Hydrogen production by dark fermentation process from pig manure, cocoa mucilage and coffee mucilage

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Abstract

The aim of this study was the evaluation of the biochemical hydrogen production (BHP), by dark fermentation (DF) of 13 mixtures, composed of residual biomass derived from the agro-industry (pig manure, cocoa mucilage, and coffee mucilage) of the Santander and Cundinamarca departments. In order to contribute to the management of organic waste and the development of a bio-based economy in Colombia. Sludge from an anaerobic digester was used, pretreated by thermal shock as an inoculum and a thermophilic environment of 55 ° C. The variables were concentration (2, 5 and 8 g COD/l), C/N ratio (25, 35 y 45), coffee and cocoa ratio in three levels 1:3, 2:2 y 3:1, that would allow determining its impact on the process. The experimental design used was Box-Behnken type, to mathematically model the system. Also, a mathematical model MARSplines (Multivariate adaptive regression splines) was used as an alternative method to corroborate the optimal points|. It was determined that the best mixing conditions with the maximum research concentration of 8gCOD/l and C/N 45. Likewise, it was determined that the RSCFM: CCM has low participation with concerning to the other independent variables, a factor that favors the country's scope, due to the crops are not permanent throughout the year.

Keywords: Bio-hydrogen, Biomass residual, Dark Fermentation, Volatile Fatty Acids

1. Introduction

Fossil fuels are the energetic suppliers of more than 80% of the world demand [1]. Their use has caused global warming due to the emissions of CO_2, CH_4, and N_xO, in addition to the concern about the oil reserves depletion [2]. Due to this situation, many energetic alternatives had been investigated to guarantee the world energy supply and to develop sustainable systems that apply the circular economy concept using part of their residues to produce energy [3, 4].

Colombia is a country with high potential to produce energy transforming the biomass generated in the agricultural sector. In 2016 the Colombian agricultural industry generated more than 7,5 million tons of organic residues, with an increase of 0,5% since 2015 [5]. Cocoa and coffee are the primary crops in the country and the ones with higher export incomes [6]. However, there are not implemented alternatives for the treatment and valorization of the agricultural residues, and the producers are losing the profits and benefits from processes like anaerobic digestion and dark fermentation [7].

This last technology produces hydrogen as a main energetic product. Hydrogen is energetically attractive due to its energy potential per unit of weight, corresponding to 120 MJ/kg, one of the highest about other fuels [8]. Additionally, its combustion produces mostly water; and in the production process, different products of interest for the chemical industry can be obtained [7]. Likewise, one of the most striking features of bio-hydrogen production lies in the consumption of residual biomass for its production through biological processes and the possibility of complementing other production systems [9].

In order to close the local production cycles, it is essential to determine the bio-hydrogen production potential from residual biomass of agro-industrial systems in Colombia. Coffee and Cocoa represent 9% and 7.6%, respectively, of the national agricultural Gross National Product –GNP-, generating more than 800 thousand direct and indirect jobs [10–12]. Consequently, the present study focuses on the production of bio-hydrogen using as substrates CCM and CFM. Also, PM is used as a support in the biological process, being the primary nitrogen source, allowing to reach the adequate C/N ratio for the planned tests [13]. The main goal was to find the most workable mixture in terms of hydrogen production through the development of a Box Behnken design, where different operative variables were evaluated; and the optimization by the mathematical modeling of the system constructed with the data found. Additionally, provide information to contribute to overcoming the challenges that, today, are faced with giving dark fermentation laboratory studies a perspective on an industrial scale for its final application in the generation of energy [14].

2. Materials and methods

2.1. Substrates

The substrates selected were pig manure (PM), collected at the Marengo Agricultural Research Center of the National University of Colombia, located in Mosquera (Cundinamarca). Cocoa mucilage (CCM) – This residue was simulated in the laboratory from different references found of this industry and using cocoa bunches from private crops in Santander. The Coffee Mucilage (CFM) came from private farms in the departments of Cundinamarca. All substrates kept at -4°C to avoid biochemical degradation by external agents.

2.2. Inoculum

The inoculum came from an anaerobic digester of the company Alpina Productos Alimenticios S.A. located in Sopó (Cundinamarca). The digester works with waste from the dairy industry and contains a wide range of anaerobic microorganisms in an aqueous medium, at neutral pH and a specific temperature range [15]. The inoculum was stored at 4° C before the experiments and brought to thermal shock, at 97° C for 30 minutes, to inhibit the growth of methanogenic microorganisms [15].

