



Effect of alkaline pre-treatment on the anaerobic biodegradability of coffee husk

Efecto del pre-tratamiento alcalino en la biodegradabilidad anaerobia de la cáscara del café

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CITE THIS ARTICLE AS:

K. De la Cruz, Y. Gómez, Y. Santander, D. J. Catagua, J. C. Dustet, I. Pereda, “Effect of alkaline pre-treatment on the anaerobic biodegradability of coffee husk”, *Revista Facultad de Ingeniería Universidad de Antioquia*, no. 92, pp. 36-41, Jul-Sep 2019. [Online]. Available: <https://www.doi.org/10.17533/udea.redin.20190516>

ARTICLE INFO:

Received: February 20, 2019
Accepted: May 17, 2019
Available online: May 17, 2019

KEYWORDS:

Anaerobic digestion, alkaline pre-treatment, methane yield, lignocellulosic biomass, polyphenols

Digestión anaerobia, pre-tratamiento alcalino, rendimiento de metano, biomasa lignocelulósica, polifenoles

ABSTRACT: The present paper deals with the mono-digestion of coffee husk during the operation of batch reactors and semi-continuous stirred tank reactor (S-CSTR) using both pre-treated residual at 3% Ca(OH)₂ and without pre-treatment. The semi-continuous operation was carried out in 4 stages at different organic loading rate (OLR, [g_{VS} L⁻¹ d⁻¹]) and hydraulic retention time (HRT, [d]) (OLR:HRT) ratios (0.1:90, 0.2:90, 0.2:45 and 0.2:30). As a result of the pre-treatment, up to 223% of the organic matter solubilisation was obtained. However, pre-treatment did not provide better results in biodegradability, observing a negative trend in methane yield when operating in S-CSTR, due to the accumulation of compounds from the fractionation of the lignin as is the case of polyphenols. The operation was further developed using the residual without pre-treatment. The highest methane yield was obtained at 45:0.2 when using the residual without pre-treatment being with 277 mL_{CH₄} g_{VS}⁻¹, for a methane productivity of 1.1 L L⁻¹ d⁻¹. When implementing the 30:0.2 ratio, a biomass washout was observed in the system, so it is recommended to operate at low OLR and high HRT when treating solid coffee wastes.

RESUMEN: La biodegradabilidad de la cáscara del café durante la operación de reactores en discontinuo y en tanque agitado semi-continuo, se evaluó empleando el residual pre-tratado con Ca(OH)₂ al 3% y sin pre-tratar. La operación en régimen semi-continuo se llevó a cabo en 4 etapas empleando relaciones de carga orgánica volumétrica [COV, [g_{SV} L⁻¹ d⁻¹]] y de tiempo de retención hidráulico [TRH, [d]] (COV:TRH) de 0,1:90; 0,2:90; 0,2:45 y 0,2:30. Como resultado del pre-tratamiento se obtiene hasta 223% de solubilización de la materia orgánica. Sin embargo, el pre-tratamiento no brindó mejores resultados en la biodegradabilidad, observándose una tendencia negativa en el rendimiento de metano en la operación en semi-continuo, debido a la acumulación de compuestos resultantes del fraccionamiento de la lignina como es el caso de polifenoles, por lo que se decidió continuar empleando el residual sin pre-tratar. El mayor rendimiento de metano se obtuvo a 45:0,2 cuando se utilizó el residual sin pre-tratar obteniéndose 277 mL_{CH₄} g_{SV}⁻¹, para una productividad de metano de 1,1 L L⁻¹ d⁻¹. Al implementar la condición de 0,2: 30 se observó un lavado de biomasa en el sistema por lo que se recomienda operar a bajas COV y elevados TRH al tratar residuales sólidos cafetaleros.

Abbreviations:

OLR: organic loading rate
HRT: hydraulic retention time
S-CSTR: semicontinuous stirred tank reactors
CC: coffee husk without pre-treatment

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ISSN 0120-6230

e-ISSN 2422-2844



CC-p: coffee husk pre-treated

TS: total solids

VS: volatile solids (SV, in Spanish)

BMP: biochemical methane potential

VFA: volatile fatty acid

TIC: total inorganic carbon; **w/w**, wet weight; **w/v**, weight/volume; **d.b.**, dry basis.

1. Introduction

The consumption of coffee as a stimulant beverage is a global paradigm, its use has increased over time mainly in industrialized or developed countries. The coffee industry releases enormous amounts of by-products that are rich in carbohydrates, proteins, pectin and bioactive components that result in low-cost renewable resources [1].

