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Preliminary Study of Biomethane Production of Organic Waste based on their Content of Sugar, Starch, Lipid, Protein and Fibre

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The biomethane potential (BMP) of five organic sources with different proximal composition have been investigated during anaerobic digestion (AD). The organic sources have been selected among different Ecuadorian biomass residues according to the criteria, that each one has a high content of one parameter of interest. Therefore, the five organic sources are: blackberries as a residue high in sugars, avocado for high content in lipids, soybean representing high protein content, green plantain peel for starch and sugarcane bagasse for high fibre content. The experimental setup was developed in batch reactors at the mesophilic regime (35 °C). All experiments are prepared with a reactive mixture composed of inoculum, basal nutritive medium (BM) and the organic material (OM) diluted in water with approximate 10% total solids. The experiment of each biomass was performed in three sequential times. The feed and the final reactive mixture were analysed according to the following parameters: chemical oxygen demand (COD), total solids (TS), total volatile solids (VS), pH, volatile fatty acids (VFA) and nitrogen (N). The biogas production is measured by the water displacement method, while the methane content was analysed by GC-TCD / FID. The results showed that the first experiment with high protein content showed the highest cumulative biogas production with 2300 mL in 21 days, while the three experiments with high sugar content produced in average the most biogas (23 mL/day). The high fibre experiment presented the highest methane content as well as the lowest concentration of VFA with 2667 ± 103 ($mg_{CH3COOH}$/L). Since the VFA profiles presented higher values for the different concentrations, which was also related with low final pH values of reactive mixture, the lowest pH value (4.6 ± 0.8) was observed for reactors charged with high sugar content.

1. Introduction

The agribusiness in Ecuador is an important contributor to the economy. This sector contributed in 2016, with the 6.4 % to the annual GDP (Banco Central del Ecuador, 2016). Therefore, large quantities of agricultural waste are produced annually from the agribusiness and food industries. Also the fast increase in population growth, with an annual growth rate of 1.3% (Instituto Nacional de Estadistica y Censos, 2017), has led to an increase in food demand and, therefore, in waste generation.

Though, agricultural residues are an important feedstock for the generation of biofuels, which could supplement or replace the current non-renewable fossil fuels (Paudel et al., 2017). Bioenergy can be produced from diverse biomass feedstock including food waste, agricultural and livestock residues. These feedstock can be used to produce electricity or heat, or to generate gaseous, liquid or solid fuels (Toklu, 2017). Anaerobic digestion (AD) is one of the most interesting and cost-effective waste management and remediation technologies available for the energetic utilization of residual biomass (Paudel et al., 2017). AD is a biochemical process where the conversion of biomass has the objective to transform solid or semi-liquid materials, with low energy concentration into fuels and fertilizers through four main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (García et al., 2012). During this process the nutrient content of the substrate is an important criterion for the successful performance of each step. It is required that the reactive mixture contains a balanced C/N ratio between 20 – 30 to 1 and that the pH value lies at a neutral regime (6.0-7.5) (Xu et al., 2017). Also a stable temperature is desired during the process since the microorganisms are very sensitive to small changes in temperature (Xu et al., 2017).

Therefore, previous studies have investigated AD process by varying different parameters as temperature regimes for one specific residue (Almeida Streitwieser, 2017; Meng *et al.*, 2016), increasing TS content (Yi et al., 2014) or the effect of pH-value (Kumanowska et al., 2017). Also, the biomethane potential of a wide variety of different materials have been studied (Hoo et al., 2017; Ge et al., 2014; Motghare et al., 2016). It is possible to find studies with substrates high in lipids (Cirne et al., 2007) or carbohydrates (Li et al., 2017), but these results are difficult to compare between each other because the anaerobic digestion process is very sensitive to the different operational conditions like dilution, inoculum source, variations in temperature, nutrients content, among other. (Ge et al., 2014; Morero, Vicentin and Campanella, 2017; Zhang et al., 2017). Xu et al studied the interaction of nutrient balance and microbial community structure (Xu et al., 2017). However, a detailed study of the correlation between the nutrient content on the biomass sources and the biogas production in AD is not available. Therefore, it is important to investigate methodically the difference in the degradation rate and the biogas production during anaerobic digestion of biomasses with significant different proximal composition.