2.3. Experimental design

An empirical Box-Behnken model was designed to evaluate the effect of independent variables on H_2 production. Three independent variables were established: coffee and cocoa ratio mucilage (RS CFM: CCM), substrate concentration, and C/N ratio; each with three own levels, as shown in **Table 1**. Colombia produces coffee and cocoa, but both are seasonable and the availability of the CFM and CCM changes during the year, that is the main reason to include the RS CFM:CCM due the results obtained will be used to scale up the process. Each mixture presented different combinations of the independent variables. For the calculation of the C/N, the PM was taken as the primary source of nitrogen, and the CFM and CCM as the primary source of carbon (as carbohydrates).

Table 1 Experimental design

Combination	**RS CFM:CCM** (gCOD CFM/gCOD CCM)	**Concentration (g COD/L)**	**C/N**
1	3:1	2	35
2	1:3	2	35
3	3:1	8	35
4	1:3	8	35
5	3:1	5	25
6	1:3	5	25
7	3:1	5	45
8	1:3	5	45
9	2:2	2	25
10	2:2	8	25
11	2:2	2	45
12	2:2	8	45
13	2:2	5	35

The initial concentration and the C/N were adjusted according to the physicochemical characteristics of each substrate, and its proportion in the mixture. The Box-Behnken experimental design evaluates 13 mixtures as can be seen in Table 1. However, only 13 mixtures were assessed since two of them evaluated the same central points. Each combination was performed by triplicate for a total of 39 reactors plus three targets, working through a discontinuous regime system. The required amount of each substrate and inoculum were placed in 250ml amber bottles, the working volume (200ml) was completed with distilled water, and 10 ml of 5,5 buffer solution. HCl 37% were used to adjust the pH to the reactors, which started with a pH of 5.5 ± 0.3. The reactors were placed in a thermostated bath to maintain a thermophilic environment (55 °C ± 0.2) during the experimental time. The production of hydrogen was measured through the volume displacement of amber bottles with a capacity of 1l, with a 0.5M NaOH solution, which functions as a CO_2 trap [16].

The test was monitored continuously to verify that there was no production of CH4 in the biogas; This was done by daily monitoring with a Biogas 5000, a portable gas analyzer. The test was allowed to run until the hydrogen production rate decreased. Finally, the information collected within the design of experiments was analyzed to determine the mixtures with the highest biohydrogen production potential, using the Box-Behnken model and the mathematical model of the MARSPLINES (Multivariate adaptive regression splines).

2.4. Statistical analysis

The experimental design described above enabled the construction of second-order polynomials in the independent variables and the identification of statistical significance in the variables[17]. The polynomial model used was of the following type:

$$Z = a_0 + \sum_{i=1}^{n} b_i x_{ni} + \sum_{i=1:j=1}^{n} d_{ij} X_{ni} X_{nj} \qquad (i < j)$$

Where Z and X_{ni} denote dependent and normalized independent variables, respectively, a_0 is a constant and b_i, c_i, d_{ij} are the regression coefficients obtained from experimental data. Independent variables were normalized (X_n) by using the following equation:

$$X_n = \frac{(X - X_{mean})}{\left(\frac{(X_{max} - X_{min})}{2}\right)}$$

Where X is the absolute value of the independent variable concerned, X_mean is the average value of the variable, and Xmax and Xmin is their maximum and minimum values, respectively.

2.5. Regression analysis

Multivariate adaptive regression splines (MARSplines) is a nonparametric regression model, used for the analysis of time series proposed by Friedman. This model makes predictions about non-linear relationships of the independent variables of the system, to obtain the modeling of the dependent variable with low error, using base sets of activation functions and their coefficients [18]. The MARSplines model has the form:

$$\hat{f}(x)=a_o+\sum_{Km=1} f_i(X_i)+\sum_{Km=2} f_{ij}(X_i,X_j)+\sum_{Km=3} f_{ijk}(X_i,X_j,X_k)+\cdots$$

The first stage contemplates the sum of all functions related to a single variable; the next sum involves all the essential functions that involve two variables and successively [19].

2.6. Analytical methods

The characterization of the substrates and the digested mixtures of the experimental design were carried out as follows: The pH measurements were completed using an Edge Model HI2002 potentiometer. The total solids (TS) by following the method 2540B APHA SM, in which the samples were taken to 105°C for 24 hours. The Volatile solids (VS) were determined on a wet basis applying ASTM D3174, where the sample was ignited at 550°C for 1 hour. Kjeldhal total nitrogen was performed following ASTM D1426, performing three intervals of 125°C (20 min), 300°C (30 min) and 400 ° C (60 min); H2SO4 98% was used for digestion and subsequently, it was distilled with concentrated NaOH and titrated with 0.5 N hydrochloric acid. The AGVs and alkalinity analyzes were developed according to the 5560D APHA SM and 2320B APHA SM respectively and the analyzes for CODs using ASTM D1252-06 employing a Hanna reactor.

