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Hydrogen Production by Dark Fermentation Process: Effect of Initial Organic Load

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The treatment of residual biomass through the dark fermentation (DF) process is presented as a sustainable alternative, where bio-hydrogen is produced, which has a high energy potential, and bio-based sub-products. Consequently, this work aimed to determine the maximum organic load for the production of bio-hydrogen, from residual biomass available in Colombia. An experimental design was constructed to determine the biochemical hydrogen potential (BHP) of mixtures composed of three substrates: pig manure (PM), cocoa mucilage (CCM), and coffee mucilage (CFM). The design managed two independent variables, the organic load (10 gVS/l, 20 gVS/l, 30 gVS/l, 40 g VS/l, and 50 gVS/l), which were determined according to the physicochemical characterization of the substrates, and the S/X evaluated on two levels 1:1 and 1:2. The experiments were carried under mesophilic conditions (±35°C), a C/N ratio of 35, and a pH of 5.5 was fixed. As a result, five mixtures were evaluated, plus five targets. The reactors were erlenmeyers of 250mL, hermetically sealed, heated, and stirred in a hot plate. The tests were allowed to run until the detection of CH_4; daily monitoring with a Biogas 5000® portable gas analyser did this. Following the determination of the physicochemical characteristics of the effluent, total solids (TS), volatile solids (VS), proteins, and chemical oxygen demand COD were analysed for each of the reactors. The results show that the organic load of 10gSV/L and the S/X of 1 has the highest production rate with 232 mL of H_2, with a production of volatile fatty acids (VFA) of 4040mg COD/L, and a VS removal percentage of 84%. The removal of VS and the VFA allows suggesting secondary processes associated with bio-refinery schemes, for a more significant elimination of volatile solids and the obtaining of other value-added by-products.

1. Introduction

Biofuels are one of the alternatives to reduce greenhouse gas emissions, contributing to the mitigation of impacts that accelerate climate change (Killeen et al., 2011). Currently, Colombia produces biofuels such as biodiesel and bioethanol. Biomass exploitation is evidenced in the commercialization of diesel, for a gallon of diesel produced, 10% is biodiesel (“Federación Nacional de Biocombustibles de Colombia,” n.d.). On the other hand, more than 2% of the total national gross domestic product is derived from the Colombian agro-industrial sector (Perfetti et al., 2017).

The residual biomass can be used and transformed into value-added products, through biological processes such as dark fermentation (DF). Additionally, there are previous researches on bio-hydrogen production from food waste (Hallenbeck and Benemann, 2002; Kanchanasuta et al., 2016). In this perspective, the use of residual biomass has suffered limitations concerning to initial approaches with biotechnologies, since the values of energy produced compared to the benefits of conventional energy production are still among the lowest (Levin et al., 2004). Waste from the agricultural and livestock sectors in the national territory, such as CFM, CCM, and PM, have the potential for the production of bio-hydrogen by dark fermentation processes (Hernandez M. et al., 2018).

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In Colombia, the estimated coffee production is 14 million bags per year; This production places the country among the largest producers in the world, making this crop contribute to economic development and has become a national brand ("Informe Gerente General," n.d.). The CFM is a waste that is generated in the coffee benefit process; after the washing stage, it can be extracted traditionally or mechanically (Chanakya and De Alwis, 2004). There are few references in the literature about the potential to produce hydrogen from this residue. (Hernández et al., 2014) report a bio-hydrogen production from coffee mucilage in co-digestion of 7.6 NL H_2/Ld.

On the other hand, the population of pigs in the country is approximately 6,473,525 animals; in Cundinamarca, there is a population of 578,734 animals ("Instituto Colombiano Agropecuario - ICA," n.d.). The PM has high availability, and it is a substrate with high levels of carbon and nitrogen that can produce bio-hydrogen under controlled conditions in dark fermentation processes, obtaining yields of 318 mL H_2/h (Hernández and Rodríguez, 2013).

Regarding the CCM, it is worth noting that in Colombia, 56,867 tons of cocoa are produced per year, and there are 2 million hectares suitable for expanding production ("Fedecacao - Federación Nacional de Cacaoteros," n.d.). Although there is a significant production, there is not much background on how to obtain value-added products from the waste generated during the industrial cocoa process. It is expected that with the treatment of cocoa residues, the added value can be increased throughout the production chain, thus improving the quality of raw materials and final products (García-Cáceres et al., 2014).

