CHEMICAL ENGINEERING TRANSACTIONS

VOL. 65, 2018

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Eliseo Ranzi, Mario Costa

ISBN 978-88-95608- 62-4; **ISSN** 2283-9216

Influence of the Use of Co-Substrates on the Anaerobic Co-Digestion of Municipal Solid Waste, Cocoa Industry Waste and Bottled Beverage Industry Waste

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Colombia generates large quantities of organic waste that are not used or deposited in the correct way. Therefore, this project seeks to harness the organic load of these residues using anaerobic co-digestion, which is a biological process that allows the recovery and the biochemical potential of methane (BPM) from waste, thus, achieving adequate treatment for the control of environmental pollution. In this project, three residues were evaluated: Municipal solid waste (MSW), cocoa industry waste (CIW) and bottled beverage industry waste (BBIW). To these residues, a physical-chemical characterization was realized prior to the BMP test, in which the following was determined: values of solid volatile particles, total solids, organic matter and nitrogen Kjeldahl. With the characterization obtained, we prepared the mixtures in which the carbon nitrogen (C/N) ratio was varied in three levels: 25, 35 and 45. The number of grams of volatile solids (gVS) was also varied in three levels: 0.5, 1.25 and 2. Likewise, the co-substrate was varied in two: sewage sludge and pig manure. It was found that mixtures having a C/N of 35 grams of volatile solids of 0.5 and using as co-substrate the pig manure generate a greater production of methane (364 mLCH4/gSV). The anaerobic co-digestion technique allows the efficient development of the process due to the synergistic behavior of the co-substrates used, which compensate for the shortcomings that each presents when performing the process separately. It is also important to mention that the mixtures that have cocoa industry waste and bottled beverage industry waste increase the biochemical potential of methane. Although this may change when the bottled beverage industry waste is replaced with the municipal solid waste since the composition is the same.

1. Introduction

Anaerobic co-digestion involves a process in the absence of oxygen, in which microorganisms decompose their biodegradable material passing through four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Cassendra et al., 2017). The process is governed by different microbes with varied rates of specific cell growth, the consumption capacity of the substrate and co-substrate and, preferred environmental conditions, such as pH and temperature (Cassendra et al., 2017). For this project, as co-substrates pig manure and sewage sludge were used. Studies carried out on pig manure have shown that it exerts negative effects on the environment, despite having a wide variety of nutrients necessary for bacterial growth (Toma et al., 2016). Manure is a two-fraction mixture of urine, feces, and water. The liquid fraction principally contains nitrogenous compounds (including ammonia, ammonium compounds, nitrates), and organic matter (Bertora et al., 2008). The solid fraction principally has phosphoric compounds, which occur in inorganic form (74–87% of the total P content) and organic compounds. Also, it has a high amount of ammonia, which helps the system to be more efficient, however, it is important to bear in mind that ammonia can be toxic to microorganisms (Murto et al., 2004). That is why it is recommended to perform the co-digestion with other kinds of organic waste such as municipal solid waste or organic waste, which are rich in carbon and give balance in the carbon/nitrogen ratio (C/N), thus improving the bacterial growth and reducing the risk of inhibition and

acidification from ammonia. On the other hand, sewage sludge also represents an environmental problem as in Colombia 80% of the wastewater generated in global urban cycles, both agricultural and industrial, return to the natural environment without being treated or reused. It is worth noting that the problem is significant since only 31% of urban areas in Colombia have water treatment systems. Notwithstanding, not all substrates are optimal to produce biogas, an optimal characterization and analyses of these substrates is important to ensure the suitability of the resulting mixtures. The importance of a proper usage and disposal of waste, due to the accelerated overpopulation and urbanization over the years, has forced human kind to the recovery of "garbage" to make it not a waste but on the contrary a raw material, which recirculates back into the environment in a sustainable and manner. One of the great advantages of anaerobic co-digestion is the capture of products and by-products (gases and liquids) generated by degradation, thereby is little loss of nutrients, it also has benefits such as dilution of toxic compounds, synergistic effects of microorganisms, as well as an increase in the load of biodegradable organic matter, and in general a better production yield form biogas (Rodríguez et al., 2017). Likewise, it allows for the use of nutrients from different waste sources and achieve the balance of the bacterial community to optimize the performance of the process. It is important to keep in mind that biogas allows to diversify the energy sources that are now available. Added to the advantages that it represents for the environment, biogas can be used for the generation of electricity or heating, thus contributing to minimizing the current dependence on fossil fuels (Li Y et al., 2016).
The BPM (Biochemical methane potential) method was used in the development of experimentation to optimize the anaerobic digestion which allows to find which substrate has the highest potential and in this way to determine the methane production of different mixtures. This project focuses primarily on evaluating methane production through the process of anaerobic co-digestion using some of the different substrates available in Colombia and on which it exists little information about its performance. And it serves as a preliminary study that allows to advance in the field of the valorisation of the waste that has been evaluated through anaerobic co-digestion.

