

Methane production from coffee crop residues

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Abstract

The increase in global energy demand promotes the use of energy crops and agro-industrial residues for biogas or methane production. In agro-business, the coffee waste as mucilage is a major source of carbohydrates mainly galactose and glucose. The aim of this study is to optimize the anaerobic digestion process of coffee mucilage for methane production. An experimental design 2³ full factorial with center points was set, generating 20 treatments, considering factors such as pH, temperature and initial sugar concentration. Optimal conditions for the methane production from coffee mucilage estimated by statistical software were pH 8.2, temperature 37 °C and sugar concentration 27 gL⁻¹. Through this research project, the experimental optimum conditions for the production of methane from coffee mucilage were used, which were pH 8.2, temperature 37 °C, sugar concentration 25.5 gL⁻¹ and 313 mL methane (atmospheric pressure), the last exceeded the estimated optimal condition.

Keywords: anaerobic digestion, coffee mucilage, methane, biogas.

1. Introduction

Biogas is considered a renewable energy fuel produced from biomass; it has become important because of the environmental benefits it offers [1]. The substrates used for anaerobic digestion are energy crops, organic waste and animal manures [2]. After oil, the second most traded product is coffee, therefore, to have high demand for this product, large waste quantities are obtained in agro-business, mainly: pulp, mucilage and coffee husk. These residues are rich in sugars and compounds with functional properties that most often do not receive adequate treatment so they become sources of river contamination, mainly [3]. Coffee cherry consists of pulp, mucilage, green coffee and husks, with 40.6 %, 21.5 %, 20% and 17.9 % respectively. In the case of coffee mucilage fraction, it is attached to the grain on the wet processing after pulping without enzymatic degradation [4]. The mechanical extraction allows the separation and concentration of this fraction to be utilized as a substrate in anaerobic digestion for the content of simple sugars. According to Esquivel et al., the coffee mucilage is composed of water (84.2 %), protein (8.9 %), sugar (4.1 %), pectin (0.91 %) and ash (0.7 %) [5]. The harvested area of coffee cherry in Mexico in 2014 was 699,307.33 hectares, with a production of 1,166,025.82 tons. Chiapas was the state that most coffee cherry was produced with 402,099.78 tons. If we consider that 21.5 % of coffee is mucilage the production would be 86,451.45 tons. The response surface methodology (RSM) is a set of mathematical and statistical techniques for the design of experiments used to model and analyze problems, in which a variable of interest is influenced by others. The goal is to

optimize the variable of interest; this is achieved by determining the optimum operating conditions of the system. The two designs most used in RSM are central composite design (CCD) and Box-Behnken design (BBD). The CCD is ideal for series of experiments and that throws a good amount of information to prove the lack of adjustments [6, 7].

2. Materials and Methods

2.1 Substrate

Coffee mucilage was used as substrate, which was obtained by mechanical extraction of coffee bean in the town of Chicomuselo, Chiapas, Mexico. The extraction was done using a pulper with demucilating, a liter of water was added for a kilogram of coffee cherry, the mucilage extracted was stored in bottles at -20 °C to prevent degradation due to the sugar content it has. The concentrations of sugars in the experimental design were 5 (72, 65, 50, 35 and 27 gL⁻¹) starting the highest concentration of 72 gL⁻¹, diluted with water to the desired concentrations, measured by HPLC. Once these concentrations were reached, it pasteurized at 65 °C 30 min and 20 min on ice. Subsequently the required volume was transferred to previously sterilized serological bottles.

2.2 Inoculum

For the tests it was used as inoculum methanogenic sludge from an industrial anaerobic wastewater treatment reactor, generated during the processing of confectionery factory located in the industrial area of the city of San Luis Potosí, México [8]. The inoculum was stored at 4 °C for 5 days to preserve it before use.