A method called "benefit" is used to obtain the grain. This can be done in two ways: dry and wet. Most producing countries, including Cuba, use the wet method to obtain clean grain. However, this route generates large volumes of liquid and solid residuals, which are a source of severe pollution because of the high pollution load they have [2]. In recent years, there has been increased interest in the comprehensive use of this waste through alternatives that contribute to environmental protection [3]. Anaerobic digestion emerges as an effective way to obtain alternative or renewable sources of energy and also significantly reduces greenhouse gas emissions into the atmosphere.

Due to the complex chemical composition of the coffee cherry (content of lignin, hemicellulose, cellulose, polyphenols, caffeine and tannins, etc.), the solid residues of this fruit are characterized as lignocellulosic materials, which limits its biodegradability and compromises the proper development of anaerobic digestion. These wastes can produce energy in three forms: liquid fuels such as bioethanol, gaseous fuels such as biogas and electricity by combustion [4].

The application of a pre-treatment emerges as a resource to fractionate the lignocellulosic material. When a greater solubilisation of the organic matter present is achieved, an increase in methane yield would be expected by stimulating the hydrolysis process [5]. However, the application of a pre-treatment frequently produces the degradation of compounds that can act as inhibitors: organic acids (acetic, formic and levulinic), furans derivatives (furfural, among others) and phenolic compounds [6], affecting above all cellular physiology and often decreasing viability and productivity [7].

Among the pre-treatments that have the greatest influence due to their nature against biomass with refractory compounds are chemical pre-treatments, thermal and the

combination of these. Alkaline pre-treatment is typically used in lignocellulosic materials with a high lignin content [8]. Therefore, the aim of the present paper is to assess the anaerobic biodegradability of the pre-treated and not pre-treated coffee husk during the operation of both batch and semi-continuous stirred tank reactors (S-CSTR).

2. 2. Material and methods

2.1 Feedstock and inoculum

The raw material used was coffee husks from fruits of the *Coffea arabica* variety, harvested manually in the 2017 coffee harvest at the Sabanilla pulper, Artemisa, Cuba. This residue was milled and screened to a diameter of less than 10 mm. The inoculum comes from the industrial wheat straw processing plant of the EBR German company, with a TS content between 24-32%. It was kept refrigerated at 4°C until its use. The active biomass was then solubilized, bio-suspended and hydrolysed in the laboratory using a patent-protected procedure. The determination of total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) and soluble COD (sCOD) were performed following the standards established by APHA-AWWA-WEF standardized methods [9]. The lignocellulosic composition (cellulose, hemicellulose and lignin) of the coffee husk was determined according to ANSI/ASTM D1103-77 and ANSI/ASTM D1106-96 [10, 11].

2.2 Experimental set-up

Discontinuous system (Biochemical Methane Potential (BMP) test)

The assembly of the anaerobic digesters was developed according to the German Standard [12], under mesophilic regime (37±1°C) with a digestion time of 15 days, using as ratio g_{VS} substrate/ g_{VS} inoculum equal to 0.1/1. Two variants were studied, one with untreated coffee husk (CC) and the other pretreated (CC-p) with 1 L $Ca(OH)_2$ /100 g_{TS} of the residue, for 6 hours at 50°C. The concentration of the alkaline solution was 3% (w/v). Each case study was developed in duplicate in 500 mL capacity glass containers occupying an effective volume of 320 mL.

The specific methanogenic capacity (SMC) test was used to account for methane production. Through a system of calibrated glass eudiometer tubes with liquid displacement operating under vacuum conditions, the gas ascends in contact with an alkaline solution of (15% NaOH). The displacement occurs when the biogas, being in contact with the solution, the content of CO_2 present dissolves and finally the CH_4 succeeds in replacing it. This event is recorded with the NaOH volume variation observed in each measurement and the result obtained is normalized

according to VDI 4630 [12]. The reactors were manually stirred before making the measurements of the volume of liquid displaced in the columns. Methane production was reported as average values of each experience performed in triplicate.

Semi-continuous system

The S-CSTR was operated at $37 \pm 1^\circ\text{C}$ using a 6 L capacity reactor in which an effective volume of 4L was occupied. This reactor has a mechanical agitation system and a coil in charge of maintaining the temperature in the medium. The feed was supplied daily manually and the experimentation was divided into four stages, taking into account the OLR [$\text{g}_{\text{VS}} \text{L}^{-1} \text{d}^{-1}$]:HRT [d^{-1}] ratio of 0.1:90; 0.2:90; 0.2:45 and 0.2:30. The experimental period began with the feeding to the CC-p and OLR system that varied between 0.1-0.2 $\text{g}_{\text{VS}} \text{L}^{-1} \text{d}^{-1}$, with the change of substrate to CC the system was maintained with OLR of 0.2 $\text{g}_{\text{VS}} \text{L}^{-1} \text{d}^{-1}$.

