Pilot-Scale Assessment of Biohydrogen and Volatile Fatty Acids Production via Dark Fermentation of Residual Biomass

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This article determines the hydrogen and volatile fatty acids production from a pilot plant's dark fermentation (DF) process, using residual biomass available in Colombia. Previous studies over the biochemical production of hydrogen (BHP) allowed determining the optimum operative conditions to scale the process. The following biomass was used as substrates: pig manure (PM), coffee mucilage (CFM), and cocoa mucilage (CCM). The inoculum was pre-treated by thermal shock to eliminate the methanogenic microorganisms. Based on the physicochemical characterization of the substrates and the inoculum, the experiment was carried out using an organic load of 10 g VS, substrate/inoculum ratio (S/X) of 1:1, and a carbon/nitrogen ratio (C/N) of 35. The pilot plant was operated under mesophilic conditions (35 °C), a pH of 5.5, and a working volume at 80% of the reactor capacity, corresponding to 4 L. The cumulative bio-hydrogen production (CHP) was 3,674.021 mL H_2, corresponding to 91.85 mL H2 g VS^{-1}, and the amount of VFA was 4,952 mg COD L^{-1}. The removal of VS was 84.4 %; this, together with the production of VFA, allows suggesting secondary processes associated with biorefinery schemes.

1. Introduction

Current estimations of the 2050 energy matrix point to the inevitable decrease of fossil fuels usage; therefore, renewables might supply a share of 52 – 67 % of primary energy (Masson-Delmotte et al., 2022). Thus, biofuel research has grown over the influence of the growing energy demand and the necessity to supply clean energy soon (Osman et al., 2020). Biofuels can be obtained through biochemical processes, such as dark fermentation and anaerobic digestion, using residual biomass as the primary hydrogen and methane production (Argun & Dao, 2018; Hallenbeck & Benemann, 2002; Łukajtis et al., 2018). Where the use of residual biomass makes more attractive its production, due to the profitability of the different products that could be obtained, in a close-loop biorefinery approach, and the high calorific value of hydrogen (120 - 142 MJ kg-1) and methane (50 - 55.5 MJ kg-1) as fuels (Chandrasekhar et al., 2020; Sarangi & Nanda, 2020).

Among the hydrogen conversion technologies, DF is regarded as the most promising method as it uses microorganisms to convert the organic matter into hydrogen; However, there are several requirements for the raw materials and the process itself to guarantee an efficient biohydrogen and value-added products generation (De Bhowmick et al., 2018; Liu et al., 2018). Countries like Colombia can benefit from this process since part of the national territory is destined for agribusiness, representing 2 % of gross domestic product (GDP) (Perfetti et al., 2017).

Colombia has enough residual biomass coming from the agriculture and livestock sectors. Some residues, such as PM, CFM, and CCM, have the potential to produce biohydrogen, biomethane, and value-added products (Hernandez M. et al., 2018). It is noteworthy that the country is among the largest coffee producers, with an estimated production of 980,000 tons per year (Federación Nacional de Cafeteros - Sitio de la Federación Nacional de Cafeteros, s. f.). Likewise, Colombia produces 56,867 tons of cocoa per year (Fedecacao - Federación Nacional de Cacaoteros, s. f.), increasing yearly.

Colombia has approximately 6.5 million pigs, and PM is highly available; it is a substrate with high carbon and nitrogen levels that favor BHP under controlled conditions in DF processes. Also, residual biomass such as cocoa and coffee mucilage is not being used in the Colombian industry, despite being rich in sugars and carbohydrates and susceptible to being employed in fermentation processes (Hernandez M. et al., 2018; Hernández et al., 2014).

Due to the large availability of CFM, CCM, and PM across Colombia and the need to obtain energy in rural areas, this study evaluated bio-hydrogen production in pilot plant batch reactors starting from a mixture of inoculum CFM, CCM, and PM. As an additional consideration, physicochemical parameters of the effluents of the dark fermentation process are reported, considering that they have secondary metabolites and other products that can be isolated and evaluated, which promotes the economic viability of the process for the estimation of biorefineries schemes.