2. Materials and Methods

In this study, the anaerobic co-digestion process was carried out using discontinuous reactors of 250 mL, under mesophilic conditions of 34 °C. An analysis was made of the biochemical potential of methane (BPM) of the following three substrates: municipal solid waste (MSW), cocoa industry waste (CIW) and waste from the bottled beverage industry (BBIW), and were used as co-substrate pig manure (PM) and sewage sludge (SS), to which previously, a physical-chemical characterization was carried out, evaluating volatile solids, total solids, organic matter, Kjeldahl nitrogen and chemical oxygen demand (COD). Each substrate was evaluated under static condition tests. Municipal solid waste came from homes in the city of Bogotá, where the organic fraction composed of vegetables, fruits and processed foods were taken; cocoa industry waste was obtained from a private farm located in the department of Santander - Colombia, where the shell and internal cocoa fibers were used, the cocoa species used in this trial was trinitario; this species originated from the crossing of two other cocoa species (Federación Nacional de Cacaoteros, 2013), and is characterized by a wide variability of shapes, sizes and behavior, being the predominant type of cocoa in the country (Federación Nacional de Cacaoteros, 2013). Regarding bottled beverage residues, these were simulated beginning with the fruit consumption of a factory of bottled fruit drinks in Colombia, using the percentage per weight of waste generated by each fruit. In this way, it was determined that the greatest proportion of residues that were produced came from mango, banana, passion fruit, blackberry and lulo. From Bananas mainly the husk was used and for the others, husks, seeds and bran were used. These wastes went through a process of size reduction and liquefaction. The pig manure was obtained at the Marengo Agricultural Research Center (C.A.M) part of the National University located in the municipality of Mosquera (Cundinamarca). This waste comes from animals fed with commercial concentrate and, finally, the sewage sludge was obtained from the PTAR 1, aqueduct, sewer and toilet company of Madrid, Cundinamarca. The inoculum used for all the tests carried out was sludge from a biodigester located in the wastewater plant from Alpina S.A., in Sopó, Cundinamarca (Colombia). This mud was selected because it comes from a functioning system, it was already stabilized and gave us a guarantee of obtaining biogas production. All the residues were preserved at a temperature of -4ºC to preserve their physicochemical characteristics.
The BPM method used in this study is based on the principles described by Owen et al (1979) and Angelidaki et al (2009). The tests were carried out in 250 mL bottles in triplicate, with a working volume of 80%. The S/X ratio (Volatile solids of the substrate/inoculum) was set at 3 (Cabeza et al., 2016). The total volume of work mentioned above was completed with distilled water. Then the pH of the liquid contained in each of them was measured, which had to be in a range of 6.3-7.8, with an optimum pH of 7 (Kondusamy & Kalamdhad, 2014) continuing the process, the bottles were closed with plastic lids and sealed with silicone. Once sealed, the bottles were placed in a thermostatic bath with automatic controller without agitation at a constant temperature

of 34°C for 20 days (at the time of execution of the study, no reactor was fed). The production of methane was monitored daily by volume displacement, where the carbon dioxide present in the biogas was retained by bubbling the biogas in a solution of NaOH with alkaline pH, pH>9 (Cendales Ladino, 2011). The study of anaerobic co-digestion in discontinuous regime was carried out by monitoring 8 mixtures. 4 of these mixtures had a ratio of C/N 35 taking as difference the number of grams of volatile solids (2gVs-0.5 gVS) and the type of co-substrate that all these contained (PM and SS). The other mixtures were tested with 1.25 gVS with a respective C/N ratio of 45 and 25 (see Table 1). Each of the mixtures was constituted by 3 samples, as shown in table N.2, where different combinations were obtained by varying the C/N, gVS and co-substrates of the organic residues in the experiment, this was done in order to determine which combination of residues generated a greater production of methane.