A recirculation in fraction 1/3 of the effluent was applied from the beginning of the use of CC. Methane production was obtained by displacing a 19.5 cm diameter hood with a total volume of 3.94 L. The volume displaced in the hood is the daily production of methane. Before the gas reaches the hood, it passes through a bottle containing a 15% NaOH solution to remove the CO_2 .

Sampling began when the S-CSTR reactor was started-up. The reactor was previously shaken for 10 minutes before the biomass sampling. The effluent was extracted from the surface of the reactors with a syringe. The liquid and solid phases were separated by centrifugation for 10 minutes at 10,000 rpm and the supernatant was filtered through a microfiber filter (0.45 μm). Both phases were stored in polyethylene tubes at 4°C . The extracted effluent was analysed by measuring: pH, temperature, TS, VS, organic matter content inside the reactor and volatile fatty acids to total inorganic carbon (VFA/TIC) ratio.

2.3 Determination of total polyphenolic content

In order to determine the content of total polyphenols in the extracts, the Folin-Ciocalteu colorimetric technique was applied [13]. A spectrophotometer (Rayleigh, model VIS-7236, China) was used to measure absorbance at 765 nm. The results were expressed in gallic acid equivalents per gram of total solids ($\text{mg GAE g}_{\text{TS}}^{-1}$). The value of the total polyphenolic concentration was determined from a calibration curve adjusted to a linear model.

2.4 Determination of VFA/TIC ratio

The ratio of the volatile fatty acids to total inorganic carbon (VFA/TIC) is an alkalinity ratio at two-point titration measurement. This ratio was determined following the method described by [14]. The samples were first titrated with 0.1 N sulphuric acid to pH 5.0 and then further to pH 4.4. The volume of sulphuric acid consumed to reach the respective pH values is equivalent to the amount of volatile fatty acids (VFA) and the amount of total inorganic carbon (TIC). The ratio of VFA to TIC provides information on the stability of the fermentation process. A ratio of ≤ 0.3 is recommended for a stable anaerobic fermentation.

3. Results and discussion

3.1 Characterization of the raw material

Characterization of inoculum and substrate (coffee husk) not pre-treated and pre-treated with alkaline solution was determined based on volatile solids and total solids content (Table 1).

Table 1 Characterization of CC and CC-p and inoculum

	CC	CC-p	Inoculum
TS (g/g w/w)	0.13	0.09	0.02
VS (g/g w/w)	0.12	0.08	0.01
%VS (d.b.)	91.62	88.42	56.02

Other authors who worked with coffee residuals reported results very similar to those shown in table 1 with %VS between 90 and 93 [2, 15, 16]. Moreover, when comparing these results with those of other lignocellulosic materials, such as corn straw containing 91.6 % VS or bagasse with 91.9 % [4], it can be concluded that this residual presents similar characteristics to other agro-industrial residues.

When applying the pre-treatment to the substrate (CC-p), a slight decrease in the volatile solids of the residual was observed, since organic compounds that were not accounted for in the gas may have been volatilized. With respect to the inoculum, it presents a volatile solids content higher than 50% recommended for use as a source of active biomass to the system [12].

The determination of the lignin, cellulose and hemicellulose content in coffee husks would confirm the lignocellulosic character of this residual (Table 2). As observed, the lignin content is in accordance with what has been reported by other authors for this substrate [1, 2, 17] that refer values of $17 \pm 1.5\%$. This value may contain alterations in the same crop species influenced by factors such as growth stage, genotype and environmental conditions, which may influence both the yield and efficiency of the process [18].

Table 2 Lignocellulosic fibre content of coffee husks

Component	%
Ligning	16.5
Cellulose	36.5
Hemicellulose	19.0

3.2 Effect of alkaline pre-treatment on the biodegradability of coffee husks. Biochemical Methane Potential assessment

The sCOD of the pre-treated coffee husk (CC-p) increased by 223% when applying the alkaline pre-treatment. As a consequence, when performing the anaerobic test in batch assays, the maximum methane yield value (274 mL_{CH₄} g_{VS-1}) was attained in the reactor containing CC-p, with an increase of 7.3% compared to the reactor containing the residue without pre-treatment with significant differences (p-value <0.5) (255 mL_{CH₄} g_{VS-1}).

