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Evaluation of Bio-Hydrogen Production by Dark Fermentation from Cocoa Waste Mucilage

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The problems caused by a large amount of waste produced every day become one of the main concerns of world nations. Then, a question is how various actions arise that seek a transformation in the habits and mentalities. One way to contribute to this cause is to change the sources of polluting energy for sustainable energy, such as hydrogen based. It has a high energy efficiency, and its combustion does not generate polluting emissions. Likewise, as a source for the generation of clean energy, residual biomass is used, which turns out to be a feasible option since its availability is abundant. In the cocoa industry, one of the residues that stands out for its quantity is the cocoa mucilage. This residue can act as a substrate to provide biogas, and that contains high levels of sugars, fibers, proteins, and nutrients. However, in this study, the possibility of producing hydrogen from cocoa mucilage is evaluated by dark fermentation processes. The evaluation is carried out on a laboratory scale, by tests in batch reactors made of 250 ml amber glass and with a 220 ml headspace. The temperatures evaluated during the experiment were 35 °C (mesophilic) and 55 C (thermophilic). The organic loads evaluated during the process were 4, 8, and 12 grams of volatile solids per liter (gVS / L), respectively. The experimental testing duration was subject to the presence of methane in the gas produced. For determining the methane presence, a portable analyzer was used. The gas determination was done daily. The evidence of the reaction rate in the tests at 55 ° C is higher than at 35 ° C, and the presence of methane at thermophilic conditions occurred at five days, while the mesophilic conditions occurred at 23 days. It is found that, for tests at two temperatures, the best results are detected when the organic load is 12 gVS / L, and the maximum hydrogen production (703 mL H_2) is reached when the temperature is 35 ° C. The maximum production suggests that under mesophilic conditions the process can be maintained in hydrolysis for longer times.

1. Introduction

The negative environmental impacts caused by productive human activities are generating great concern around the world. Pollution, both atmospheric and ecological, has increased exponentially for reasons such as demands for energy sources, unchecked production of goods, population growth, among others. The current energy model boosts air pollution as the primary source of energy is fossil fuels (coal, oil, natural gas). The burning of fossil fuels, as well as all those processes necessary for their extraction, causing atmospheric pollutants such as carbon dioxide (CO_2) nitrogen oxides (NO and NO_2), sulfur oxides (SO_2 and SO) or suspended particles to be generated. One of the most significant contributors to air pollution is the case of cars because they emit the three primary pollutants, carbon dioxide (CO_2), carbon monoxide (CO) and nitrogen oxides (NOx), as well as unburned hydrocarbons, compounds of lead. During the burning, hydrocarbons react with nitrogen oxides through sunlight and at elevated temperatures, which causes ozone at ground level, which can cause eye irritation, coughing, gasping, respiratory failure, and permanent lung disorders. Also, nitrogen oxides (NO_X) contribute to the formation of ozone and acid rain, as well as affecting water quality and finally carbon monoxide, which is a lethal colourless gas that reduces oxygen flow in the bloodstream and can affect brain function and vision (Perez, 2017). Considering the above, the World Health Organization estimated that one in nine deaths worldwide is the result of air pollution-related conditions

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(Quaderi, 2018). So, the depletion of fossil fuels, the increase in the value of extraction and use of their derivatives, as well as the global warming associated with greenhouse gas emissions resulting from their uncontrolled use, have generated considerable interest in the search for alternative sources of energy. On the other hand, an additional problem with the use of fossil fuels lies in the amount of waste produced daily. Such waste generation causes a big concern, and a series of actions are proposed to mitigate its impact, which seeks to transform habits and mentalities. These actions include the use of residual biomass as a source for clean energy generation, such as hydrogen. Hydrogen as a clean fuel does not generate emissions from exhaust pipes, as in the case of internal combustion vehicles (since it generates only water). It also has a high energy performance: 2.75 times higher than fossil conventional or hydrocarbons (Han and Shin, 2004), that's why hydrogen could also reduce dependence on hydrocarbons and may become essential in the future (Anjos et al., 2017). Hydrogen energy has been used in the food and household companies, leading to the growing need for large-scale production, which would be sustainable and economically viable (Kapdan & Kargi, 2006). The hydrogen production can be through various processes such as gasification, ion exchange, among others. However, it is essential to note that most methods of obtaining are costly. Given the demand for production and, as mentioned above, an alternative to hydrogen production as a source of clean energy is residual biomass. Since, during agricultural, livestock, and forestry activities, a large amount of waste is available, which turns out to be sources for obtaining clean and renewable energy, what turns out to be a feasible option. Organic waste is generally used for composting and as a dietary supplement for animals. They have enormous potential for obtaining biofuels, as is the case with biogas, and some of these residues have all the necessary elements for the production of hydrogen. As is the case of cocoa residue that compared to other substrates, this reaches much more productive levels, resulting in more significant benefits and excellent stability, since, in the process, there is no more considerable variability (Hernández et al., 2018). If it looks at the cocoa industry, one of the residues that stand out for its quantity is the mucilage of cocoa. This residue can act as a substrate to provide biogas and contains high levels of sugars, fibers, proteins, and nutrients that make it a promising residue. Therefore, during the investigation, the transformation of mucilage for hydrogen production is analysed.

