

The effects of the inoculum, organic load, and temperature on biohydrogen production by dark fermentation with Colombian biomass.

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Currently, the demand for energy has grown exponentially and is mainly satisfied with fossil fuels; this has generated severe environmental pollution problems (Baykara et al. 2018). These effects are a motivation to look for clean, renewable energy sources. However, the application of reliable energy alternatives requires significant research efforts. One of the options is the hydrogen generation, which can be used as a clean fuel because it is an energy carrier without CO₂ emission, which generates water as a byproduct after combustion, which implies decreasing adverse effects on the environment in the long-term and has high energy potential. Since the last decade, hydrogen has been considered an attractive solution (Niño-Navarro and Chairez 2020). There are several methods used for hydrogen production from biomass, classified as second-generation biofuels such as gasification, pyrolysis, thermal, photochemical, photoelectrochemical, photofermentation, and dark fermentation. (Ghimire and Frunzo 2015). The treatment of residual biomass through the dark fermentation (DF) is an anaerobic process in which microorganisms produce biohydrogen, carbon dioxide, and soluble metabolites during the decomposition of organic compounds. The soluble metabolites resulting from this process include volatile fatty acids (VFA), i.e., acetic acid, butyric acid, propionic acid, and other products such as lactic acid and solvents. This process is environmentally friendly and sustainable because it uses renewable resources; it can consume various organic wastes as substrates, including agricultural and food waste (Lo and Chen 2008). In this context, the choice of the fermentation mixture is a crucial parameter for scaling to chemical and biochemical reactors. In the same way, it must be homogenized the medium where the culture develops to achieve a uniform distribution of nutrients and avoid pH gradients in the liquid phase to improve growth and bacterial activity. Contrary to conventional anaerobic digestion, dark fermentation requires a pH control due to the accumulation of VFA during the reaction leading to a decrease in pH and, finally, the inhibition of biohydrogen production (Hitit and Lazaro 2017). This work aimed to determine the effect of various variables such as the type of inoculum, load, and temperature to produce bio-hydrogen from residual biomass available in Colombia.

Materials and methods

The experiments were carried out in 250 mL batch reactors and with an operating volume of 200 mL. Two experimental designs to determine the biochemical potential of hydrogen by dark fermentation were raised. For the first experimental design, mixtures composed of two selected inoculums and industrial cocoa mucilage were used. For this case the digestion temperature was 55 °C and the organic load of 4 gVS / L. Once this test was completed, an inoculum was chosen, and three different organic loads (4 gVS / L, 8 gVS / L and 12 gVS / L) and mesophilic (35 °C) and thermophilic (55 °C) temperatures were tested. For both experimental designs, the temperature was constant and using thermostatic baths. To determine the hydrogen production, the quantification through the method of displacement of an alkaline solution (0.5 M NaOH) was performed. For experimentation, an acetic acid-acetate buffer solution (pH 5.5) was used, with which the pH was adjusted in each of the reactors. During the experimentation, the presence of methane was determined daily using the BIOGAS 5000 portable analyzer. The appearance of methane defined the duration of the process. The substrate chosen during the study was the industrial cocoa mucilage (ICM), which came from a private industrial farm, located in Huila (Colombia). For fermentation, two types of inoculum were used: on the one hand, an inoculum from an anaerobic digester (ADI) of the Alpina company located in Sopó (Cundinamarca) and, on the other hand, a primary sludge from the PTAR El Salitre (PSS) in Bogotá (Colombia). Also, the selected inoculums were subjected to thermal pretreatment for 15 min at 120 ° C, in order to support the hydrogen-producing microorganisms. Each reactor contained a substrate / inoculum ratio of 2 (g VS substrate / g VS inoculum). All tests were done in triplicate.

Results

After fermentation with the ADI and PSS inoculums, the average biohydrogen productions were 48.67 mL H₂ and 30.44 mL H₂, respectively. The statistical analysis allows it to be affirmed that the difference between both inoculums is not significant, i.e., the inoculums used do not affect the production of H₂. Based on the previous results, in the following test, the loads and temperatures were evaluated. The IMA was used as inoculum because, in the initial test, it has a little more biohydrogen production was obtained. Once the tests have been carried out and after the respective statistical analysis of the data, the results allow to infer that there is no significant difference in the substrate concentrations by similar volumetric hydrogen productions will be obtained (16.33 mL, 53.67 mL,

703, 67 mL for 35 ° C) and (48, 67 mL, 72.42 mL, 313.33 mL for 55 ° C) for loads 4, 8 and 12 gSV / L. respectively. In test of 55 ° C, the growth rate and metabolic pathway of the microorganisms was higher, which was evidenced in the presence of methane at 5 days of experimentation. However, under mesophilic conditions, the hydrolysis is maintained longer. Once both the loads and the temperature have been analyzed, it can be thought that the ideal combination is 35 ° C and 12 g VS / L, because the highest production of H₂ was obtained. However, it can be interesting to make tests with higher loads, thinking about the scaling of the process.

Conclusions

The importance of choosing an inoculum to favor the conditions for the production of hydrogen by dark fermentation makes it necessary to use an inoculum that favors the bacterial activity of producers and non-consumers of hydrogen. Although the difference between the inoculums used was not significant, this does not show that those chosen can be excluded when thinking on a larger scale. Likewise, the work allows recognizing that in the case of loads, it would also be relevant tests were increased the carbohydrate content in the reactors to achieve higher productions.

Reference

- Baykara, S.Z., 2018. Hydrogen: a brief overview on its sources, production and environmental impact, *Int. J. Hydrogen Energy*, 43, pp. 10605-10614. <https://doi.org/10.1016/j.ijhydene.2018.02.022>
- Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R. Lens, P.N.L., Esposito, G. 2015. A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products, *Appl. Energy*, 144 pp. 73-95, <https://doi.org/10.1016/j.apenergy.2015.01.045>
- Lo, Y.-C., Chen, S.-D., Chen, C.-Y., Huang, T.-I., Lin, C.-Y., Chang, J.-S., (2008), Combining enzymatic hydrolysis and dark-photo fermentation processes for hydrogen production from starch feedstock: a feasibility study, *Int. J. Hydrogen Energy*, 33, pp. 5224-5233, <https://doi.org/10.1016/j.ijhydene.2008.05.014>
- Niño-Navarro, C., Chairez, I., Christen, P., Canul-Chan, M., García-Peña, E.I., 2020, Enhanced hydrogen production by a sequential dark and photo fermentation process: Effects of initial feedstock composition, dilution and microbial population, *Renewable Energy*, Volume 147, Part 1, Pages 924-936, <https://doi.org/10.1016/j.renene.2019.09.024>.
- Hitit, Z.Y., Lazaro, C.Z., Hallenbeck, P.C., 2017. Increased hydrogen yield and COD removal from starch/glucose based medium by sequential dark and photo-fermentation using *Clostridium butyricum* and *Rhodospseudomonas palustris*, *Int. J. Hydrogen Energy*, 42 (2017), pp. 18832-18843 <https://doi.org/10.1016/j.ijhydene.2017.05.161>