2. Materials and methods

Previous studies over PM, CFM, and CCM biohydrogen potential (Rangel et al., 2020) were used to determine the following operative conditions: organic load 10 g VS, S/X ratio 1:1, mesophilic state (35 °C), C/N ratio 35, pH 5.5 and stirring speed of 80 rpm. The experiments were based on the physicochemical characterization of the substrates. The experiment was performed by triplicate, with a target and daily follow-up.

2.1 Substrates and inoculum

The substrates were obtained from Colombian agricultural production systems. The CCM was collected from a medium-scale traditional farm placed in Carmen de Chucurí, Santander, during the obtention of the seed. The CFM was collected during the mechanical demucilaging process at a private farm established in Las Herreras, Teruel, Huila. The PM was obtained from Agriculture Research Center Marengo (C.A.M) of the Universidad Nacional de Colombia located in Mosquera, Cundinamarca. The substrates were frozen at -4 °C to avoid biological degradation after de assays.

The inoculum was obtained from the wastewater plant of Alpina S.A., Sopo, Cundinamarca. As a methanogenic granular sludge, a heat shock treatment consisted of boiling during the inoculum for 30 minutes to guarantee the best conditions during the dark fermentation process.

2.2 Analytical methods

A physicochemical analysis was performed to establish the volumes of substrate and inoculum to be added to the reactors. The characterization of the substrates and the effluent were carried out as follows: the pH measurements were completed using an Edge Model HI2002 potentiometer. The total solids (TS) followed method 2540B APHA SM. The volatile solids (VS) were determined wetly by applying ASTM D3174. Total Kjeldahl Nitrogen (TKN) was performed following ASTM D1426. The VFA and alkalinity analyses were developed according to the 5560D APHA SM and 2320B APHA SM.

The quantification of the volume of biohydrogen produced was performed by RITTER flowmeters (MiligasCounter - RIGAMO software), which allows the total gas measurement in real-time.

3. Results

The physicochemical characterization of the substrates was carried out to calculate the volume of substrates and inoculum to be added in the reactor; in Table 1, the characterization results can be observed.

Table 1: Physicochemical characterization of substrates and inoculum

	PM	CCM	CFM	Inoculum
TKN (%w)	2.07±0.15	0.56±0.20	0.058±0.050	-
TS (%w)	29±0.3	19.7±0.5	2.1±0.87	5.6±0.56
VS (%w)	22.92±0.34	19.19±0.43	1.82±0.75	4.94±0.38
gVS added	31,0562	9,3613	0,6846	-

The pilot-scale performance was evaluated in terms of H_2 and VFA production. After a batch operation of 11 days, the CHP was 3674.02 mL, corresponding to 91.85 mL H_2 g VS^{-1}. Fig. 1 shows the cumulative hydrogen production after the fermentation time; a system stabilization is distinguished, as the standard deviation decreases with the days (9 to 6 percent) due to microorganisms growing efficiency at the consumption of the substrates. The hydrogen production is considered necessary, given its increase during the last five days. This can be associated with the pH stability during the process, which was 5.5 until the previous day.
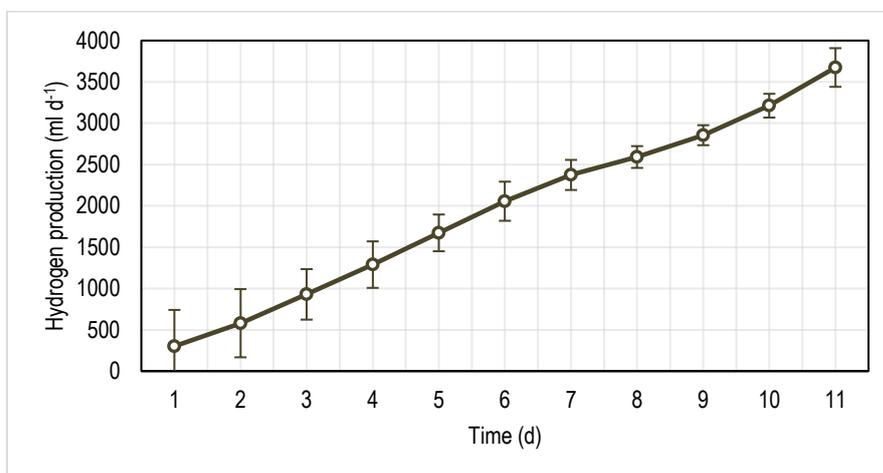

