CHEMICAL ENGINEERING TRANSACTIONS

VOL. 65, 2018

A publication of AIDIC

The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Eliseo Ranzi, Mario Costa

ISBN 978-88-95608-62-4; **ISSN** 2283-9216

DOI: 10.3303/CET1865042

Assessment of the Biohydrogen Production Potential of Different Organic Residues In Colombia: Cocoa Waste, Pig Manure and Coffee Mucilage

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Colombia has a great potential to produce biofuels based on biomass residues due to the importance of agroindustry in the country's economy. The diversity of this industry guarantees the availability of different residues which are susceptible to be valorised through biological processes. There are several works, in the country, focused on biomass use to produce bioethanol and biodiesel. Nevertheless, the biohydrogen production using residual biomass has been scarcely studied. Dark fermentation and anaerobic digestion processes allow the degradation of substrates such as agricultural biomass and agroindustrial waste in order to produce hydrogen and methane, respectively. These residues are highly biodegradable, have low cost and have high availability. In this study, an assessment of the biohydrogen production potential by various organic residues (coffee mucilage, cocoa waste and pig manure) was carried out. The biohydrogen production potential of each residue was conducted in batch reactors (250 mL bottles). In order to maintain a mesophilic condition, an immersion thermostatic bath was used. Biological methane potential was evaluated to compare the energy recovery through hydrogen and methane for each substrate. Soluble products as volatile fatty acids and total reducing sugars were measured to characterize the dark fermentation process. The batch experiments were evaluated until the production rate of hydrogen and methane was reduced substantially. In all cases, the biomass demonstrated its versatility and capacity to produce biohydrogen. Maximum production was reached during dark fermentation process of coffee mucilage with a cumulative hydrogen production of 338.9 mL and a maximum hydrogen production rate of 47.0 mL/d. However, the dark fermentation experiments showed instability with high standard deviation for production values. Despite the instability, the energy recovery from coffee mucilage through dark fermentation was 10 times above the energy produced by anaerobic digestion.

1. Introduction

Currently, the world energy demand is based on fossil fuels such as coal, oil and natural gas which have a wide market and distribution. However, their reserve levels are scarce, variable and unevenly distributed throughout the planet (Harsono et al., 2015). Alternative technologies for biomass use, as energy source, establish a promising perspective to address environmental problems and to supply renewable energy (Bentsen et al., 2014). Biofuels produce lower emissions of SO_2, NO_X and soot than conventional fossil fuels because they have an insignificant content of sulfur, nitrogen and ash (Bilgen et al., 2015). Additionally, biomass, as resource, has a high geographical distribution in comparison with the fossil fuels reserves; they represent an advantage for their use (Asadi et al., 2017).

The biomass use through biological processes has been a proven alternative even with industrial installations (Wandera et al., 2018). The main biological process is anaerobic digestion, being the route that allows biomass treatment and energy obtaining. Likewise, dark fermentation produces hydrogen as an energy source with great use potential (Levin and Chahine, 2010). However, it does not achieve the complete biomass

Please cite this article as: Hernandez M., Gonzalez A.J., Suarez F., Ochoa C., Candela A.M., Cabeza I., 2018, Assessment of the biohydrogen production potential of different organic residues in colombia: cocoa waste, pig manure and coffee mucilage, Chemical Engineering Transactions, 65, 247-252 DOI: 10.3303/CET1865042

treatment but it generates by-products that can be used in chemical or biological processes (Urbaniec and Bakker, 2015). The application of biological processes is still under development since each biomass has specific characteristics that must be evaluated before running a project. The evaluation of these biological processes, within the biorefineries framework, is sought to value the generated by-products and to increase the systems versatility (Sawatdeenarunat et la., 2016).

Colombia, an agricultural country, where agricultural and livestock activity are the most important economic activities with a share of 7.7% of GDP (DANE, 2017), has the priority to advance in technological alternatives which take advantage of the existing potential from residual biomass. Agricultural activities are a constant source of residual biomass that does not have an added value during the harvesting and throughout the raw material processing industry. The livestock sector has 76% of the total area related to agricultural activities. This area is distributed by 40% cattle, 40% poultry, 10% pork and 10% remaining activities which involve low scale process. It generates a large amount of residual biomass that can affect water bodies, soil and air in different ways (ICA, 2016). Colombia has a population of 5,094,664 pigs, distributed in 218,698 farms located mainly in the departments of Antioquia (34.42%), Cundinamarca (10.17%), Valle del Cauca (8.18%), Meta (4.64%), Boyacá (4.62%) and Cauca (3.77%) (ICA, 2016).