In this study, the preliminary results of the biomethane potential of five different biomass sources are investigated at the mesophilic regime (35°C) in 1L batch reactors and evaluated according to the specific biogas production and the rate of degradation. With this purpose the feed and the reactive mixture after the AD process were analysed according to the following parameters: chemical oxygen demand (COD), total solids (TS), total volatile solids (VS), volatile fatty acids (VFA), nitrogen, protein, phosphorus and total organic carbon (TOC).

2. Materials and Methods

2.1 Sample selection, collection of biomass species for the study

Different agricultural residues were collected from local agribusinesses in Quito and nearby places to the region. Ten biomasses were pre-selected, but the five sources with the highest content in nutrients were selected and investigated for the AD process. The five selected biomass species include residues of blackberry (*Rubus fructicosus*), soybean (*Glycine max*) and avocado *(Persea americana*), green plantain peel (*Musa spp*), and sugarcane bagasse (*Saccharum officinarum*). Their composition could be compared to the data reported at (USDA, 2017), (Arun et al., 2015), and (Valiño et al., 2004).

2.2 Proximal characterization

The characterization of the residual biomasses was realized according the following methods. Chemical Oxygen Demand (COD) is determined by colorimetric method with the Test'n Tube vials, based on the procedures presented by Almeida, (Almeida Streitwieser, 2017). Moisture and ash content were determined according to the AOAC methods 4.1.10 (AOAC, 1990) by drying to a constant weight at 105 ^{o}C and calcinating at 550°C. Lipids were extracted in a solvent recovery extractor model Det-gras N – VELP Scientifica for 2h, using 50mL of hexane (95%). The extracted lipids were heated at 105 °C in an oven for 1h and determined by weight difference (VELP Scientifica, 2016). Total nitrogen content (N) was determined by Kjeldahl method, where samples were digested using the DK 6 Unit – VELP Scientifica and later distilled with UDK 129 Distillation Unit – VELP Scientifica. The final value of percentage of nitrogen (N) in the sample was determined by colorimetric titration (VELP Scientifica., 2015b). Crude protein was expressed as 6.25 x N. Starch contents of biomass were determined by the Total Starch (AA/AMG) test kit based on AOAC method (996.11) and AACC method (76.13.01) by spectrophotometric at 510 nm (Megazyme, 2017). Fibre amount was analysed according to AOCS-AOAC method 962.09 (AOAC, 1990). Finally, reduced sugar content was determined by volumetric method Lane-Eynon according to AOAC method 923.09 (AOAC, 1990).The results were compared to values reported in literature and expressed as the mean ± standard deviation. In some samples a non-measurable result was obtained, which is expressed as N/A in tables.

2.3 Experimental Setup

Five hermetically sealed borosilicate glass batch reactors (Fisherbrand) with a working volume of 800 mL and a biogas exit line were installed at a heating bath with a constant temperature of 35 ± 1°C. The daily biogas production was obtained by volume displacement of a solution 0.05N H_2SO_4, which was measured in a 2L volumetric cylinder. Each experiment had a duration of 24 days and was repeated three times for each biomass. The feed for each experiment was prepared by diluting the organic material with water until a total solid content of approximately 10% was obtained. Then 50 ml of basal nutritive medium (BM) were added to the feed to overcome possible nutrient deficiencies according to the medium suggested by (Owen et al., 1979). Before the mixture was filled into the flask, the pH value of the feed was adjusted to 7.2 – 7.6 with a solution of NaOH 0.2M and the inoculum was added to the mixture in absence of air. Finally the flasks were purged with nitrogen for 3 minutes to achieve anaerobic conditions (Mshandete et al., 2005). The inoculum used for the first run was obtained from a continuous anaerobic digestion process at the Institute for Development of Alternative Energies and Materials (IDEMA) at USFQ. The composition of this first inoculum is presented in Table 1. While for the two repetitions the reactive mixture from the previous experiment was used as inoculum, so that microorganisms were already adapted to the composition of the new medium.