In the present study, the mixture of CFM, CCM, and PM was evaluated due to the complementary properties of these substrates that favour dark fermentation so that bio-hydrogen production can be supported (Gómez et al., 2009; Hernandez M. et al., 2018). As an additional consideration of the present study, 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. This condition encourages the economic viability for the evaluation of bio-refineries schemes (Herrero Garcia N. et al., 2018; Liu et al., 2008; Noike, 2002; Yesil et al., 2014).

2. Methodology

An experimental design was carried out, where the initial organic load and the S/X ratio were used as variables. The temperature, stirring speed, initial pH, and C/N ratio were fixed.

2.1 Inoculum

The inoculum used in all the tests carried out comes from the wastewater treatment plant of the private company Alpina S.A., located in Sopó, Cundinamarca (Colombia). Since the inoculum comes from an anaerobic digestion reactor, a heat shock treatment was carried out to eliminate the methanogenic consortia. The treatment consisted of boiling the inoculum for 30 min.

2.2 Substrates

The substrates under study are residual biomass from three of the agro-industrial sectors: CFM, CCM, and PM. The waste was obtained from Colombian production systems and was stored in a freezer at -4 ° C, to avoid microbiological degradation before the tests. The CCM came from a traditional, medium-scale farm, located in Carmen de Chucurí, Santander (Colombia). PM was obtained from the Agricultural Research Center of Marengo (C.A.M) of the National University of Colombia, located in Mosquera, Cundinamarca. For its part, CFM was collected during the traditional de-mucilagination process on a private farm, located in Las Herreras, Teruel, Huila (Colombia).

2.3 Experimental setup

As mentioned earlier, the control variables that were taken into account in the experimental design were temperature and agitation. The temperature was set at 35°C (Guo et al., 2015). A stirring speed of 70 rpm was set (Srirugsa et al., 2019). Both temperature and stirring were monitored using a heating plate with magnetic stirring.

In all the tests that were carried out, an initial pH of 5.5 was set, in order to favor the production of hydrogen and delay the appearance of methane (Wang and Yin, 2019). A C/N ratio of 35 was also set, which was adjusted based on the characterization of the substrates (Argun et al., 2008). In all the mixtures analyzed, the primary source of nitrogen was PM. As independent variables, the initial organic load and the S/X ratio were studied. The initial organic load analyzed were 10 g VS/ L, 20 g VS/L, and 30 g VS/L, 40 g VS/L, and 50 g VS/L. The S/X ratios considered were 1:1 and 1:2.

The DF process was carried out in 250 mL erlenmeyers with 200 mL working volume. Biogas production was measured by the displacement of a 0.5 M sodium hydroxide solution, using one-liter amber bottles. The

hydroxide is intended to retain the CO_2 produced, reducing the error in the measurement of the hydrogen produced given the presence of other components in the biogas. The composition of the biogas obtained (CO_2, CH_4, and O_2 %) was determined by means of a portable gas analyzer BIOGAS 5000® Landtec. Once the presence of methane in the reactors was detected, the process was stopped.
In total, five treatment combinations were analyzed, each in triplicate and with their respective targets. After the days of the process, the physicochemical characterization of the resulting effluent was performed.

2.4 Analytical methods

The characterization of the substrates, inoculum, and effluents was carried out as follows. pH measurements were completed using an Edge Model HI2002 potentiometer. Total solids (TS) following the 2540B APHA SM method. Volatile solids (VS) were determined on a wet basis by applying ASTM D3174. Total Kjeldhal Nitrogen (TKN) was performed following ASTM D1426. Volatile fatty acid (VFA) and alkalinity analyses were performed according to APHA SM 5560D and APHA SM 2320B, respectively, and COD analyzes using ASTM D1252-06 using a Hanna reactor.

3. Results and discussion

The results obtained for the five treatment combinations evaluated are presented below. In all the experiments, it was observed that the bio-hydrogen producing microorganisms responded to constant agitation, reporting a production that was maintained during the days of execution of the experimental design.

3.1 Biochemical hydrogen potential

The cumulative production of bio-hydrogen recorded in each of the treatment combinations evaluated was quite similar. Figure 1, shows the tendency of each mixture and the variation in time, which covered a period between 6 days and 11 days before the presence of methane was evidenced.