2. Material and methods

The evaluation of bio-hydrogen production through the dark fermentation process was carried out in batch-type reactors of the total volume of 250 mL and an operating volume of 200 mL Also, the experiments in thermostatic baths at constant temperatures were put. The temperature conditions for experimentation were 35 °C and 55 °C. The reactors contained a substrate/inoculum ratio of 2 (g SV substrate/g SV inoculum). The organic loads 4, 8, and 12 gSV/L in the experiment were evaluated. The blank samples (without mucilage) with the amount of inoculum used for the different loads were added. All experiments were done in triplicate. For the quantification of the biohydrogen produced, the displacement method was used, using an alkaline solution of sodium hydroxide (NaOH 0.5M) (see Figure 1). Reactors with samples were adjusted pH with a buffer solution or acetic acid-acetate regulator with a pH of 5.5. The experimental development time was subject to the presence of methane in the produced gas.

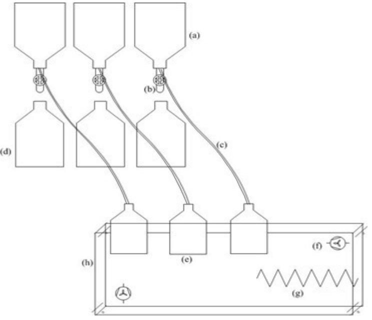

Figure 1. Scheme of experimentation in thermostatic baths (Mosquera et al., 2018).

The BIOGAS 5000 portable analyzer was used to determine the presence of methane. Measurements daily were made, and when it was detected 0.5%v or more methane presence in the biogas, the experiment was stopped. The substrate used during the experimentation was the Industrial Cocoa Mucilage (ICM), obtained from an industrial process of a private regime farm in Huila (Colombia). The inoculum used came from an anaerobic digester from the company Alpina, located in Sopó (Cundinamarca). In order to use the inoculum in the experimentation, it underwent a thermal pretreatment using a temperature of 120 °C for 15 min. Before the process, a physicochemical characterization for both the substrate and the inoculum was realized. The content of volatile solids (SV), total solids (ST) and organic matter (MO) were determined, through the following standard methods, 2540B (APHA) for total solids, The American Society for Testing and Materials (ASTM) D3174 and Nitrogen Kjeldahl (NTK) according to D1426-15 of the American Society for Testing and Materials (ASTM). The data obtained were analyzed through a variance analysis (ANOVA) using the SPSS statistical tool.

3. Results and discussion

3.1 Physicochemical characterization of the substrate

Table 1 present the physicochemical characterization of the chosen substrate. The results obtained were compared with characterization reported by Rangel et al., 2019, and the biomass and residue database- Phyllis2 (Efficiency,2015). There are similarities in the ranges of all the variables analysed. In the characterization is observed, the substrate has a high content of organic matter that can be consumed by microorganisms, which favors the process development.

Table 1. Characterization of industrial cocoa mucilage[a]

	%Humidity	%Total Solids	%Organic Matter	%Volatile Solids	%NTK	COD (g/L)
Industrial Cocoa Mucilage	10.85	89.15	85.13	75.90	3.08	8.46
Standard deviation (%)	0.16	0.16	0.44	0.49	0.04	0.01

(a) The analysis is reported on a wet basis

3.2 Cumulative hydrogen production

Figure 2 shows the accumulated volumetric production for the loads studied (4 gVS/L, 8 gVS/L, and 12 gVS/L), at defined temperatures (35 °C and 55 °C). Similarly, cumulative production for the respective blank can be observed.