2.3 Experimental design

CCD was applied for determining the optimum conditions, the number of experiments obtained was: 8 star points ($2^k = 2^3$), 6 axial points (2^k) and central points (6 replicates). A 2^k full factorial design with five levels leadings to 20 sets of experiments was made to evaluate the effect of pH (factor Y_1), temperature (factor Y_2) and the initial sugar concentration (factor Y_3) as independent variables of the fermentation. The following equation was used to build response surface graphs for the model and for predicting the optimal value:

$$Z = \alpha_0 + \alpha_1 Y_1 + \alpha_2 Y_2 + \alpha_3 Y_3 + \alpha_{11} Y_1^2 + \alpha_{12} Y_1 Y_2 + \alpha_{13} Y_1 Y_3 + \alpha_{22} Y_2^2 + \alpha_{23} Y_2 Y_3 + \alpha_{33} Y_3^2 \quad (1)$$

where, Z is the predicted response corresponding to the methane production at anaerobic digestion, Y_1 , Y_2 , and Y_3 are independent variables, α_0 is an independent term, α_1 , α_2 and α_3 are linear effects, α_{12} , α_{13} and α_{23} are interaction terms and α_{11} , α_{22} and α_{33} are quadratic coefficients. The model for methane production (MP) was evaluated with good fit, significance and the coefficient determination. The optimum value was obtained by solving the regression equation. The ANOVA, response surface and the optimal conditions were got using the commercial software (Statgraphics centurion XVI.II Manugistics Inc., Rockville, MD). The significant effects on dependent variables were determined by t-test with a probability value smaller than 0.05 (P-value <0.05).

2.4 Anaerobic batch digester

The anaerobic batch digestion was conducted in serological bottles of 110 mL with a working volume of 100 mL, it was added 98 mL of coffee mucilage, 1 mL of inoculum and 1 mL of buffer solution. To control the pH the following compounds in (mgL^{-1}) were used: Na_2HPO_4 , 11,900; $\text{NH}_4\text{H}_2\text{PO}_4$, 4500; K_2HPO_4 , 125; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 100; ZnCl_2 , 75; $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$, 25; $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 15; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 12.5; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5; $\text{CoCl}_2 \cdot 8\text{H}_2\text{O}$, 3 [9]. The pH of each test was adjusted with 2N NaOH. This process was according to experimental design conditions with constant agitation using an orbital shaker with controlled temperature at 200 rpm during 108 h, samples were taken with a syringe for each 12 h. The validation of optimal conditions was assessed in triplicate.

2.5 Analysis

The determination of methane produced was performed by the method of volume of water displacement, with a 2N NaOH trap [10]. The anaerobic digestion medium samples were taken in the tests, it was centrifuged at 10,000 rpm for 10 min at 5 °C. The supernatant was filtered through a 0.22 μm filter (Millipore, Bedford, MA, USA) to determine sugar consumption and concentration of volatile fatty acids by high-performance liquid chromatography (HPLC), using a Phenomenex column eluted at 60 °C with H_2SO_4 0.0025 M at a flow rate of 0.5 mLmin^{-1} and having a refractive-index detector [11].

2.6 Substrate characterization

The main compounds observed in the coffee mucilage are monosaccharides such as galactose and glucose, as well as lactose as the disaccharide, protein, and acetic acid (table 1), with an acidic pH of 4.5. Acetic acid formation may be due to handling in shipment and storage of coffee mucilage, this was kept at freezing temperatures, in order to limit the fermentation caused by the content of microorganisms capable of consuming the sugars present in the coffee mucilage, as mentioned by Jackels, S. and Jackels, Ch. [12].

Table 1. Characterization of sugars and acids available

Compound	Concentration (gL^{-1})
Galactose	37.67
Glucose	36.65
Lactose	1.4
Acetic acid	0.67
Protein	0.12

3. Results and discussion

3.1 Experimental design

The MP from coffee mucilage was conducted under a distribution of 20 tests defined in the experimental design, 6 tests were central points at pH 6 at 37 °C, and sugar concentration of 50 gL^{-1} , this was done to reduce the experimental error and accuracy of estimating the variance for the points having the same distance in the central region [13].