Table 1: Characterization of the inoculum used for the start-up of the experiments.

Analysis	Abbreviation	Value	Units
Total Solids	TS	5.15 ± 0.06	(%)
Organic Total Solids	oTS	3.92 ± 0.03	(%)
Chemical Oxygen Demand	COD	38900 ± 174	(mg/L)
Alkalinity	Alk	1048 ± 41.8	(mg$CaCO_3$/L)
pH value	pH	7.08 ± 0.05	(-)
Volatile Fatty Acids	VFA	10640 ± 27.8	(mgCH_3COOH/L)
Nitrogen	N	0.08 ± 0.01	(%)

The biogas composition has been analysed by gas chromatography. Each bioreactor had a gas tight cap with an outlet equipped with a sampling septum to take the biogas samples (Mshandete et al., 2005). Methane and carbon dioxide concentrations are evaluated in a Thermo Scientific GC Traces 1300 with two detectors: FID and TCD, in a split/splitless injector (Mshandete et al., 2005). Samples are injected manually in a TG-BOND Q column with specifications provided by ThermoFisher. The carried gas used for the biogas analysis is Helium grade 5, purity 99.999% provided by The Linde Group.

2.4 Characterization methods for the resulting reactive mixture

The characterization of the effluent is performed according to the methods explained for the characterization of the biomass and feed. Total solids (TS), organic total solids (oTS), volatile fatty acids expressed as acetic acid equivalent (VFA) and chemical oxygen demand (COD) were measured as presented in Almeida (Almeida Streitwieser, 2017). Total nitrogen content (N) was determined based on VELP SCIENTIFICA methods (VELP Scientifica., 2015b). Crude protein was expressed as 6.25 x N.

3. Preliminary Results

3.1 Biomass and feed composition

The proximate composition of the five biomasses who reported high values of the different nutrient content is summarized in Table 2. All the chemical analyses for the characterization of the pure biomass are expressed as means ± standard deviation.

Table 2: Proximate composition of the pure biomasses.

Raw material	Moisture (%)	Ash (%)	Organic Matter (%)	Reduced sugar* (%)	Protein* (%)	Total lipid* (%)	Starch* (%)	Fibre* (%)
Blackberry	87.6 ± 0.27	0.48 ± 0.07	11.9 ± 0.2	4.88 ± 0.8	1.36 ± 0.07	0.11 ±0.09	N/A	5.04 ± 1.5
Soybean	8.83 ± 0.04	4.25 ± 0.01	86.9 ± 0.5	N/A	46.4 ± 2.1	21.6 ±0.08	N/A	19.18 ± 0.8
Avocado	74.4 ± 0.01	0.97 ± 0.03	24.6 ±0.3	1.2 ± 0.6	4.79 ± 1.6	57.0 ±0.08	0.02 ± 0.1	30.28 ± 1.8
Green plantain peel	87.3 ± 0.50	1.40 ± 3.70	11.3 ± 0.6	0.4 ± 0.3	12.0 ± 1.8	3.78 ±0.48	37.6 ± 1.6	41.85 ± 2.1
Bagasse	28.3 ± 1.10	13.8 ± 2.28	57.9 ± 2.5	N/A	1.63 ± 0.5	0.16 ±0.01	N/A	91.68 ± 0.3

*Results are in dry matter

3.2 Composition of the reactive mixture and degradation

The composition of the final reactive mixture and the percentage degradation of key components will provide important information on the state of the anaerobic digestion process (Table 3). The percentage degradation in COD as well as the reduction in TS show that for the experiments with high sugar content (blackberry) and protein (soybean), a higher COD degradation rate is observed, as well as a higher reduction in total solids.