Coffee is an important and representative crop since Colombia is the fourth coffee producer after Brazil, Vietnam and Indonesia. The activity is distributed throughout several regions in the national territory. Coffee mucilage can be separated using mechanical demucilaging of the ripe harvested beans which uses less water than traditional separation process. This residue, rich in carbohydrates, has been studied as a raw material for the production of ethanol, lactic acid and as a substrate to improve anaerobic digestion yields, among others (Neu et al., 2016). According to information provided by the Colombian Federation of Cocoa (FEDECACAO), cocoa planted area has risen by 35% in the past eight years, from 115,800 hectares in 2007 to 157,000 hectares in 2014. This suggests the relevance to establish concrete solutions to the management and recovery of wastes, once it constitutes the main source of income for about 30,000 peasant families.

In order to advance in the knowledge, this study focuses on the evaluation of the biohydrogen production through dark fermentation using three complex substrates available in Colombia: coffee mucilage, cocoa waste and pig manure. This evaluation was carried out applying the biochemical potential procedure to each biomass type for hydrogen and methane production. Both biological processes were evaluated to compare the energy recovery by hydrogen against methane. Volatile fatty acids (VFA), total reducing sugars, metabolites and others parameters were analysed to evaluate the response of each system.

2. Materials and Methods

2.1 Inoculum

Methanogenic granular sludge was obtained from the sewage plant of Alpina S.A. in Sopo, Cundinamarca (Colombia). It was used directly as inoculum in anaerobic digestion; meanwhile a heat shock treatment which consisted in boiling the inoculum during 30 min was used before being added to dark fermentation process.

2.2 Substrates

Cocoa waste (CW), pig manure (PM) and coffee mucilage (CM) were the residual biomass evaluated in this study. Residues were kept in a freezer at -4 ºC to avoid microbiological degradation before the assays. Cocoa waste was collected directly in a medium scale farm during fruit processing to obtain the cocoa seed. The species employed comes from a crossing process of several species and is called trinitarium. Pig manure was obtained in the Agriculture Research Center Marengo (C.A.M) of the Universidad Nacional de Colombia located in Mosquera – Cundinamarca. The feeding of the animals was based on a commercial concentrate. Coffee mucilage was collected during the mechanical demucilaging process on a farm located in Cundinamarca. It was separated physically from coffee grains, pulp and other thick solids using a standard sieve. Substrates were diluted with tap water to achieve the chemical oxygen demand (COD) required.

2.3 Biohydrogen and biomethane potential

Dark fermentation (DF) and anaerobic digestion (AD) processes were evaluated to establish the hydrogen and methane potential of biomass selected. The experiments were carried out in 250 mL bottles, by triplicate, with a total working volume of 200 mL. The substrate and inoculum ratio (S/X) was fixed as 1 for both processes in order to avoid inhibition in anaerobic digestion related to high VFA concentrations. The inoculum volume used for each bottle was 30 mL, and distilled water was used to complete the total working volume. All the bottles were sealed with rubber stoppers and silicone. The bottles were kept in a thermostatic bath at mesophilic condition (35 ºC) during 37 days. Methane and hydrogen were determined by volume displacement using a NaOH solution (pH>10) as CO_2 trap. In order to assure hydrogen production, additional tests were conducted collecting gas in Tedlar® bags and using BIOGAS 5000® Landtec to verify gas composition. C/N ratios were

54.0, 55.9 and 9.1 to coffee mucilage, cocoa waste and pig manure, respectively. These values correspond to the pure substrates without any complements addition. Buffer solution (10 mL) was added in order to keep pH condition of 5.5 and 7.0 for dark fermentation and anaerobic digestion tests, respectively.

2.4 Analytical methods

Volatile fatty acids and metabolites were analysed by high performance liquid chromatography (HPLC) – Model 1200, Agilent, coupled with UV detector (210 nm); Aminex HPX-87H column (300 mm x 7.8 mm, Bio-Rad). The mobile phase was 5 mmol H_2SO_4 with 10 µL sample injection volume and 0.6 ml/min sample flow rate at 50°C. Carbohydrates (glucose, xylose, arabinose and cellobiose) were analysed using the refractive index detector (IR). The total reducing sugars (TRS) were determined by colorimetric analysis using dinitrosalicylic acid (DNS) reagent. The chemical oxygen demand, VFA, alkalinity (ALK) and total solids (TS) and volatile solids (VS) were measured according to standard methods (APHA, 2005). CHONS elemental analysis, to evaluated C/N ratio, was developed through a methodology which uses stoichiometric ratios and chemical composition supported in carbon, nitrogen and sulphur analyses developed by CalderonLabs®.