Furthermore, the design consists of 6 tests called axial or star points, these points were under the following input factors; pH ranging from 3.7 to 8.2, temperature 29 °C to 44 °C; sugar concentration of 27 gL^{-1} to 73 gL^{-1} . Finally it consists of 8 tests called factorial points, pH values of 4.5 and 7.5, temperatures were 32 °C and 42 °C; sugar concentrations; 35 gL^{-1} and 65 gL^{-1} (table 2).

Table 2. Experimental design

Test	Independent variable			Dependent variable		
	Factor Y ₁	Factor Y ₂ (°C)	Factor Y ₃ (g L ⁻¹)	MP (mL)	Y _{MP P/S} (mol CH ₄ mol ⁻¹ S ⁻¹)	PR _{MP} (mmol CH ₄ h ⁻¹)
C1	6	37	50	173	0.26	0.41
C2	6	37	50	188	0.28	0.48
C3	6	37	50	188	0.28	0.48
C4	6	37	50	180	0.27	0.44
C5	6	37	50	162	0.24	0.36
C6	6	37	50	171	0.25	0.40
A1	3.7	37	50	5	0.01	0.00
A2	6	29.38	50	45	0.07	0.03
A3	6	37	72.87	140	0.14	0.18
A4	8.29	37	50	269	0.40	0.99
A5	6	37	27	272	0.74	1.87
A6	6	44.62	50	90	0.13	0.11
F1	4.5	42	35	6	0.01	0.00
F2	7.5	32	65	178	0.20	0.33
F3	4.5	32	65	6	0.01	0.00
F4	4.5	42	65	6	0.01	0.00
F5	7.5	32	35	203	0.43	0.80
F6	7.5	42	65	172	0.19	0.31
F7	4.5	32	35	78	0.16	0.12
F8	7.5	42	35	168	0.35	0.55

C: central points, A: axial points, F: factorial points, Y₁: pH, Y₂: temperature, Y₃: sugar concentration, MP: methane production, Y_{MP P/S}: yield product-substrate, mol CH₄mol⁻¹S⁻¹: mol of methane per mol of sugar, PR_{MP}: production rate process, mmol CH₄h⁻¹: milli mol of methane per hour

3.2 Analysis of response surface and optimization

Analysis of variance showed a significant statistical effect on pH, temperature and concentration of sugars by having values 0.0134, 0.0004 and 0.0037, respectively (probability value > 0.05) taking a confidence level of 95%, the quadratic term of Y₁² is presenting the greatest effect, followed by linear terms Y₃ and Y₂.

By using a quadratic model it proved the equation 2:

$$MP = -15.615 + 0.818Y_1 + 0.826Y_2 - 0.069Y_3 - 0.058Y_1^2 + 0.0029Y_1Y_2 + 0.0016Y_1Y_3 - 0.012Y_2^2 + 0.0009Y_2Y_3 + 0.0001\alpha_{33}Y_3^2 \quad (2)$$

In the equation (2), Y₁, Y₂ and Y₃ are variables coded to pH, temperature and initial sugar concentration, respectively. The regression coefficient was 0.96, which indicates that the points are near the line, having a favorable confidence interval.

Figure 1 displays the effects of pH, temperature and initial sugar concentration with regard to MP. The combined effect of pH with temperature, MP effect was observed at pH 8, and temperatures around 37 °C.