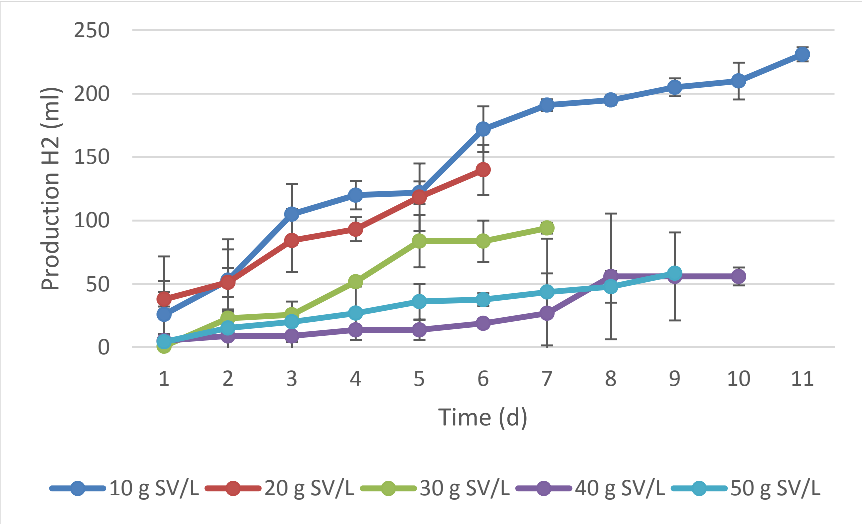

Figure 1: cumulative bio-hydrogen production for each treatment mixture.

(Argun and Dao, 2017) report the use of low S/X ratios for dark fermentation processes because these conditions favor the production of hydrogen by restricting the excessive presence of microorganisms that limit the metabolic pathway. In the same way, in the present work, it was observed that the S/X with the highest BHP was the 1:1 used in the tests of 10, 20, and 30 gVS/L of initial load. Likewise, the lower organic load (10 g VS/L) reflected stability over time and a cumulative production higher than the other concentrations studied; this was concluded after observing that it was the mixture that lasted more days without methane production and reached a higher cumulative production of H_2.

3.2 Organic load effect

In Figure 2 is observed that the highest cumulative production of H_2 is achieved when the organic load is 10 g VS/L, with a bio-hydrogen production of 232 ml H_2 in 11 days (23.2 mL H_2/gVS). It is followed by the combination with an initial load of 20 g VS/L whose cumulative production was 139 L H_2 in 6 days (6.95 mL

H_2/g VS) and the combination with an initial load of 30 g VS/L with 94 mL H_2 in 7 days (3.13 mL H_2/g VS). On the other hand, the performance of the combinations with an initial organic load of 40 and 50 g VS/L was quite low, with values of 56 mL H_2 in 10 days (1.4 mL H_2/g VS) and 59 mL H_2 in 9 days (1.18 ml H_2/g VS).
These results are consistent with those obtained in studies that used melon and watermelon as substrates, at a temperature of 36ºC and pH values between 5.5 and 6.0, riching a cumulative production of 151 mL H_2/L (Turhal et al., 2019). The low production in the tests with 40 g VS/L and 50 g VS/L of initial organic load is due to an oversaturation of the medium as a study concluded in 2019, where they stated that the higher organic load produced an overload in the system and a decrease in bio-hydrogen production (Kongjan et al., 2019).

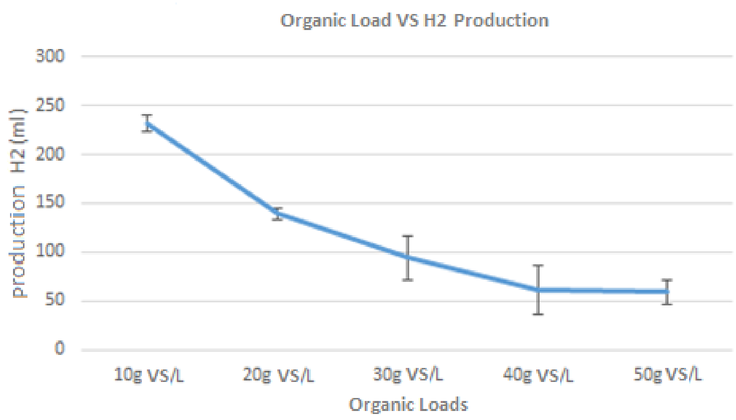

Figure 2: cumulative bio-hydrogen production and organic load.

Previous researchers reported a cumulative production of bio-hydrogen around 562 mL for the co-digestion of crude glycerol and palm oil (Kanchanasuta et al., 2017). In addition, previous works evidenced production rates of 251 mL H_2/g xylose, and 15.1 L H_2/ L day, and they affirmed that the highest organic load produced an overload in the system and a decrease in bio-hydrogen production (Kongjan et al., 2019). In our case, the above reflected an inversely proportional relationship concerning the amount of organic load expressed in volatile solids, and the production of bio-hydrogen, under higher the organic loads, the production of bio-hydrogen decreased.