Figure 1: Cumulative hydrogen production after the 11 days of fermentation

Regarding the VFA production, there is a positive progression over the production until day 10, raising above 4,900 mg COD L^{-1}, decreasing to 4,400 mg COD L^{-1} (see Fig. 2). The target also presented a decrease in VFA production related to the beginning of the methanogenesis phase.
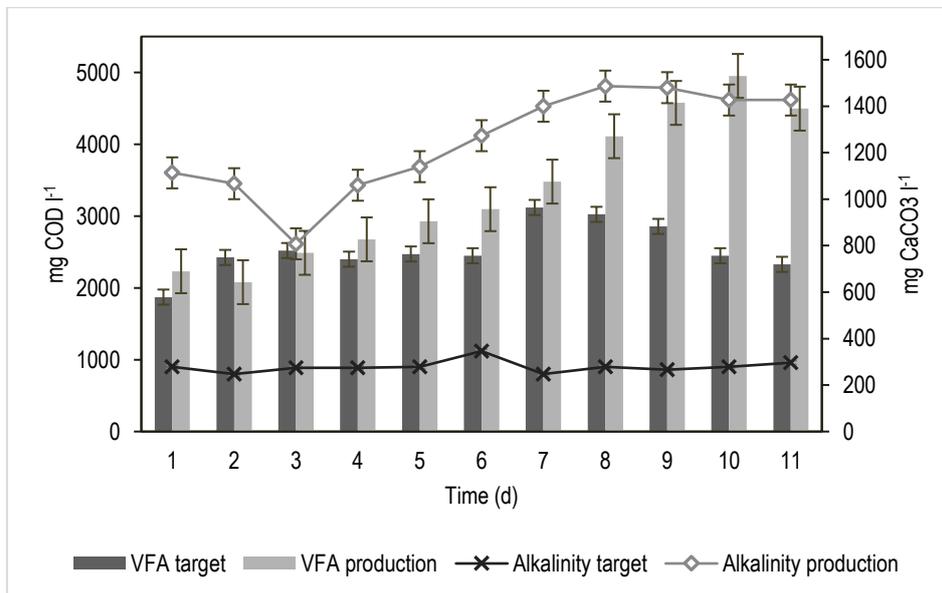

Figure 2: Volatile Fatty Acids production during the fermentation time

Figure 3 shows the VFA and metabolites found in the effluent from the reactors. The main metabolic pathway developed in the process was acetate, with a prevalence of acetic acid more significant than 50% (2,171 mg mL^{-1}), followed by valerate and butyrate, with 15 % and 18 %, respectively. It is observed that the production of lactic acid was the lowest, which represents a low consumption of hydrogen in the process.
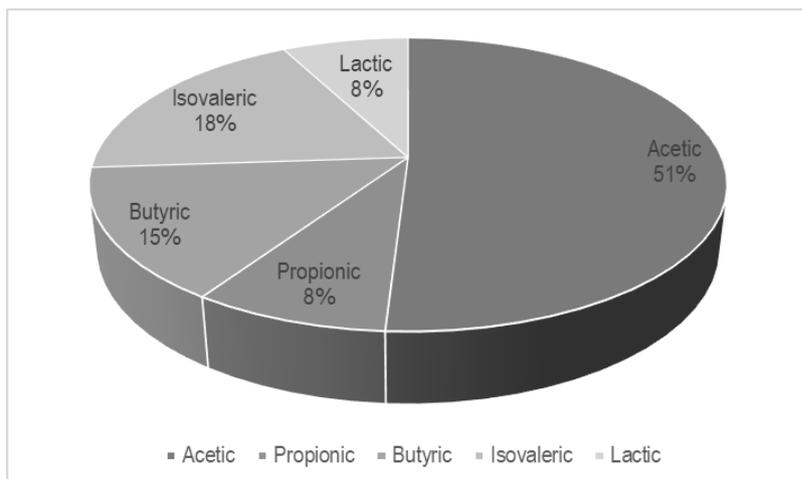
Figure 3: Volatile Fatty Acids present in the effluent