The reason for the difference could be that this biomass contain compounds such as free sugars, oligomers and organic acids, which are readily degradable (Ge et al., 2014). Also, the highest concentration of volatile fatty acids is observed for the experiments loaded with blackberry and soybean, and the lowest values of pH are for reactors loaded with blackberry and bagasse. A high VFA and low pH value are clear indications that the first steps of AD hydrolysis and acidogenesis have taken place fast, while accumulation is observed in the last step of methanogenesis due to limitations in the degradation of short chain fatty acids by the microorganisms.

Table 3: Analysis of reactive mixture.

Raw material	COD Degradation (%)	TS Reduction (%)	pH (-)	VFA ($mg_{CH3COOH}$/L)
Blackberry	65.0 ± 18.4	42.9 ± 24.1	4.6 ± 0.8	14427 ± 3761
Soybean	72.3 ± 4.20	52.2 ± 27.6	5.3 ± 0.2	12863 ± 2208
Avocado	44.3 ± 26.0	24.0 ± 8.89	5.0 ± 0.3	13924 ± 7342
Bagasse	26.6 ± 29.1	45.9 ± 35.8	4.8 ± 0.4	2667 ± 103
Green plantain peel	22.5 ± 19.1	24.4 ± 19.3	5.2 ± 0.5	5440 ± 2304

* Average values reported for the three experiments.

3.3 Biogas production and composition

The biogas production is analysed as its cumulative value for the duration of each experiment and its methane content. The production is measured as water is displaced daily. The results can be observed in Figure 1. For experiment 1 (Figure 1 a) soybean, the residue high in protein, obtained the highest cumulative biogas production of 2300 mL, followed by blackberry with 820 mL. This was significantly higher than those of avocado, bagasse and green plantain with a cumulative biogas production of less the 500mL in 24 days. In the following experiments, the biogas production of soybean reduced to 630 and 780 mL. For the experiments with high sugar content, the second experiment showed almost the same results with 650 mL, but it dropped drastically for the third experiment below 100 mL, indicating an inactivation of the microorganisms caused by the decrease in the pH value and the increase in VFA from the previous experiments. The other biomasses remain with low biogas production during the replicates. This could be explained with the consequent reduced activity of the inoculum that was collected from reactors of experiments 1 and 2. In Figure 1 b), the maximum biogas production, is for the biomass with high protein content, 783 mL, values are not reported for clarity of data.

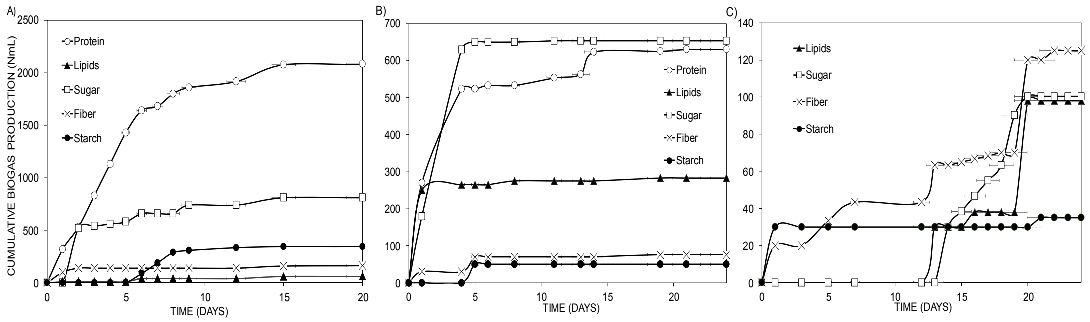

Figure 1: Cumulative biogas production: a) experiment 1, b) experiment 2, c) experiment 3.