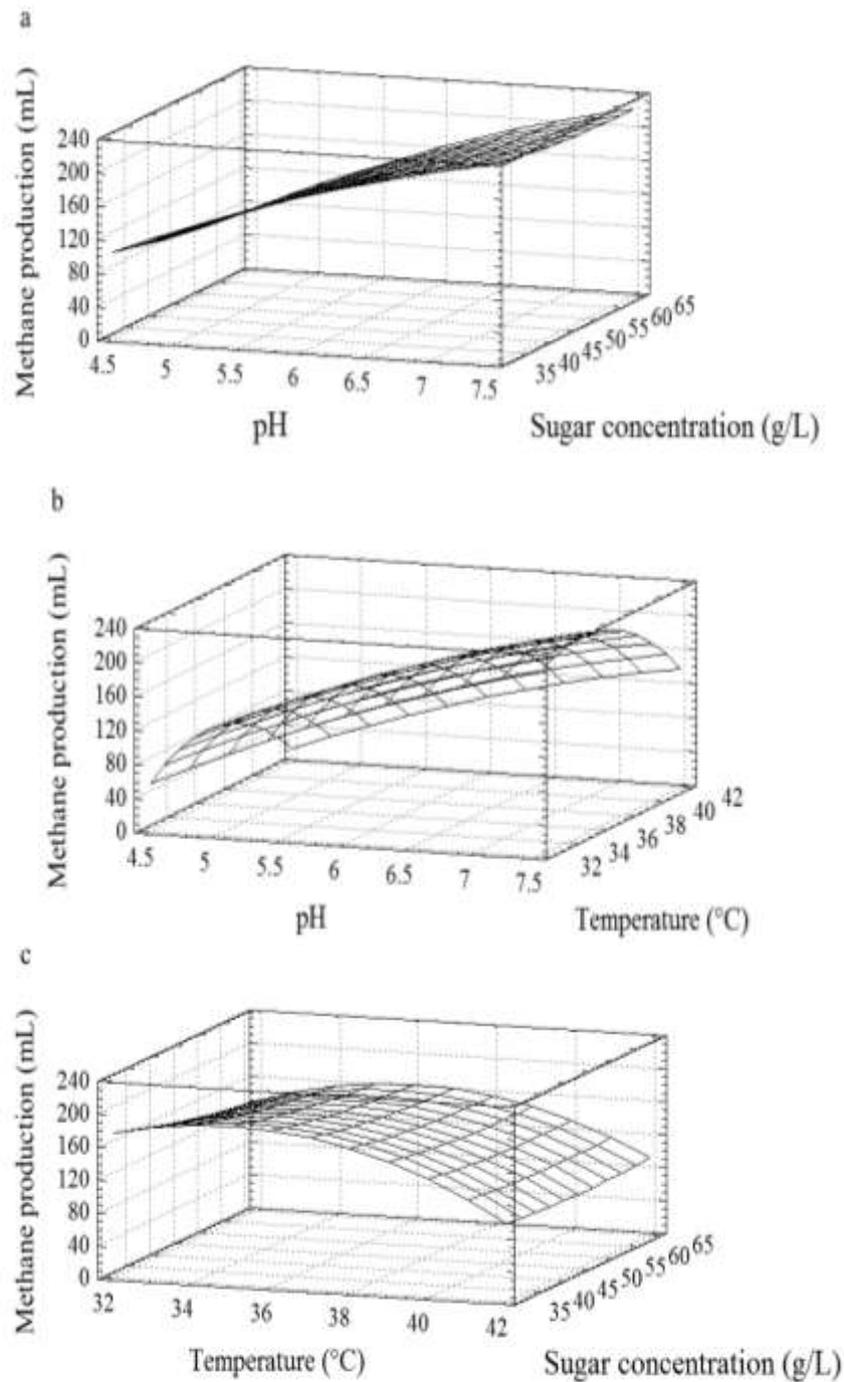


Figure 1. Response surfaces that describe the model predicted with the representation of the dependence of MP on pH, temperature and initial sugar concentration. (a) Effect of pH with respect to initial sugar concentration, (b) Effect of pH with respect to temperature, (c) Effect of temperature with respect to initial sugar concentration.

Under the analysis of each input variable in the experimental design, the optimal variables for producing methane from coffee mucilage is obtained, which are shown in table 3. The obtained methane production was 311 mL of methane, compared to the theoretical data of

293 mL of methane, with a sugar consumption of 40% (figure 2), the experimental variables confirms the viability of methane production.

