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Production of biogas from acid and alkaline pretreated cocoa pod husk (*Theobroma cacao L.*)

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Abstract : The aim of this work was applied acid and alkaline pretreatments to cocoa pod husk (CPH) in order to increase the potential of biogas production by anaerobic digestion. Different ruminal fluid (RF) / pig manure (PM) relationships were studied (2:1, 1:2 and 1:0). The inoculum selected was RF:PM = 2:1 ratio to obtain a biomethanation potential value of 0.120 m3 CH_4/kg VS. The effect of acid and alkaline pretreatment in CPH using H_2SO_4 and NaOH was evaluated. The alkaline pretreatment showed the best results in reducing the lignin content, reaching a value of 43.78 %. Anaerobic digestion process using as substrate the pretreated CPH and the inoculum selected, with an organic load of 1, 2 and 3 g $VS_{Inoculum}$/g $VS_{Substrate}$ were analyzed. The results show the potential of the CPH as lignocellulosic substrate for the production of biogas, which improves the value to these products in the agricultural sector.
Keywords : cocoa pod husk, biogas, anaerobic digestion, lignocellulosic biomass, pretreatment.

Introduction

By 2050 the department of Bolivar could become the main cocoa producer of Colombia. The levels of production in the region could account for a significant portion of the total production and exportations in the country. In fact, Bolivar is currently one of the 3 departments that contribute the most to the sector[1].

Cocoa represents an important crop at an industrial level due to the fact that its beans and processed products are the main ingredients of chocolate[2]. However, large amounts of residues are generated during the production of beans, since they only represent up to 30% of the fruit, while the remaining 70% corresponds to process waste in the form of cocoa pod husk[3].

Nevertheless, the use of the husk as a renewable energy source seems promissory. It has been found that the husk presents a high heating value (17,0 MJ/kg), which evidences an energy resemblance to other biomasses such as rice husk, rice straw and bagasse[3]. In addition, its high content of lignocellulosic compounds[3,4,5] can be considered as a potential substrate for the anaerobic digestion for biogas generation.

Anaerobic digestion has been used as a prime example of biological process for the generation of methane with satisfactory results. Quintero and Rondon performed a preliminary study on the production of biogas by anaerobic co-digestion of coffee mucilage (S) with pig manure (I). They achieved a yield of 0,73 m3 CH_4/kg VS of added substrate, and a VS removal of 52,1%[6]. Digestion of fique bagasse (FB) was similarly evaluated, using ruminal fluid (RF) and pig manure (PM)[7]. The results showed that FB was successfully degraded by using both microbial consortia. This phenomenon is attributed to the hydrolytic and methanogenic activity of RF and PM, and a yield of 0,3 m^3 CH_4/kg VS[7].

Recent investigations on biogas production have been focusing on the use of lignocellulosic residues as raw material, thanks to their availability as organic residues and to the fact that they are not completely exploited in the current schemes of agro-industrial production[8,9]. This lignocellulosic biomass is mainly constituted by three types of polymers: cellulose, hemicellulose and lignin. Both cellulose and hemicellulose are suitable for energy production[10,11,12]. However, the structural and mechanical properties of the biomass hinder the enzymatic and microbial degradation. The lignin within the biomass confers rigidity to the material, it is non-water-soluble and acts as an optically-inert material. It also represents the most recalcitrant component of the cell wall and thus, the main drawback when trying to use these materials in bioconversion processes. This set of properties makes it difficult for high-lignin-content materials to be chemically or biologically degraded[13,14]. Hence, anaerobic digestion processes require the application of pre-treatments that aim to reduce the structural barriers of the biomass, and expose the fractions of cellulose and hemicellulose to microbial attack. This would lead to an increase in the rates of degradation and the yields of biogas production[15]. Hydrolysis is recognized as the limiting stage for anaerobic digestion processes[16]. Therefore, the use of pretreatments on the biomass improves the rate at which the material is degraded[17,18,19,20,21].