3.3 Effect of alkaline pre-treatment on semi-continuous anaerobic digestion

The semi-continuous systems operated over a digestion period of 112 days. Figure 1 shows the behaviour of methane yield for each volumetric organic load and the different substrates.

The initial organic load selected was 0.1 g_{Vs} L⁻¹ d⁻¹ and was maintained throughout 27 days. During this period, pH value was 7.3±0.3, which indicate a regulatory capacity of the hydrogen potential of the anaerobic system despite this is not a control parameter to take immediate solutions if acidification occurs.

In Figure 1, stage I corresponds to the start-up of the system. In stage II, the OLR was increased to 0.2 g_{Vs} L⁻¹ d⁻¹ and remained at this value during the rest of the experimental period. Due to the characteristics of the solid coffee residues, it is very important to observe the development of the anaerobic process, since it is possible to transform some compounds into toxic substances that would partially or totally inhibit the biodegradation process. Previous unreported experiences of this research group demonstrated that higher OLR prompt the system to fail.

During the addition of CC-p, in the second stage, an average value of 163.3 mL_{CH₄} g_{Vs-1} was obtained, a lower result than that obtained in a discontinuous regime with a decrement of 41%. For this reason, it was decided to swift to untreated coffee husk (CC). With this change, a 34% increase in methane yield was observed, although it is 14.5% lower than expected one for this residual.

It should also be noted that with the change to CC (stage II-III) the methane yield began to show an increasing trend, that remained stable in the range of 190.0 – 365.0 mL_{CH₄} g_{Vs-1} with an average of 277.0 mL_{CH₄} g_{Vs-1} over the course of stage III. Accordingly, the methane yield was increased by 8.6% in relation to the discontinuous test. With the increase in the solubilized organic matter and fractionating lignin, an increase in methane yield was expected, due to microbial biomass would have access to cellulose and hemicellulose to hydrolyse it.

However, this result is similar to the one reported by [19] which applied an alkaline pre-treatment with NaOH in a semi-continuous assay. Consequently, the reactor showed an inhibition by the accumulation of compounds resulting from the fractionation of the lignin with the pre-treatment. Other authors report that lignin degradation leads to the release of phenolic compounds at higher concentrations. These phenolic compounds have a negative impact on the hydrolysis of a substrate [6]. Investigators [1] point out that a high concentration of polyphenols inhibits the fermentation process. These elements are present in coffee and its residues.

The characterisation of the effluents in the different stages, report polyphenols content when using CC and CC-p of 23.1 and 58.4 mg_{GAE} g_{TS-1}, respectively. There was a higher accumulation of these compounds when the pre-treated residual was used as a sole substrate, which can be attributed to the generation of toxic substances while pre-treated or hydrolysed during the AD. As effect, methane yield during the CC-p feed stage was lower than the one obtained for CC substrate. The alkaline or thermal pre-treatments can increase the concentration of phenolic compounds, i.e. p-coumaric acid, ferulic acid, vanilic acid and 4hydroxybenzoic acid, to inhibitory levels [6].

In stage IV, the strategy led to reducing the solids content and the inhibitory effect with a higher dilution to induce a decrease in the concentration of toxic substances in the system. During the experimental period, the methane productivity rate (PCH₄) increased over time with values ranging between 0.4 and 1.6 L L⁻¹d⁻¹ (Figure 2). This shows the way in which the active biomass responds to the daily addition of substrate to the system, that is, the rapidity with which the microorganisms consume the volatile solids. Other authors reported similar values when they treated fresh pulp with manure, with productivities that ranged between 0.5 and 1.1 L L⁻¹d⁻¹ [20].

The best operational condition during experimentation was for the combination of 45 days of HRT and 0.2 g_{Vs} L⁻¹d⁻¹ (stage III) with an average production of 277.0 mL_{CH₄} g_{Vs-1} and a productivity of 1.1 L L⁻¹d⁻¹. These results