Table 3. Optimum variables

Factors	Optimum conditions	Experimental conditions
pH	8.29	8.2
Temperature	36.22 °C	37 °C
Initial sugar concentration	27 gL ⁻¹	25.5 gL ⁻¹
Methane production	293 mL	311 mL

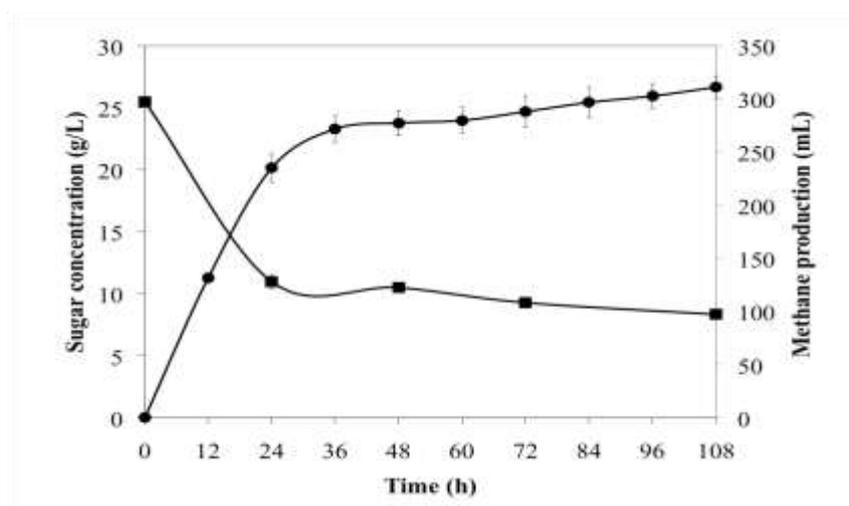


Figure 2. (●) Methane production and sugar consumption (■) in optimum experimental condition

Seeing that the sugar concentration remained constant after 48 hours, the methane production was continued in this lapse. This is due to the inhibition caused by volatile fatty acid accumulation. The quantification of volatile fatty acids (VFA) was performed and mainly butyric acid, lactic acid and acetic acid were detected, figure 3 shows the behavior of these compounds.

Optimal conditions for the methane production from coffee mucilage estimated by the software were pH 8.2, temperature 37 °C and sugar concentration 27 gL⁻¹. Through this work, the experimental optimum conditions for the production of methane from coffee mucilage were identified, which were pH 8.2, temperature 37 °C, sugar concentration 25.5 gL⁻¹ and 313 mL methane, the last exceeded the estimated optimal condition.

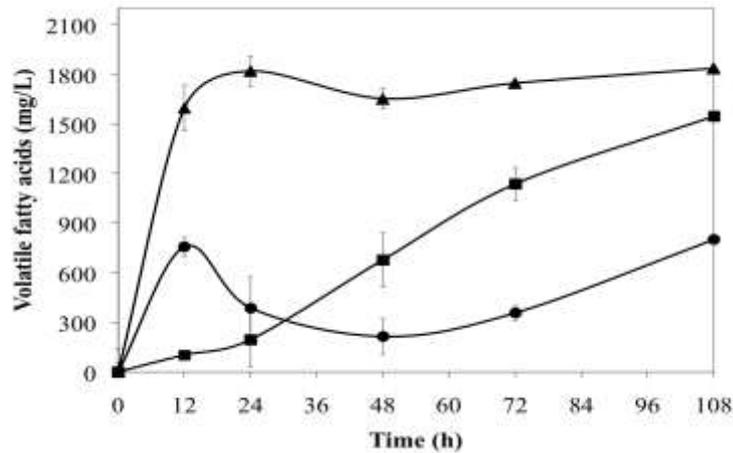


Figure 3. Quantification of volatile fatty acids generated with time during the optimum experimental conditions (▲) Butyric acid; (■) Lactic acid and (●) Acetic acid

Optimizing the process improved the methane production system under optimal conditions, so that the methane production yield also improved. Tests with pH values close to 7, showed a MP above 100 mL, similar to that reported by Charles *et al.*, which indicates that values between 6.8-8.2, optimize methane production by digestion [14]. Appels *et al.* noted that the optimal values are close to 6.5-7.5 [1]. Bagi *et al.* indicated that the optimum pH is above 7, it is reached by adjusting buffer solutions during the digestion process [15].