Table 1: Mixture used in experimentation

Co-substrate	Mixture	C/N	gVS
PM	1	35	0.5
	2	35	2
	3	45	1.25
	4	25	1.25
SS	1	35	0.5
	2	35	2
	3	45	1.25
	4	25	1.25

Table 2: Combinations of organic waste

Sample	Organic waste
1	BBIW and MSW
2	BBIW and CIW
3	MSW and CIW

The relation of substrates was established in such a way that the C/N ratio was reached and the amount of gVS were fixed considering the physicochemical characteristics of the substrates used. The parameters analyzed for the substrates were the following: Total Solids (ST) per 2540B from the Standard Methods (APHA), Volatile Solids (SV) per D3174 from the American Society for Testing and Materials (ASTM), Chemical Oxygen Demand (COD) per D1252-06 from the American Society for Testing and Materials (ASTM), And Kjeldahl Nitrogen (NTK) per D1426-15 from the American Society for Testing and Materials (ASTM).

3. Results and Analysis of Results

The organic waste used as substrates were subjected to a physicochemical characterization to establish the nutrient content and other parameters necessary for the functioning of the system.

Table 3: Physicochemical characteristics of substrates

SUBSTRATES	**PARAMETERS**				
	TS (%)[b]	**VS (%)[b]**	**COD (g/L) [a]**	**NTK (%)[a]**	**OM (%)**
Pig manure	22,83	19,79	4,98	1,88	82,30
Cocoa industry waste	21,09	15,26	2,88	0,99	72,38
Municipal solid waste	19,42	18,95	7,94	1,56	97,62
Bottled beverage industry waste	7,09	6,85	4,05	1,11	96,58
Sewage sludge	23,88	8,69	1,35	1,83	36,38

a. Sample on dry basis / b. Sample on wet basis

Table 3 shows the results of these parameters. The total solids content is the combination of the solids that can be broken down by bacteria and the solids that will never degrade. Volatile solids are the solids that can be decomposed with a biological treatment (Chastain et al., 2003), the volatile solids and the total solids also allow to determine the amount of organic matter (OM) in the substrate, being this greater than 70% it is possible to infer that there will be a good biological digestion in the initial stage of each test (Rodríguez et al., 2017). Likewise, another important factor to consider is the pretreatment carried out in the different substrates, in this investigation the reduction of the size of each waste was carried out since a decrease of the particle will allow the large organic polymers (hydrocarbons, lipids, proteins) of the substrate to be easier to biodegrade by the microorganisms in the different stages of the anaerobic digestion especially in the hydrolytic stage because it is the one that limits the global speed of the process (Veeken et al., 1999).

The BPM results of the mixtures in which pig manure was used as co-substrate are shown in Table 4. Mix 1 with fruit and cocoa residues have the highest methane production with 364 mL CH4/gVS, presumably owed to the carbon/nitrogen ratio (C/N) and the amount of gVS. Pig manure has a low C/N ratio. For this reason, it should be combined with residues containing low levels of nitrogen for the process to be stable. In this way bacteria in anaerobic digestion use carbon 25-35 times more than nitrogen, which implies that, for a better digestion, the relationship between carbon and nitrogen should be given in a range of 30: 1 in the substrate (Rodríguez et al., 2017). Regarding volatile solids, some studies indicate that the appropriate ratio in order to obtain optimal results is between 0.23 and 2.09 gVS (Nielfa et al., 2014), based on the tests carried out. It was found that when the amount of gVS is lower, methane production is greater. Hence, when the load is higher, there is a risk of inhibiting the process, making it unstable (Bosch Martí, 2011). Table 5 shows the results of the mixtures in which sewage sludge was used as co-substrate. Mix 1 with urban solid waste and cocoa have the highest methane production with 275.33 mL CH4/gVS. As in the previous results the C/N is an important factor for the generation of methane. The addition of a carbon-rich co-substrate to the sewage sludge that has a low C/N allows an optimal C/N (Wickham et al., 2016). The importance of an optimal C/N is subject to the fact that a low C/N ratio generates problems of toxicity in the reactors (Fierro et al., 2014).