Additionally, anaerobic digestion (AD) represents a biochemical process that is used for the degradation and stabilization of complex organic matter by a number of microorganism consortia[19]. The main product of the process is a gas mixture known as biogas, and an aqueous solution, which contains the microorganisms that are responsible of degrading the biomass. Different investigations have evaluated the effect of the mixture of inoculums on biogas production processes. Rueda and Sanabria showed that a mixture of RF-PM acted as a suitable inoculum for the degradation of fique bagasse thanks to its high acidogenic and specific methanogenic activity[22]. On the other hand, Ye et al. used rice straw (RS) as an attempt to improve the production of biogas from kitchen waste (KW) and pig manure (PM). Their results evidenced that a 674,4 L/kg VS yield can be achieved at an optimal proportion of KW/PM/RS (0,4:1,6:1)[23].

Henceforth, the main objective of this research was focused towards the assessment of a biogas production process by cocoa pod husk digestion by inoculums (RF and PM). The influence of pre-treatments (acid and alkaline) regarding biogas production is also studied.

Materials and Methods

A Cocoa pod husk was acquired from a town in the north of Colombia. It was washed with water in order to eliminate impurities, and then stored at room temperature for further use. Pig manure (PM) and ruminal fluid (RF), which were used as inoculums for this research, were gathered from the waste products that are generated at a local farm and industry.

Characterization.

Cocoa pod husk (substrate) was subjected to tests that sought to determine the content of lignocellulosic compounds (lignin, cellulose, hemicellulose) within its structure, as well as VS, TS, carbon and nitrogen content. On the other hand, the inoculums were subjected to VS, TS, carbon and dioxide content tests.

Selection of the best inoculum for biogas production

In order to stablish the most appropriate FR:PM ratio, the biomethanation potential (BPM) of cocoa pod husk was evaluated using different RF:PM inoculum ratios (1:0, 1:2, 2:1) and a fixed amount of substrate. All the tests were performed in duplicate.

Cumulative methane volume was set as the response variable for the determination of the BMP of the substrate. This volume was calculated by alkaline displacement method[24]. Biodigestion was evaluated for 15 days at room temperature, in order to determine the displaced volume and BMP.

Pretreatments.

The residual biomass from cocoa pod husk was chemically and mechanically pretreated with the purpose of evaluating the effect of such stage on the yield of biogas production. The mechanical pretreatment consisted on drying the samples by sun exposure for 8 hours[25]; and then grinding them homogeneously using a mortar. The sample was subjected to two different chemical pretreatments, which were conducted as follows:

The husk (20 g) was pretreated with 200 mL of an aqueous solution of NaOH at 0,75 % w/v and 121 °C for twenty minutes in an autoclave. The mixture was filtered with the purpose of separating the solid residues, which were then washed until reaching neutral pH (7,0).

The mechanically pretreated material was subjected to acid pretreatment at a concentration of solids of 5 % (w/v) and room temperature using a 1 % w/v solution of H_2SO_4 for 3 hours. The suspension was taken to the autoclave at 121 °C for 60 min. At the end of the treatment, the content was filtered and the solids were washed until reaching neutral pH (7)[26]. The pretreated biomass was characterized in order to determine lignin, cellulose, hemicellulose, TS and VS content prior to the anaerobic digestion test. The remaining material was sored for further DA[27].

Influence of the Inoculum/Substrate mixture.

The assessment of the effect of VS concentration on methane production was carried out in a batch process consisting on nine 500 mL bio-digesters. Three different inoculum/VS substrate ratios were used (ISR1 = 1:1, ISR2 = 2:1, ISR3 = 3:1), as well as a constant concentration of inoculum. The ISR values were established as optimum values from literature[28,29].

Three series of bioreactors were set for this stage. Each one of them contained samples of mechanically, acid and alkaline pretreated husk, respectively. These vessels were then filled with N_2 in order to ensure that anaerobiosis took place, and they were sealed with butyl tape with the purpose of determining the amount of produced biogas by alkaline displacement method[30]. This evaluation stage was executed during a 15-day timeframe at room temperature.