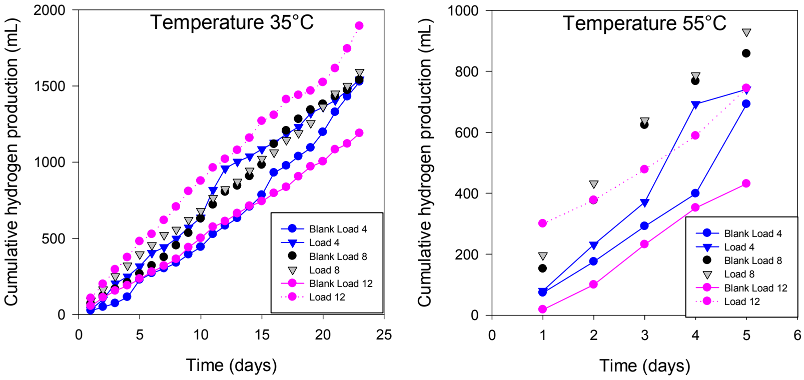

Figure 2. Cumulative hydrogen production with different loads (4, 8 and 12 gVS/L) and temperatures (35 °C and 55 °C)

Almost all the experimentation development is evident that the production of the different samples was most of the time above the blank. Besides, in some cases (load 4 and 8), at the end of the experimentation, time production was minimal, which may indicate the depletion of the organic material to degrade. Since the inoculum cames from a productive reactor, it has organic matter that can produce hydrogen. For 4 gVS/L load, the differences in the target with the sample are not significant (16.33 ml and 48.67 ml for temperatures of 35°C and 55°C, respectively). The contrast with the 12 gSV/L load, the productions are 44 and 7 times higher, for the respective temperatures. For the case, the temperature of 35°C highlight that the production process on the day of completion of the experimentation (23 days) not finished yet.

When analyzing the cumulative hydrogen production for day 5 in both temperatures (Table 2), the production results when the test temperature corresponds to 55°C, are doubled. The results indicate that, at higher operating temperatures, there is an increment in the system's entropy. The increment is due to the activation of thermophilic species. These species can produce higher amounts of hydrogen compared to mesophilic species (Balachandar et al.,2013). The observation confirms that the reaction rate observed at 55°C is higher than 35°C in the first five days.

*Table 2. Cumulative production on day 5**

Temperature (°C)	Cumulative production (ml)		
	4 gVS/L	8 gVS/L	12 gVS/L
35 °C	316	395	479
55 °C	756	972	766

*The results showed calculated without subtracting the cumulative production of the respective blanc

However, it is essential to clarify that in the case of the temperature of 55 ° C, from day 5, there is evidence of methanogenic activity, which involves that hydrogen started to consume. Then, if it is considered the accumulated production until the moment when the presence of methane is detected (that is when the consumption of hydrogen inside the reactor begins), the process at a temperature of 35 ° C is better; since the presence of methane can be noted under thermophilic conditions at 5 days. Meanwhile, in mesophilic conditions amount after 23 days of experimentation, not yet. It is essential to highlight the positive effect of the ratio (substrate/inoculum=2) on the specific speed. In some studies, show the specific speed increases when the ratio (substrate/inoculum) is higher (Luque 2019). Finally, it is essential to mention that the pH was adjusted to 5.5, which turned out to be suitable to aid production yields, as several studies have shown that pH between 5 and 6 have been optimal for higher hydrogen production yields (Balachandar et al., 2013).

3.3 Variance Analysis (ANOVA)

From obtaining the data throughout the experimentation process, performed an analysis of 2 fixed factors; 1) Temperature with 2 levels (35 °C and 55 °C) and 2) organic load (4 gVS/L, 8 gVS/L and 12gVS/L). The analysis has allowed an approach to the most appropriate factorial combination. Table 3 presents the analysis results with the SPSS statistical tool.

Table 3. Variance Analysis -ANOVA

Origin of variations	Sum of squares	Degrees of freedom	Average squares	F	Probability	Critical value for F
Temperature	57545.281	1	57545.281	9.305	1.008E-02	4.747
Load	851883.840	2	425941.920	68.872	2.648E-07	3.885
Interaction	173090.396	2	86545.198	13.994	7.303E-04	3.885
Within the group	74214.167	12	6184.514			
Total	1156733.684	17				

According to the analysis in Table 3, the temperature factor statistically significantly affects the production of H_2 (9.305 > 4.747), the load factor if it statistically affects H_2 production (68.872 > 3.885) and there is a statistically significant interaction effect between both variables (13.994 >3.885).