The results of the characterizations of the substrates are presented in Table 2; these were obtained following the protocols established, except for the determination of carbohydrates, which was determined according to the method suggested by Albalasmeh [20].

3. Results and discussion

3.1. Substrates characterization and inoculum

Table 2 presents the results of the physical-chemical characterization. In order to validate the data obtained, these were compared with those of the database "*database for biomass and waste*"[21], finding that they are quite similar. The three substrates have high values of VS/TS as an indicator of the high organic matter content, their biodegradability, and valorization potential. Regarding carbohydrates, CCM and CFM reported high values and low content of proteins as Vriesmann [22]. On the other hand, the PM has a good percentage of proteins and will be the nitrogen source in the DF process [7].

Table 2 Characterization of the residual biomass used in the study

		Substrates			
		PM	**CCM**	**CFM**	**Inoculum**
Moisture	%	77,08±0,7	80,72±0,8	97,2±0,4	94,4
NTK	%	2,10	0,58	0,31	
Organic matter	%	71	79,6	97	96,6
N	%	2,07	0,21	0,06	
COD	g/l	23,87	10,50	21,75	
Proteins	%	22	4	6,5	
Carbohydrates	%	2,9	60,37	85,95	

* The analyses were performed on a wet basis

3.2. Box-Behnken combinations

The time in all the experiments was 144 hours, that is relatively short considering the high amount of soluble matter in the mixes evaluated but in concordance with the times reported in the literature [23–26]. **Fig. 1** reports the cumulative hydrogen production for each combination evaluated. Combination 12 reported the highest production with 155,333 ml H_2/day, showing a direct relationship between the production and the substrates concentrations [23]; combination 12 has an organic load of 8 gCOD/l, a relation CFM: CCM of 2 and a C/N ratio of 45. However, combinations 5 and 6 reported significant hydrogen productions with lower substrates concentration (5 gCOD/l) and less C/N (25). The results obtained shows that higher hydrogen productions are achieved with 5-8 gCOD/l for the substrates evaluated and when the C/N ratio is 45 or 25. According to the results, the intermediate C/N ratio does not seem to favor the process, however, that optimal results are reported later. High substrates concentrations allow for processing and valorizing higher amounts of residual biomass, avoiding the negative environmental impacts that these residues generate when they are not disposed of thoroughly.

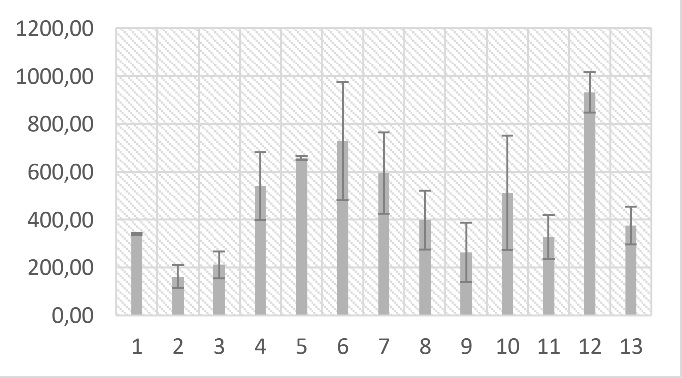


Fig. 1 Cumulative production of each of the combinations given in ml of H_2

In order to quantify the influence of these three variables on the response variable, a Pareto analysis was performed. **Fig. 2** shows that the influence of C/N and CFM: CCM is less than 10%, and the organic loading concentration is the variable with high impact in the production (93%), this agrees with that described by other authors, where higher concentrations allow better productions [13, 27–29]. This result means that the range of substrates concentrations evaluated does not cause any toxic effect during the biological process. Likewise, the C/N has a positive impact on hydrogen production, helping to increase the yield. The C/N ratio is an essential variable in DF processes because it is the measure of the presence of nitrogen in the reactor; the high or low presence of nitrogen can inhibit the microorganism [30]. A negative influence was estimated for the RS CFM: CCM, meaning that with more presence of CFM the hydrogen production decrease; the decrease in the production is because CFM has a lower presence of carbohydrates per g of COD comparing with CCM. This is an excellent find considering that the coffee and cocoa are seasonal crops in Colombia, so the availability of these two residues will change during the different months of the year.