Presence of methane within the bottles that were used as displacement system was confirmed by gas chromatography at the Chemistry and Pharmacy Laboratories of the University of Cartagena.

Results and Discussion

Characterization of cocoa pod husk and inoculums.

Table 1 shows the results that were obtained regarding substrate characterization. A comparison with literature is also included. The studied cocoa samples exhibited high levels of cellulose and hemicellulose (which are essential to biogas production) and low lignin content. The latter is considered as a recalcitrant compound that can hardly be degraded by microorganism. In the case of inoculums, Table 2 shows that PM presents a higher concentration of TS and VS, when compared to RF.

Table 1.Physicochemical characterization of cocoa pod husk.

Parameters	**Method**	**Cocoa pod husk [%]**			
		The authors	**[2]**	**[4]**	**[21]**
Lignin	TAPPI T 222 05-74	12,06	14,6	33,96	21,16
Cellulose	TAPPI T 203 05-74	18,42	35,0	30,41	26,15
Hemicellulose	TAPPI T 223 05-70	10,04	10,8 – 11,0	11,97	12,57
TS	AOAC 925.09	23,95	–	–	–
VS	Combustion at 550 °C	20,38*	–	–	–
Carbon	AOAC 972.43	5,56	–	43,87	–
Nitrogen	AOAC 972.43	0,23	–	2,23	–
C/N		24,17	–	19,67	–

Table 2.Physicochemical characterization of the inoculums.

Parameters	Method	Pig Manure [%]	Ruminal Fluid [%]
Total solids	AOAC 925.09	18,98	0,97
Volatile solids	Combustion at 550 °C	12,90*	0,72*
Carbon	AOAC 972.43	3,79	0,18
Nitrogen	AOAC 972.43	1,05	0,75
C/N		3,61	0,24
pH		6	7

*Related to the amount of Total Solids

The selected inoculums possessed suitable organic microbial loads to promote the anaerobic degradation of cocoa pod husk. In addition, the pH of both microbial consortia allows their mutual utilization by the synergetic relationship they could develop. According to Khanal, the appropriate pH value for a bio-digester (with elements being mixed altogether) must range between 6,8 – 7,4 in order to guarantee suitable process conditions[32].

Selection of the most suitable microbial consortium.

In order to select a microbial consortium, the bio-digesters were filled with 140g of cocoa pod husk, PM and FR mass. Figure 1 indicates the cumulative biogas production from cocoa pod husk at different RF:PM ratios.

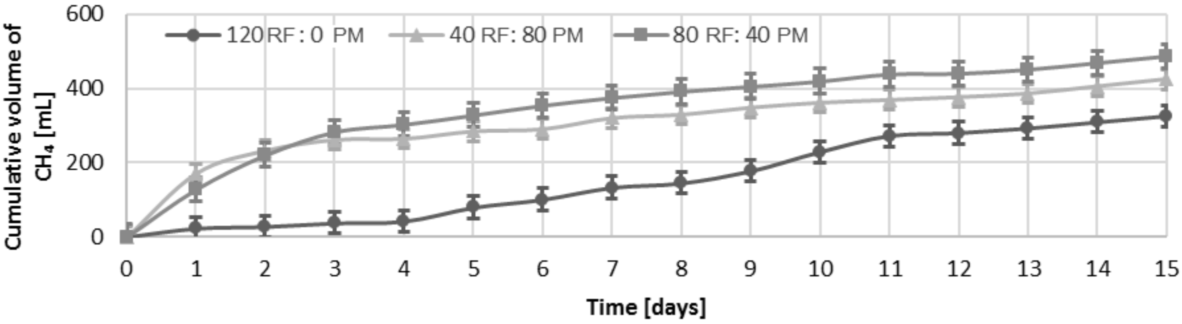

Fig 1. Biogas production from cocoa pod husk at different ratios of ruminal fluid (RF) : pig manure (PM).