3.4 Response graph

Figure 3 allows representing the interaction between the temperature factors and organic load. It also allows the optimal value for the production.

Figure 3. Response graphs of individual effects

In the response plots, it can observe the behavior of the two factors separately. The temperature plot (a) shows that at a temperature of 55° C, the production of hydrogen decreases, while in the organic load graph (b), is shown the behavior of the amount of H_2 produced with respect to the different loads, is higher when the load is 12 gVS/L.

3.5 Interaction plot (average) for H_2 production

Figure 4 allows to find a way to obtain the optimal value in process performance.

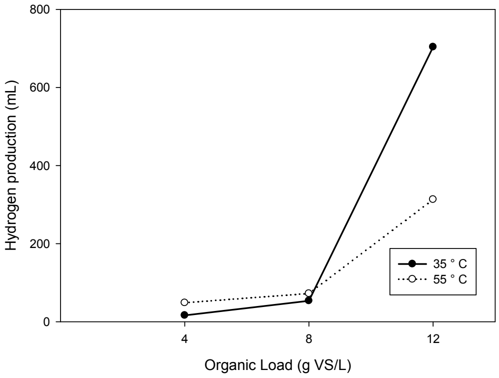

Figure 4. Interaction plot (average) for H2 production

The figure refers to the interaction between the temperature and the organic load used in the reactors. There is a lack of parallelism, which denotes that there is a strong, statistically significant interaction. The temperature of 35 °C will always produce more H_2 than the temperature of 55 °C. When working with loads 4gVS/L and 8gVS/L at either temperature, production is not very different. However, it is evident that when the load is 12 gVS/L, production increases significantly (703 mL).

3.6 Fisher's Proof of Minimum Significant Difference (LSD).

Finally, the LSD test was performed, and the simple effects of both temperature and load on hydrogen production are determined. The results are seen in tables 4 and 5, respectively.

Table 4. LSD test for simple effect of temperature

Temperature (C)	Amount	Media
35	9	258
55	9	145
Contrast	Difference	LSD
35-55	113.08	98.93

Comparing the mean of the temperature 35 °C with 55 °C, it has to 113.08>98.93; therefore, the temperature of 35 °C is different from that of 55 °C, then temperature 35 °C gives higher production of H_2

Table 5. LSD test for simple effect of organic load

Temperature (C)	Amount	Media
4	6	33
8	6	63
12	6	509
Contrast	Difference	LSD
4-8	31	80.8
4-12	476	80.8
8-12	445	80.8

Comparing the mean of organic load 4g VS/L with 8gVS/L, has to 31 <80.8; therefore, there are no differences between the two loads. When comparing load mean 4gVS/L with 12 gVS/L, it has to 476>80.8; therefore, there are differences in the two loads. When comparing the mean of load 8g VS/L with 12 gVS/L, it has to 445>80.8, so there are differences in the two loads. For experimentation, the highest mean observed was the 12gVS/L organic load, and in that, it gives the highest production of H_2. Then, from the above statistical analyses, it can be inferred that with the LSD test, the recommended temperature is 35 °C and the load of 12 gVS/L. Also, similar information recommended in the interaction graph. So based on the above results, the ideal combination to obtain the highest production of H_2 from cocoa mucilage is 35 °C and 12g VS/L.

4. Conclusions

At the end of experimentation and the analysis, it is concluded that concentrations are not crucial in obtaining hydrogen. On the other hand, the reaction rate in the tests at 55 °C is higher than 35 °C, and the presence of methane at thermophilic conditions occurred at 5 days, while at mesophilic conditions, it gives at 23 days. The maximum hydrogen production (703 mL H_2) is achieved when the temperature is 35 °C, and the organic load is 12 (gVS/L). The process can maintain in hydrolysis for a longer time, due to mesophilic conditions.

Tests with a higher organic load will be carried out to increase the carbohydrate content in the reactor and achieve better hydrogen productions. Because the production of the blank was very close to that of the reactors. with the organic loads of 4 and 8 gSV/L

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