3. Results and Discussion

3.1 Biomass characterization

The volatile and total solids ratio showed the high biodegradability of coffee mucilage and cocoa waste, which is an important characteristic for biological process (Table 1). In both cases, a high proportion of reducing sugars was found, which indicates the ability to produce hydrogen during dark fermentation process. Hydrogen is related to VFA production during acidogenesis reactions mainly. Also, reducing sugars are useful for methane production by anaerobic digestion, if VFA concentrations are below inhibition levels. The C/N ratio suggests that coffee mucilage and cocoa waste are suitable for the hydrogen production process, which required high carbon content. Meanwhile, pig manure has high nitrogen which could stabilize the anaerobic digestion process, but it decreases the opportunity to generate hydrogen. The main carbohydrates in these substrates were glucose, xylose, arabinose and cellobiose.

Table 1: Physicochemical characteristics of substrates

Substrate	TS (mg/L)	VS (mg/L)	VS/TS	TRS (g/L)	C/N
Coffee mucilage	8.5	8.5	1.00	10.53	54.0
Cocoa waste	13.8	13.7	0.99	10.16	55.9
Pig manure	23.8	19.3	0.81	1.93	9.1

The dark fermentation tests showed a VFA/ALK ratio above 1.6, which are according to a high VFA production as can be shown in Table 2. Despite this situation, and the buffer solution applied at the beginning of the experiments, pH achieved a neutral value in cocoa waste and pig manure. This situation could activate the hydrogen consumer microorganisms, which limit hydrogen release to the gas phase. (Figure 1). However, pH increase could be related to ammonia production where high concentrations inhibit the biological process. In contrast, the coffee mucilage experiments kept an acidic pH, which is a desirable condition for hydrogen production (Kanchanasuta et al., 2017).

Table 2: Effluent characterization for dark fermentation process

Condition	VFA	ALK	pH	
	mg/L		Initial	Final
DF-CM	2,120	1,280	5.5	5.7
DF-CW	2,520	1,566	5.8	7.2
DF-PM	2,960	1,633	5.8	7.8

3.2 Biomass potential for hydrogen and methane production

Dark fermentation and anaerobic digestion (Figure 1 and 2) required a lag-phase of around 15 days to present gas production activity. It was specially remarked for dark fermentation due to the heat pre-treatment used to the inoculum. However, the delay time could be associated with the acclimation time required for microorganisms to degrade disaccharides, as cellobiose, and other compounds from the complex substrates used. In the dark fermentation tests (Figure 1), coffee mucilage achieved a maximum hydrogen production rate of 47.0 mL/d against 35.7 mL/d for pig manure, which was reached just at the end of the evaluation time. Despite this behavior, hydrogen production (HP) from coffee mucilage during exponential phase could be represented by a linear regression between hydrogen production and time (HP=15.07t-203.84) with a correlation coefficient of 0.9296. In the anaerobic digestion tests (Figure 2), cocoa waste showed the best behavior reaching a maximum methane production rate of 205.3 mL/d. Meanwhile, coffee mucilage and pig manure reached 66.0 and 61.3 mL/d, respectively. In both biological processes, an additional production peak was observed at the last operation day (37). It suggests microorganisms' activity due to material solubilization from the complex substrates used.