When comparing the ratio of RF:PM inoculums during the biodigestion process, it was observed that the 2:1 ratio exhibited a higher total cumulative volume of CH_4 (487,1 mL); and a 0,120 m^3 CH_4/kg VS yield (Table 3). This ratio was selected for further stages of the research.

Table 3. Cumulative volume and production yield of methane.

Ruminal fluid / pig manure ratio (RF:PM)	Cumulative volume of methane [mL]	BMP [m^3 CH_4/kg $SV_{substrate}$]
120:0	325,6	0,080
40:80	424,8	0,104
80:40	487,1	0,120

From this result, it was observed that both microbial consortia present the required synergy for a functional biogas production. Previous studies have shown that RF promotes the digestion of lignocellulosic biomass thanks to the high hydrolytic and acidogenic activity of the microorganisms within the inoculum[23]. In a similar way, PM complements the degradative action of RF due to its high methanogenic activity (0,04 g COD – CH_4/g VS day); i.e., its enzymatic activity is efficient enough for the transformation of fatty acids (from the organic load) into methane as a desired final product of the chemical process[6].

Influence of the pretreatments (acid and alkaline) on the cacao pod husk.

The untreated cacao pod husk was subjected to two chemical pretreatments: H_2SO_4 and NaOH. The results of the characterization of each pretreated and untreated fraction of biomass are presented in Table 4.

Table 4. Percentage composition of the lignocellulosic structure of cocoa pod husk

Parameter	Pretreatment		
	Untreated	Alkaline	Acid
Lignin	12,06	6,78	9,86
Cellulose	18,42	11,91	13,64
Hemicellulose	10,04	9,06	8,63
TS	23,95	21,97	18,03
VS	20,38	19,95	17,73

The results evidenced reductions of 43,78% on lignin content, mainly due to NaOH action at the tested conditions, and 18,24% after acid pretreatment with H_2SO_4. This is attributed to the fact that NaOH is a strong delignification agent with high reactivity towards the aromatic rings of the polymer[7]. This phenomenon makes it possible for the enzymes to exert a higher degradative activity during the hydrolysis stage of anaerobic digestion. It also proves that pretreatments can be used for the depolymerization of polysaccharides and their conversion into highly degradable and soluble organic matter for subsequent hydrolysis[7]. Therefore, reducing lignin content also leads to higher biogas production[33]. According to Liew et al., biomethanation of lignocellulosic biomass is increased with reducing lignin content[34]. However, the effect of chemical pretreatment depends on the method that is applied, as well as on the substrate properties. Substrates with high carbohydrate content experience accelerated degradation and VFA, which in turn affects the performance of methanogenesis[35].

Influence of the Inoculum/Substrate Ratio (ISR).

This final stage of the process consisted on evaluating the Inoculum/Substrate Ratio (ISR), which is measured as the concentration of inoculum VS per concentration of substrate VS (g $SV_{inoculum}$/g $SV_{substrate}$) in batch reactors for the anaerobic degradation of organic matter and its further BMP. Figures 2-4 show the kinetics of biogas production from untreated and pretreated cocoa pod husk at different ISR.