4. Discussion

The physicochemical characterizations of the substrates, shown in table 1, are highly relevant. The contents of TS, VS, and moisture allow determining the viability of the process since they are parameters of biodegradability or digestibility. Likewise, choosing the percentage of carbohydrates and proteins makes it possible to establish whether there will be reasonable production rates, directly affecting the performance and characteristics of the biogas to be obtained (Kamm & Kamm, 2004; Sadhukhan et al., 2014).
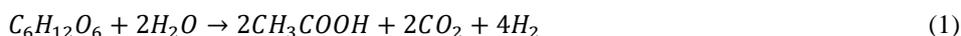
Other research indicates that agricultural residues are suitable substrates for raw materials in bioprocess because they are more abundant and low-cost resources, with high energy yields due to high carbohydrate content such as cellulose, hexose, and pentose sugars (Ren et al., 2006). As shown in Figure 1., after day 8, the target starts its methanogenic stage, it has no substrates, and the amount of carbohydrates is lower. The methane molecule has a higher mass than hydrogen and reflects an increase in biogas production. Therefore, at the end of the 11 days, the blank was more unstable and was intervened to maintain the pH at 5.5, using a higher amount of acid reagents.

Also, Vatsala et al. (2008) obtained a yield of 1.83 kg of bio-hydrogen in a 10 m^3 reactor and 21.4 kilograms of bio-hydrogen in a 100 m^3 reactor. The production of bio-hydrogen is substantial. It is economically viable due to rising prices in fuels such as natural gas, which makes it promising as an alternative energy source. These values are comparable with the present research.

Furthermore, (Lin et al., 2011) reported a bio-hydrogen production rate of 15.59 m^3/d and a yield of 1.04 mol H_2 mol^{-1} saccharose in a reactor with a working volume of 0.4 m^3, a stirring speed between 10-15 rpm, a temperature of 35 °C, and an organic loading rate of 240 kg COD m^{-3} d^{-1}, performing with saccharose as substrate. In this study, under the same thermophilic conditions and a smaller reactor, bio-hydrogen production remains positive with an increase in the last three days, recording a total output of 3674.021 mL H_2 after eleven days of fermentation. Also, from day 5, the relation of the data is close and is reflected in a standard deviation between 9 % and 6 %.

Vatsala et al. (2008) reported a VFA production of 226 kg for the 100 m3 reactor, given to soluble products such as acetate and butyrate. Besides, total production of VFA between 10,198 to 24,418 mg COD L^{-1}, composed mainly of butyrate and acetate, with 44.4 - 53.2 % and 21.3 - 26.4 %, respectively (Lin et al., 2011). (Lin et al., 2010) applied a working volume of 400 L at a temperature of 35 °C, reaching VFA production of 3,102 mg COD L^{-1}.

Despite the presence of acetic acid in the reactor effluent, it is considered a good route for the generation of H_2 because stoichiometrically, it has a better performance in terms of hydrogen production, as presented in equation one, where the stoichiometric ratio of acetic acid is shown.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \tag{1}$$

It should be noted that the stoichiometric ratio presented in the previous one is equivalent to 544 mL H_2 g^{-1} hexose at 25 °C, double what can be obtained through the butyric acid route (Ghimire et al., 2015).

5. Conclusions

Biohydrogen production using organic residues available in Colombia was evaluated at a pilot scale. Demonstrating the scalability of the process when working with an organic load of 10 g VS, S/X 1:1, C/N of 35, reaching a cumulative production of hydrogen of 4,367 mL H_2, corresponding to a yield of 109,175 mL H_2g VS^{-1}. Likewise, this load presented significant progress for VFA production (4,952 mg COD L^{-1}), being acetic acid predominant in its composition.

This process ensures waste management generates benefits from energy generation and the commercialization of value-added products. Further studies need to focus on a techno-economic assessment of the biorefinery processes approach that allows the recovery of VFA, giving added value to the process and the use of agro-industrial waste through dark fermentation.

Acknowledgments

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