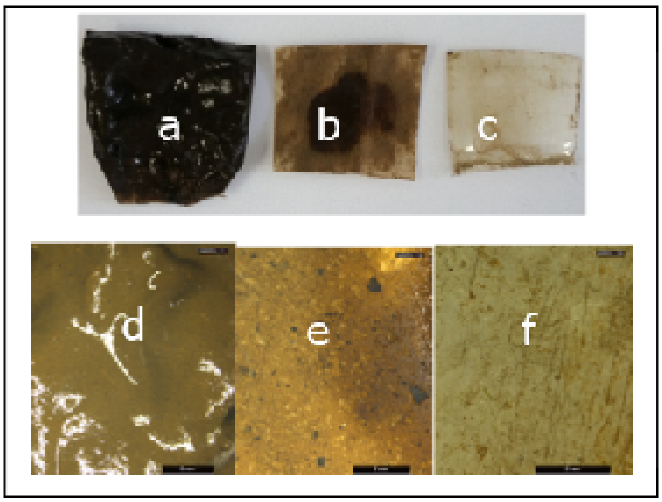
Results regarding the one-way ANOVA show a statistical significance with a p-value of 0.031, when comparing methane production between B0 and BF. If $p < 0.05$ the null hypotheses is rejected, what indicates a statistically significant difference between samples. Regarding biogas production, no statistical difference between B0 and BF could be appreciated due to the calculated p-value of 0.217. In conclusion, PET biofilm in bioreactor has a significant impact on the methane production, which is the main product of the anaerobic digestion. This could be confirmed in figure 2, where the difference between biogas production of B0 and BF was almost 50 %, while methane production was almost twice as high for BF, in comparison to B0. These results suggest that using the PET biofilm, the VFAs present in the bioreactor could be succesfully converted into methane, which is mostly synthetized by these acids.

Biofilm

Figure 6 shows the photographs of the biofilm taken at different sides of the sanded PET bottles. Figures 6d, 6e, and 6f were taken with the optical microscope. Figures 6a, 6b and 6c were taken with the digital camera. The bottles were overlapped so that the internal side of one bottle made direct contact with the external side of the other one, contributing to the accumulation of microorganisms in the space between. At the end of the experiments, the overlapped internal side of the bottles had the highest amount of adhered microbial population, like in figures 6a and 6d. The internal side of the bottles, which could not be overlapped, accumulated less microorganisms, like in figure 6b and 6e. The external side of the bottles is shown in figure 6c and 6f, which showed the fewest amount of microorganisms adhered. The sanded surfaced can be seen in figure 6c and 6f. It can be noticed that sanding the surface did not led to microorganism's adhesion, like in the case of overlapped bottles.

This images together with the results of the biogas and methane production demonstrate that the microbial population adhered on the biofilm could convert organic acids into biogas and methane more successfully. When using biofilms, no accumulation of acids took place, what suggests, that the microbial population adhered to the biofilm convert the organic acids into biogas and methane successfully.

Fig 6: Biofilm by the end of the experiments, comparison of a) overlapped internal side b) internal side c) external side.



Source: Own creation

Social impacts

The fact that vinasses are discharged in the environment without any pretreatment has many social impacts. First in health and safety because vinasses cause eutrophication of water bodies, which leads to an excessive algal and microorganism's growth. Besides, vinasses are thrown at high temperatures in lakes, what leads to death of indigenous aquatic species. A poor environmental quality of water bodies can result in health issues causing unfavorable sanitary conditions and economic deprivation for socially excluded communities. Furthermore, detrimental environmental conditions can impact employment, as investment diminish leading to scarcely job opportunities. Community cohesion and social relationship can be sensitive to environmental conditions. How the environmental pollution affects the society should be considered as a significant topic in social studies, engineering, and environmental sciences, so that students understand their place in the world and the importance to take actions which benefits the environment.

CONCLUSIONS

A biofilm carrier was successfully used in the bioreactor enhancing the availability of microorganisms that could metabolize the high organic matter, despite an unbalanced COD/N ratio, especially due to the high amount of organic oxygen demand or organic matter in vinasses. The effect of biofilms was carried out in assays digesting with cattle manure as inoculum

By day 60, when feeding was done every 7 days, cumulative biogas production had achieved similar values. Nevertheless, when the feeding took place more frequently, bioreactor with biofilm showed a more efficient AD, with a higher methane production. Biofilm bioreactor achieved stable methane values around 55 - 60 %, while control bioreactor achieved more unstable values around 34 - 44 %. In general, bioreactor with biofilms produced 40 % more biogas and 70 % more methane than control bioreactor. The amount of produced H_2S was 20 % lower for bioreactor biofilm, suggesting that biofilm carrier could be considered as a biological treatment for H_2S. Sulfide oxidizing microorganisms could degrade amino acids, responsible of the H_2S production in biogas.

The results of the biofilm assays demonstrate that PET is a low-cost and efficient alternative as biofilm carrier. It is important to consider that the overproduction of PET worldwide has become a serios environmental problem, and reusing PET bottles for AD could hinder their disposal in landfills and water.

Results suggest 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 number of microorganisms could be adhered. The results of the optical analysis showed that sanding PET bottles did not led to a higher number of adhered microorganisms. Methane production indicate that feeding could had taken place more frequently than 7 days, increasing the biogas and methane production. Considering that PET bottles are low-cost biofilm carriers, and their reutilization can avoid environmental problems, PET means as a good alternative to other materials. Furthermore, the implementation of PET bio recycling using bacteria consortia contained in the inoculum, i.e. *Ideonella sakaiensis*, which can both produce biogas and degrade PET, can be further analyzed for the simultaneous waste mitigation and production of value-added feedstock for chemicals or electricity generation. A biorefinery concept could be proposed, where PET monomers from digestate could be extracted and further used. Through this work, new alternatives are opened for a deeper analysis on the dynamic of the biofilm formation, as well as the reduction of H_2S in biogas through biofilm bioreactors.

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