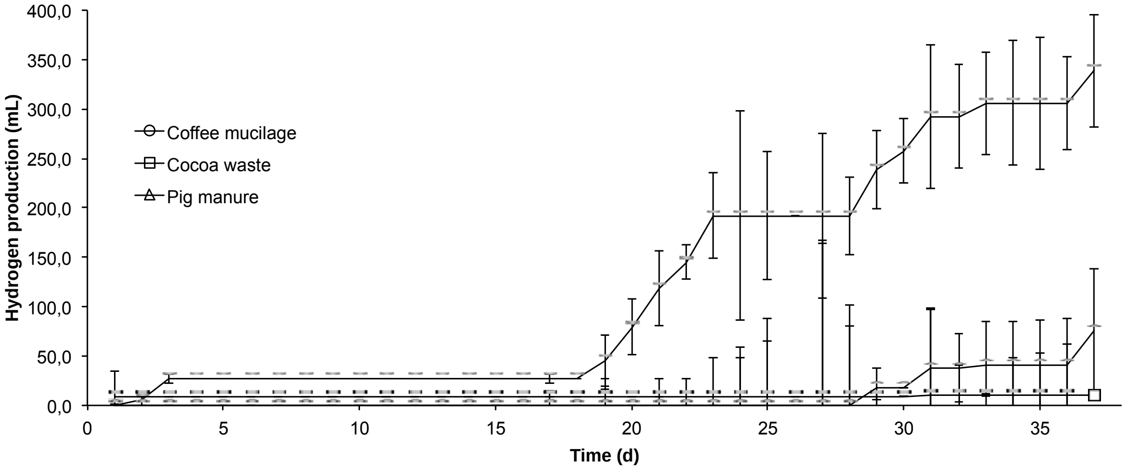

Figure 1: Cumulative hydrogen production of coffee mucilage, cocoa waste and pig manure.

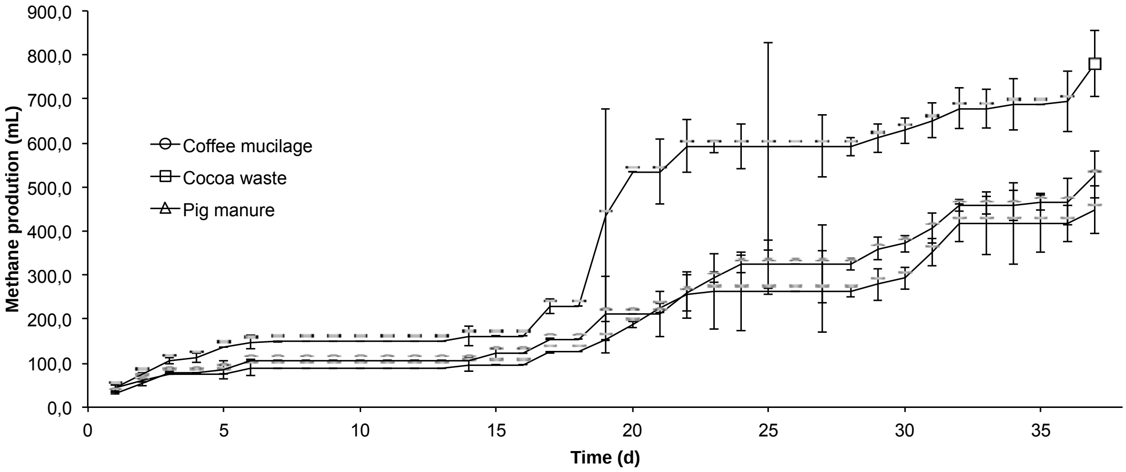

Figure 2: Cumulative methane production of coffee mucilage, cocoa waste and pig manure.

The anaerobic digestion experiments showed high stability (Figure 3) using pig manure which has a low C/N ratio against substrates with high C/N ratios. The standard deviation for the results of PM was around 18%; meanwhile it was 40 and 53% for coffee mucilage and cocoa waste, respectively. In contrast, the dark fermentation experiments showed high standard deviation values between triplicates tests. Cocoa waste and pig manure had the highest standard deviation, which confirms the issue to develop this biological process. In these conditions, co-digestion is vital to develop a stable process helping to regulate the C/N ratio (Kanchanasuta et al., 2017). The maximum production was reached through the anaerobic digestion of cocoa waste with 781.2 mL of methane, instead 338.9 mL of hydrogen reached by the coffee mucilage dark fermentation. Therefore, hydrogen production process showed some limitations, which could be related to the low S/X ratio (Florio, 2017). An increase of this ratio could improve the VFA production keeping an acidic pH condition (Table 2).