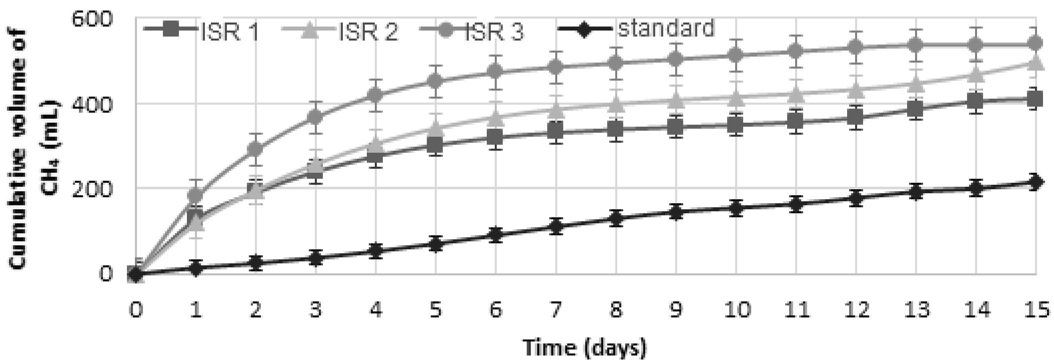

Fig. 2.Kinetics of biogas production from untreated cocoa pod husk at different ISR.

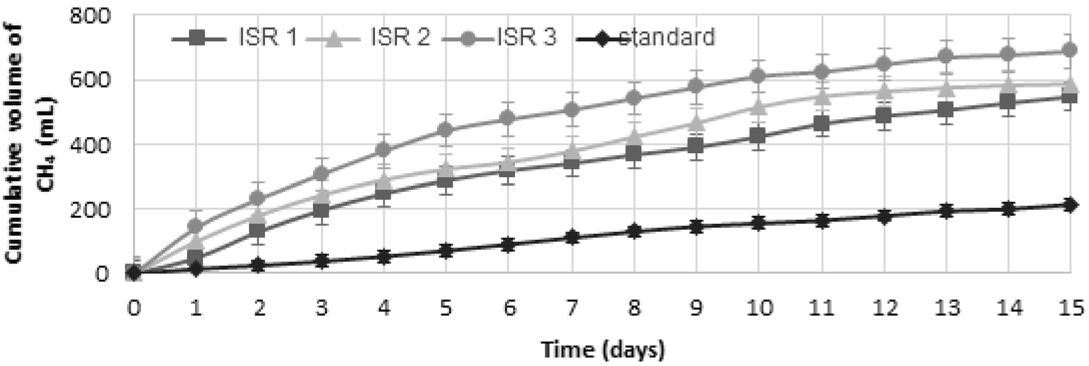

Fig.3. Kinetics of biogas production from cocoa pod husk after alkaline pretreatment at different ISR.

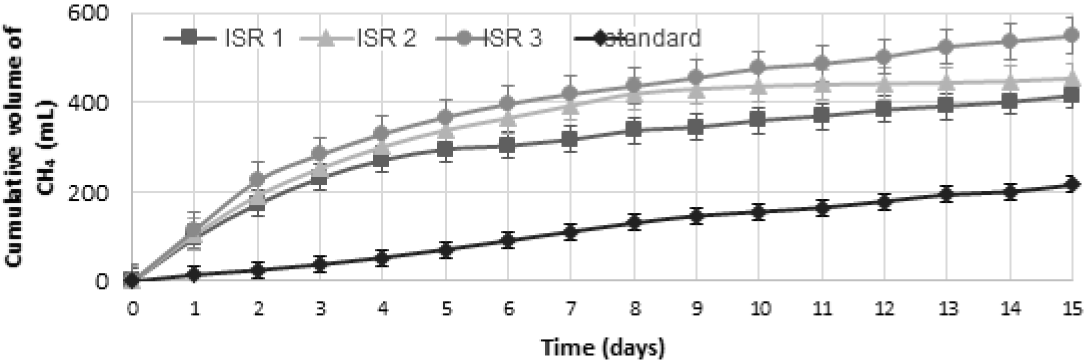

Fig. 4.Kinetics of biogas production from cocoa pod husk after acid pretreatment at different ISR.

The 3 g $VS_{inoculum}$/g $VS_{substrate}$ ratio exhibited the most suitable behavior in all cases of untreated or pretreated biomass regarding anaerobic digestion of cocoa pod husk. Total cumulative volumes of 538,84 mL (untreated), 548,14 mL (acid treatment) and 687,51 mL of methane (alkaline treatment) were obtained using this ratio. It is therefore inferred that the production of biogas is inversely proportional to the added organic load, with a significant increase on biogas production with increasing ISR.