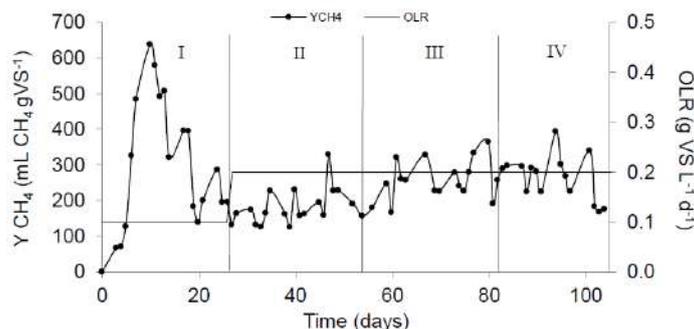


Figure 1 Coffee husk biodegradability with [CC-p) and without pre-treatment (CC). Discontinuous vertical line represents changes in feeding strategy between CC-p and CC

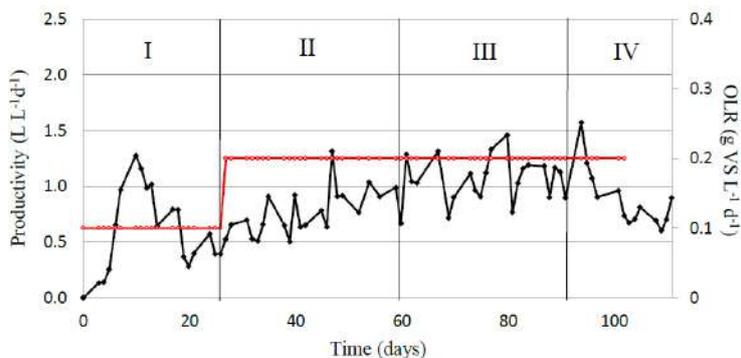


Figure 2 Methane productivity during semi-continuous assay. The red line represents the change of OLR

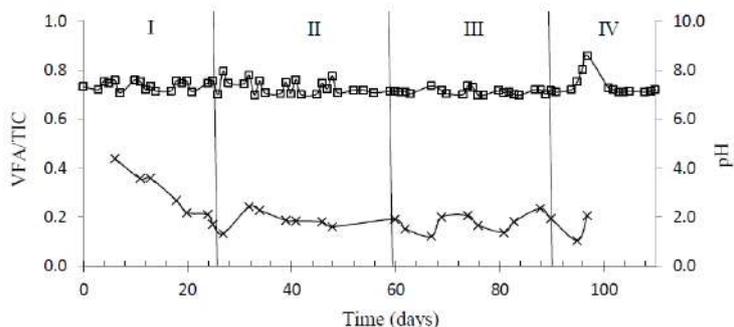


Figure 3 pH (□) values and VFA/TIC (x) ratio during mono-digestion of coffee husk with [CC-p) and without pre-treatment (CC)

are similar to those obtained for other lignocellulosic residues. In [21] is reported that maximum methane yield of corn straw digestion is $302.8 \text{ mL}_{\text{CH}_4} \text{ g}_{\text{VS}}^{-1}$ when applying $2 \text{ g}_{\text{VS}} \text{ L}^{-1} \text{ d}^{-1}$. This result was associated with the ease of degradation of other components present in maize straw that facilitate the anaerobic digestion, and the absence of inhibitory components, such as polyphenols present in coffee. Another substrate such as rice straw expressed yield values between $195.0\text{-}280.0 \text{ mL}_{\text{CH}_4} \text{ g}_{\text{VS}}^{-1}$ for $0.5 \text{ g}_{\text{VS}} \text{ L}^{-1} \text{ d}^{-1}$ [22, 23].

According to these results, pH and VFA/TIC values were in the expected values as recorded in Figure 3.

The pH was generally within the optimal range for AD process (6.5 - 8.2) [24, 25]. On the other hand, the VFA/TIC ratio ranged between 0.12-0.43 indicating an adequate conversion of the substrate to methane [6, 8, 14, 20]. In general, the behavior of this ratio remained stable during the experimental period demonstrating the stability of the process under the combination of HRT and the applied OLR.

4. Concluding remarks

- The alkaline pre-treatment applied to coffee husk expressed a 223% of solubilization in terms of COD with an increase in methane yield obtained only under discontinuous regime.
- The accumulation of toxic substances such as polyphenols were detected when the pre-treated coffee waste was added, observing a methane yield decrement of 41% with respect to the methane yield obtained under discontinuous operation.
- For the semi-continuous operation, when non-treated coffee husk was digested, the best results were for the combination of 45 days of HRT and $0.2 \text{ g}_{\text{VS}} \text{ L}^{-1} \text{ d}^{-1}$ attaining a methane yield of $277 \text{ mL}_{\text{CH}_4} \text{ g}_{\text{VS}}^{-1}$. It was confirmed that these residues require low hydraulic and volumetric organic loads.

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