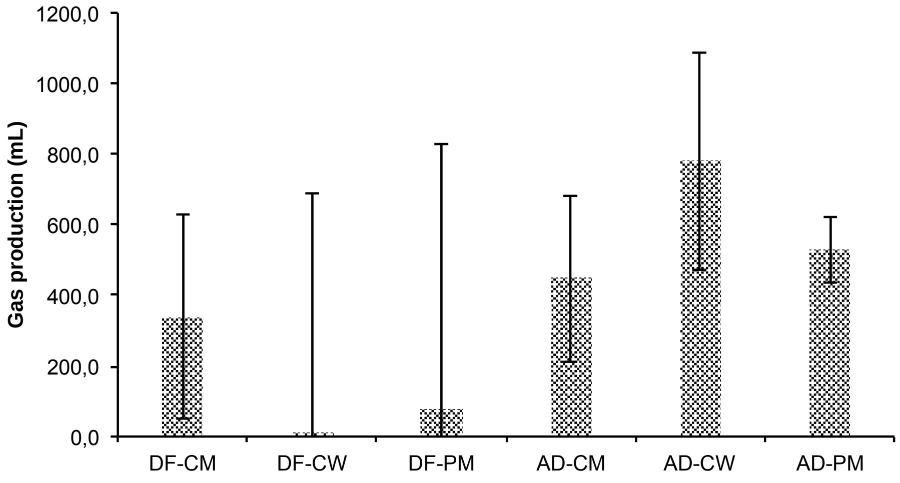

Figure 3: Total hydrogen and methane production for triplicate experiments.

3.3 Complementary results for hydrogen production

The hydrogen production yields for coffee mucilage, cocoa waste and pig manure were 2.12, 0.07 and 0.48 $L/L_{solution}$, respectively. Meanwhile, the methane production yields were 2.80, 4.88 and 3.30 $L/L_{solution}$, respectively for the same substrates. The maximum hydrogen yield is lower than that obtained for palm oil mill effluent (POME) (O-thong et al., 2008), which suggests a limitation during the biological process. Despite the high production yields for methane, the energy recovery related to the hydrogen production from coffee mucilage was higher than any other experiment. According to this, the total energy recovery from this dark fermentation test was 31.5 kJ against the maximum energy recovery from the anaerobic digestion tests: 3.1 kJ for cocoa waste. It suggests the high potential for hydrogen production even when unstable conditions dominate the process. Therefore, it is important to adjust specific parameters such as pH, S/X and C/N among others to ensure stable hydrogen production.

Main soluble products, in the experiments with coffee mucilage and cocoa waste, showed that acetate fermentation type was dominant (Table 3). Butyrate concentrations were under the detection limit suggesting a low degradation through this pathway. The acidogenic pathway is the preferential route when there are carbohydrates as xylose and arabinose (Fangkum and Reungsang, 2011). Also, ethanol or lactate concentrations were not detected in soluble phase. The VFA concentrations, represented by acetate, achieved a maximum of 35.3 mmol, which is a lower value to cause inhibition during these biological processes (Zhang, 2012).

Table 3: Soluble products during dark fermentations experiments

Test	Acetate	Propionate	IsoValerate	Furfural
DF-CM	2,118.7	616.7	-	184.0
DF-CW	1,600.0	632.0	4,185.0	178.0
DF-PM	1,459.7	319.7	12,875.7	414.0

Furfural concentrations during each experiment did not reach inhibition levels reported to dark fermentation (Akobi et al., 2017). However, furfural could limit the action of hydrogen-producing microorganisms until these concentrations are degraded.

4. Conclusions

Substrates as coffee mucilage and cocoa waste have high reducing sugars concentration, which indicates their ability for hydrogen production through dark fermentation. Coffee mucilage achieved a cumulative hydrogen production of 338.9 mL with a maximum hydrogen production rate of 47.0 mL/d. However, the high C/N ratios lead to a problem with the process stability, which reaches standard deviation above 86%. In contrast, anaerobic digestion had a cumulative methane production of 448.0, 781.2 and 527.7 mL for coffee mucilage, cocoa waste and pig manure respectively. However, the energy recovery from coffee mucilage through dark fermentation was above 10 times the energy produced by anaerobic digestion of cocoa waste.

The hydrogen fermentation route was acetate type fermentation with a maximum concentration of 2,118.7 mg/L, which was far of any kind of inhibition by the metabolites measured. Detected furfural concentrations were lower than the concentrations reported to inhibition of the dark fermentation process. These results suggest the ability of these substrates to obtain hydrogen instead of methane improving energy recovery. However, it is mandatory to regulate operative parameters even in the case of high C/N ratios which are ideal for hydrogen production.

Acknowledgments

The authors acknowledge financial support from Colciencias (Administrative Department of Science, Technology and Innovation of Colombia) - Project number FP44842-38 - 2017.

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