On the other hand, BMP was calculated for each ISR (Figure 5). The highest yield corresponded to alkaline pretreated cocoa pod husk, with a maximum value of 0,247 m^3 CH_4/kg VS using an ISR of 3.

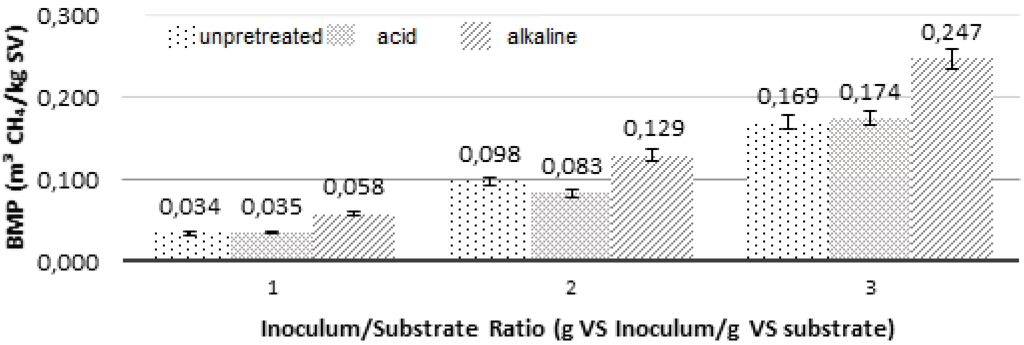

Figure 5. Yield of biogas production from cocoa pod husk at different ISR and pretreatments.

Resultsshow that alkaline pretreatment allows the rupture of the cellulose-hemicellulose matrix within the structure of cocoa pod husk, thus increasing both substrate porosity and surface area. These conditions increase the levels of enzymatic digestibility of the cellulose within the biomass[33,36].

However, acid pretreatment did not substantially affect biogas production under the used ISR when compared to mechanically pretreated biomass. According to Zheng et al., the main drawback of low pH pretreatments is the production of several inhibitors (e.g., carboxylic acids, furans and phenolic compounds); which results in low biogas production[15]. Suitable conditions (temperature, pH and time) must be selected in order to prevent inhibitor formation.

Once the most appropriate conditions for biogas production were obtained, a gas sample with ISR 3 (alkaline pretreatment) was taken from the bio-digester with the purpose of studying the presence of methane in the produced biogas. Chromatography analysis proved the presence of CH_4 at concentrations of 51,25 and 51,11 %. Some impurities were also detected: CO_2 (32,72 and 32,76 %) and N_2 (2,08 and 2,10 %).

These results evidence that cocoa pod husk represents a feasible alternative for biogas production. The implementation of alkaline pretreatment also helps increase the yield of biomass degradation processes. The use of these raw materials as alternatives for renewable energy production will contribute to the strengthening of agro-industrial chains all over the country. Several value-added products or alternative energy sources could be obtained by properly exploiting residual lignocellulosic materials.

Conclusions

The comparative study that was performed on pig manure and ruminal fluid proves that the (2:1) ratio is the most suitable for the anaerobic digestion process. This phenomenon is attributed to the synergetic effect of the mixture, which allows the utilization of both hydrolytic and acidogenic activities of ruminal fluid; and the methanogenic activity of pig manure.

A maximum yield of biogas production was reached (0,247 m^3 CH_4/kg VS) by feeding the bio-digesters with an organic load of 3 g $VS_{inoculum}$/g $VS_{substrate}$ and cocoa pod husk pretreated with NaOH at 0,75 % w/v. Chromatography analysis indicated that the final gas sample exhibited a methane concentration of 51,11-51,25 %.

This research proves that pretreated cocoa pod husk, along with ruminal fluid and pig manure can be used for the production of biogas, which would allow the utilization of these types of agro-industrial residues for energy purposes.

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