For values of temperatures near 38-42 °C, they had an average MP of 170 mL, these parameters are similar to those mentioned in Alzate *et al* [16, 17], Achou *et al.* [18] and Alastier *et al.* [13]. The effects presented in MP at temperatures below or above 38-42 °C, showing lower production values, as Goux *et al.* mentioned [19], high temperature values favor the hydrolytic activity of microorganisms. In this study, the inoculum was in mesophilic conditions, hence it behaves under inhibition effect of methanogenic consortium. The tests with pH of 3.6, temperature of 37 °C and sugar concentration of 50 gL⁻¹, showed a null methane production, this has a similarity with those reported by Slimane *et al.* [20]; Donoso *et al.* [21]; Eskicigluo and Ghorbani [22] and González *et al.* [23]. These indicate that low pH favors the development of inhibitors in the system, such as volatile fatty acids, ammonia and CO₂. Temperatures above 44 °C generates thermophilic conditions [24], which for batch tests using mesophilic inoculum are more sensitive to temperature fluctuations and require more time to adapt to a new temperature.

The product-substrate performance of each experimental test which forms the design was evaluated, CH₄ (mol) with respect to mol of sugar. It was also analyzed the production rate expressed in CH₄ mmol per hour. The highest product-substrate yield was 0.742 molCH₄ per mol of sugar, which corresponds to the test conditions of pH 6, at 37 °C, and sugar concentration of 27 gL⁻¹, this yield match the values reported by Davila and Razo, varying from 0.6 to 1.3 mol CH₄ per mol of sugar [25, 26]. The lowest yield was 0.007 mol CH₄ per mol of sugar, these three tests had conditions of pH 3.7, 4.5 and 4.5, temperatures of 37 °C, 32 °C and 42 °C, sugar concentrations of 50 gL⁻¹, 65 gL⁻¹ and 65 gL⁻¹ respectively.

Butyric acid recorded 1833 mgL⁻¹, which is the highest concentration in digestion, followed by lactic acid 1547 mgL⁻¹. A third compound formed was the acetic acid, the high formation of this acid is due to the adaptation phase of the inoculum to the medium [27], the values are 757 and 720 mgL⁻¹ respectively. The VFA can be used as substrates in forming methane [28], therefore the decrease occurs in 48 hours with a record of 213 mgL⁻¹ of acetic acid with regard to the remaining two acids which were less assimilated by methanogenic sludge. According to Fang *et al.* [29] and Fantozzi *et al.* [30], values greater than 6000 mgL⁻¹ of VFA have an inhibitory effect on the digestion process; therefore, in this investigation the VFA do not generate this effect.

4. Conclusion

The characterization of coffee mucilage can identify exploitable sugars for methane production. Using coffee mucilage as a substrate for methane production is a viable option. Optimal conditions for the methane production from coffee mucilage estimated by the software were pH 8.2, temperature 37 °C and sugar concentration of 27 gL⁻¹. Through this work, the experimental optimum conditions for the production of methane from coffee mucilage were identified, which were pH 8.2, temperature 37 °C, sugar concentration of 25.5 gL⁻¹ and 313 mL methane (atmospheric pressure), the last exceeded the estimated optimal condition. Optimizing the process improved the methane production system under optimal conditions, hence the methane production yield also improved.

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Conflict of interest

The authors declare that they have no conflict of interest.

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