3.3 Bio-hydrogen, alkalinity and volatile fatty acids

The alkalinity and volatile fatty acids (VFA) showed results for the organic loads (Figure 3), 10 g VS/L of 2014 mg $CaCO_3$/L and 4040 mg COD/L; for 20 g VS/L an alkalinity value 2434 mg $CaCO_3$/L and VFA 8800 mg COD/L; for 30 g VS/L 3154 mg $CaCO_3$/L and 10160 mg COD/L; for 40 g VS/L 1709 mg $CaCO_3$/L and 12844 mg COD/L; and 50 g VS/L generated 3550 mg $CaCO_3$/L with a VFA production of 14109 mg COD/L.
Alkalinity is beneficial for protein solubilization and degradation; also, it prevents the growth of acetoclastic methanogens and retains fatty acids (Dahiya et al., 2015). In this case, the alkalinity increases with the organic load; at a lower load presents a closer relationship, and the alkalinity values were stable.
Some studies affirm a 78% removal of suspended solids (Prasertsan et al., 2009). Besides, in an anaerobic sequence Batch reactor (AnSBR), the removal efficiency of 65.54% was observed, representing 991 mg/L, where the organic loading rate increased, the removal of solids increased (Reddy et al., 2011). The solids removal for the organic load of 10 g VS/L was 84.4% and is related to the final organic load of 1.52 g VS/L. The higher the production of hydrogen, the higher the presence of organic compounds and the removal of volatile solids, it indicates the organic degradation that determines the efficiency of the anaerobic fermentation (Yang and Wang, 2017). Consequently, it showed a high production of VFA at the stage of hydrogen production in anaerobic digestion (Yesil et al., 2014). Hernández et al (2018) that under high amounts of VFA accumulation, the production of bio-hydrogen is limited, under controlled conditions. the production of VFA is high with 4040 mg COD/L concerning the bio-hydrogen production (Hernandez M. et al., 2018). They affirmed the carbohydrate content is the main factor that influences the concentration of butyrate, plus the total production of bio-hydrogen and pH, which are factors that influence the generation of volatile fatty acids (Alibardi and Cossu, 2016). In this case, the production of hydrogen when it is lower and the organic load is higher, the amount of VFA is higher, respectively. They claimed biotechnology responds to a cost-effective and environmentally friendly approach and receives attention due to the increase in market demand (Atasoy et al., 2018).

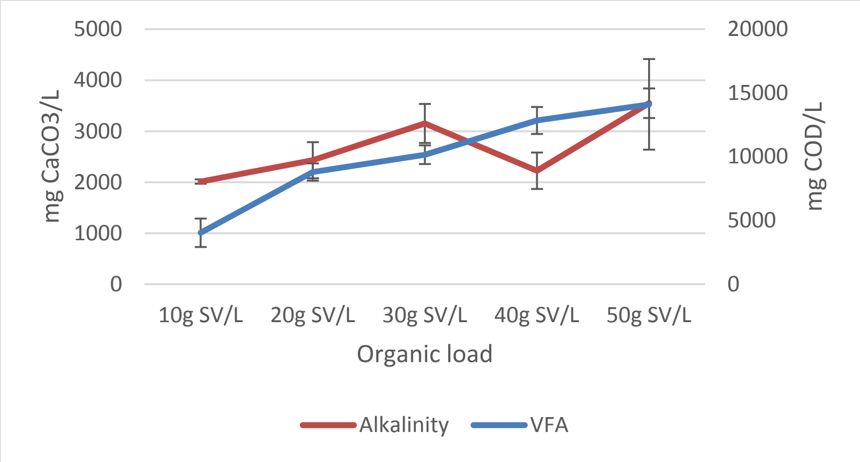

Figure 3: alkalinity and volatile fatty acids comparison for the organic load.

4. Conclusions

The most significant production of bio-hydrogen was 232 mL H_2/ 11d, with an organic load of10 g VS/L, a S/X ratio of 1:1, C/N of 35 and a temperature of 35°C, having a higher production of bio-hydrogen will have a lower production of VFAs. However, the production of VFAs represents 4040 mg COD/L, and this can lead to a bio-refinery approach for the recovery of VFA, generating value-added products in future studies.

Acknowledgments

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