Table 4: BPM of the mixtures evaluated with pig manure

Mixtures	mL CH_4/gVS		
	Sample 1	Sample 2	Sample 3
Mix 1	316	364	356.67
Mix 2	98.33	118	90.83
Mix 3	112.53	98.93	105.07
Mix 4	112	98.67	92.27

Table 5: BPM of the mixtures evaluated with sewage sludge

Mixtures	mL CH_4/gVS		
	Sample 1	Sample 2	Sample 3
Mix 1	240.33	240.67	275.33
Mix 2	118.83	102.83	94.17
Mix 3	172.93	194	198.13
Mix 4	181.87	233.87	142

Figure 1 compares the results of the best samples of Table 4 and 5, which in the case of Table 4, are the data of sample 2 and in table 5 the data of sample 3. These samples are considered so as not to have any confusion regarding the graph and the fact that the objective of the experiment was to compare with which co-substrate a greater methane production was produced. The high generation of methane by the mixtures that used pig manure as co-substrate, is since the system was not affected by the high amounts of VFAs (volatile fatty acids), due to the production of ammonia, which maintained the pH at neutral levels (Murto et al., 2004).

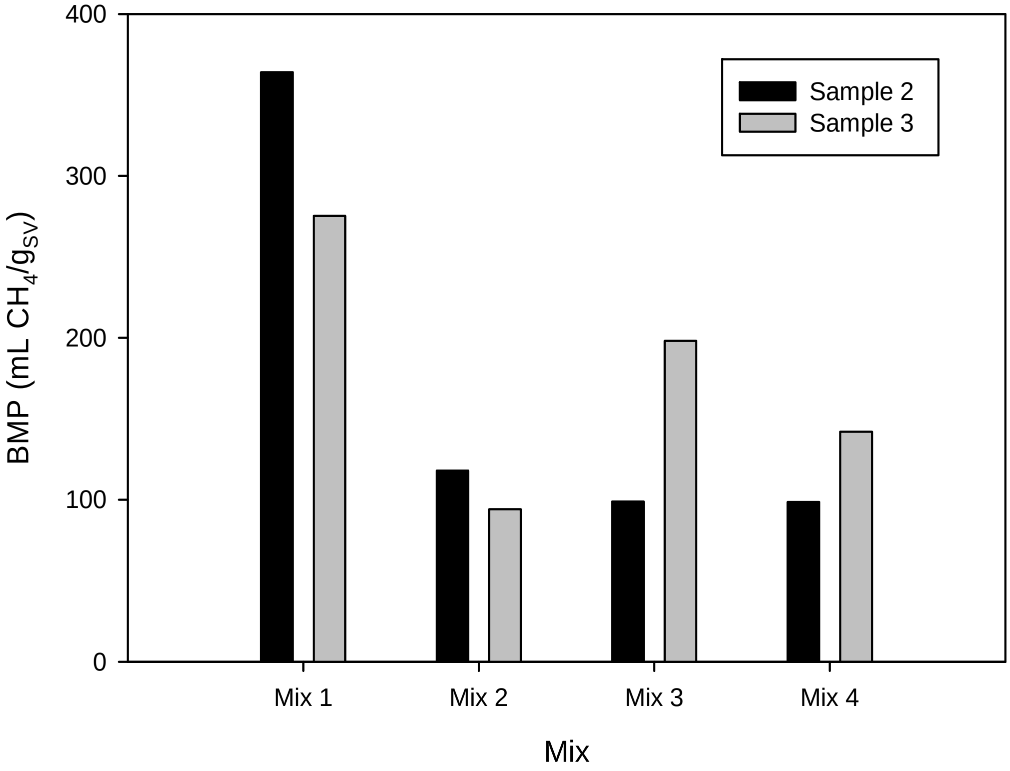

Figure 1: BPM Pig Manure Vs Sewage Sludge

4. Conclusions

According to the three residues evaluated (Municipal solid waste, cacao industry waste and botted beverage industry waste), with the different co-substrates (Pig manure and sewage sludge), we can infer that the residues that contained as co-substrate pig manure were those that produced a greater methane potential, as shown by mix 1 where the fruit and cocoa residues were used with a BPM of 364 mL CH4/gVS, subsequently, the waste that used as co-substrate the sewage sludge (municipal solid waste and cocoa), generated 275.33 mL CH4/gVS, both mixtures had a C/N of 35 and 0.5 gVS, which shows that a low amount of gVS increases methane production.
When a comparison was made between the 2 co-substrates that were used for this test, it was evident when taking the samples by mixtures that in the mix 1, which in general terms was the highest production in the two co-substrates, pig manure was more optimal, although the production trend was not maintained in the other samples since in mix 2 were very similar and in mix 2 and 3 change, the highest production was maintained in the mixtures containing mud of sewage treatment plant. Also, the two mixtures that yielded the highest production used the cocoa industry waste.

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