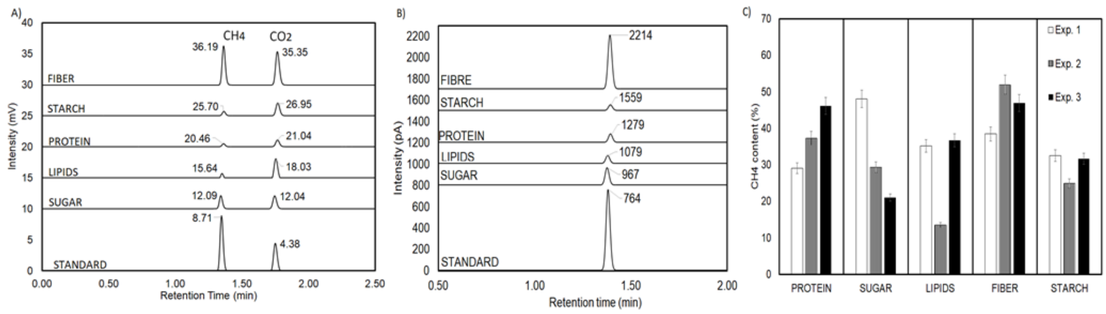

Figure 2: GC Chromatograms for the BMP experiments: a) TCD detector, b) FID detector, c) methane content for all the experiments as percentage of CH_4 / CO_2

The methane content was measured by GC/TCD-FID for each experiment. Figure 2 show the results of the GC analysis performed for the second batch only. The biogas composition was measured with the detectors TCD (Figure 2 a) and FID (Figure 2 b). The diagrams show that in all experiments both peaks are present: methane and carbon dioxide. In Figure 2 c the percentage methane production for all the experiments is shown. It can be observed that methane percentage has a significant decrease from experiments 1 and 2 with a decrease on pH values and an increase on VFA for the experiments with high lipids, sugar and starch content (Kim *et al.*, 2004). The higher methane production on average is observed on reactor with higher fibre content which could be attributed to the lower concentration of sugars and easy degradable matter. However, the reactor with high starch content had similar TS values, the inhibition on this process was due to the lower value on C/N ratios. As it was explained AD highly depends on this factor.

In Figure 2 c) the methane content of the different experiments is presented. It can be observed that the methane content of the experiments with high fibre content are above 30% and 50%. This was also reported by Ge et al where a linear correlation between CH_4 production and degradation of hemicellulose was observed (Ge et al., 2014), although the overall biogas production was lower than for the other experiments. On the contrary, for the experiments with high starch content the methane content was the lowest with 29% in average. For the experiments with the highest biogas production, that is high protein and high sugar, the methane content varies significantly. For the experiment with high protein an adaptation for the microorganisms can be supposed, since the methane content increases with the number of experiments. And for the high sugar experiment the assumption of the inhibition of microorganisms can be confirmed, since on the third experiment, the methane content drops significantly as well as the pH value (Ali Shah et al., 2014). Also, high amounts of dissolved CO_2 observed in the reactors could affect the pH of the medium and, consequently, can alter the microbial activity. Finally, for the experiments with high content of lipids the methane content on the second experiment decreases, probably due to a low relationship between C/N below 20:1. Also an accumulation of lipids could produce an inhibition on methane production due to the formation of long-chain fatty acids (LCFAs) as it is reported by Cirne et al. (Cirne et al., 2007).

4. Conclusions

This study presents the preliminary results to improve the understanding of AD of biomass with different composition. They indicate that the highest biogas production was observed for the biomass with high content of protein followed by sugars. Though, the high content of sugars increased drastically the concentration of VFA, inhibiting the microorganisms in the phase of methanogenesis. Also, a significant concentration of lipids inhibited the methane production due to the formation of LCFAs. The highest methane content was observed with the experiments with high fibre content. This could be explained by the very low availability of nutrients to the microorganisms, depleting so the concentration of VFA and so reducing the amount of free CO_2 in the system. The study also shows that the biogas production and composition can be correlated to the pH value and the VFA concentration of the reactive mixture, as well as the COD degradation rate. The next step in this study is to perform more experiments to confirm these results, and further be able to determine the main degradation mechanism as well as the main microorganisms that are degrading the material at each composition.

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