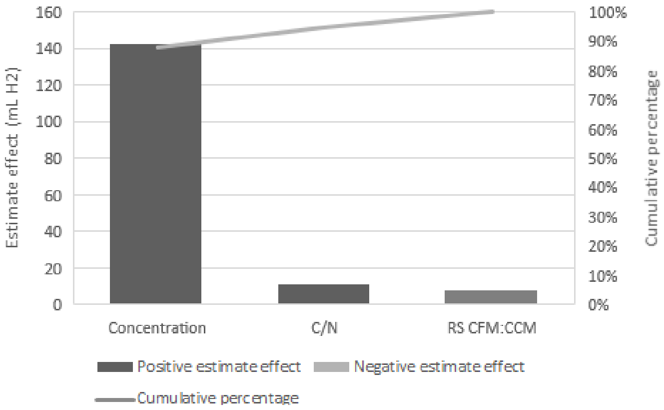


Fig. 2 Effects of the independent variables on the BHP

3.3. Effluent characterization

The pH has the most significant influence on the effluent composition of the acidogenic reactors, and an increment implies lower hydrogen production [31, 32]. All the reactors presented an increment in the pH as evidence of the lower hydrogen production at the end. The initial pH was settled at 5,5, and at the end, the values measured were between 5,8 and 7,58. This behavior is in concordance with the data reported in the literature [31, 33].

Fig. 3 presents the relationship between VFA production and hydrogen production. Combinations 1, 2, and 3 have lower hydrogen production, less than the media 19,33 ml/day, and production of VFA major than the media (1236 mgCOD/l). The results obtained are in concordance with Agler [33], who reported that VFA production is directly related to hydrogen consumption for the generation of by-products in the fermentation processes. Contrary, combinations 5, 6, and 12 reported VFA productions lower than the media.

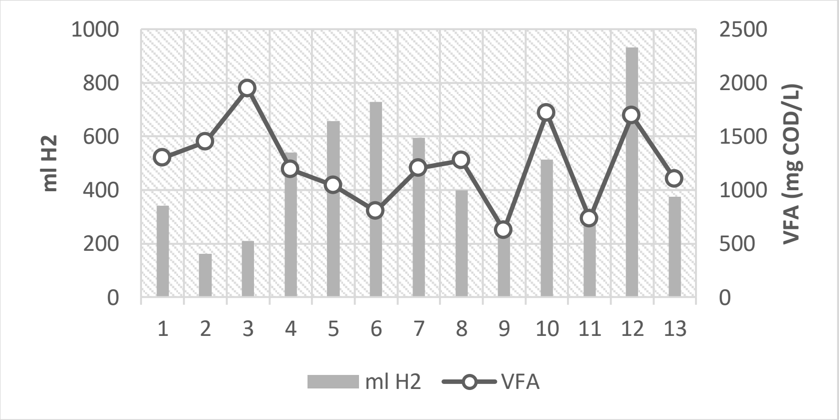


Fig. 3 Comparison between the production of hydrogen and VFA for each of the 13 combinations

The alkalinity is a desired effect between the reactors since it is an indicator of the buffer effect that the mixture possesses. Controlling the pH is fundamental since it has the most significant influence on the behavior of DF reactors; likewise, its direct influence on hydrogenase enzymes has been observed [34]. Also, high alkalinity is sought, because controlling the pH in real time, adding acidic or basic solutions, is a challenge in the real application [35].

Fig. 4 shows the relationship between alkalinity and VFA production, which indicates that in mixtures 4 and 6 there is a higher alkalinity of the mixture compared to the production of VFA, with a close VFA/alk ratio to 1.1; this is desirable because a high alkalinity favors the production of hydrogen due to the low production of VFA [36]. Regarding combination 12 is shown that it has average values of alkalinity that can endanger the behavior of the process, considering that the AGVs are also high.

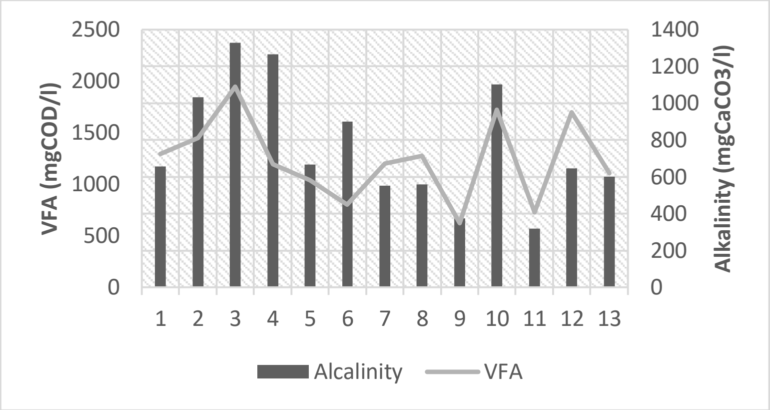


Fig. 4 Relationship between alkalinity and VFA production for each of the 13 mixtures

Besides, it was found a negative influence between C/N ratio and higher values of alkalinity; the mixtures with lower C/N ratio are the ones with more quantity of PM, and this substrate have demonstrated their buffer effect in previous research [37]. The relationship between pH and alkalinity is directly proportional because they affect the production of VFA and the consumption of hydrogen [34]. In **Fig. 5** where it is observed how pH and alkalinity have similar behavior.

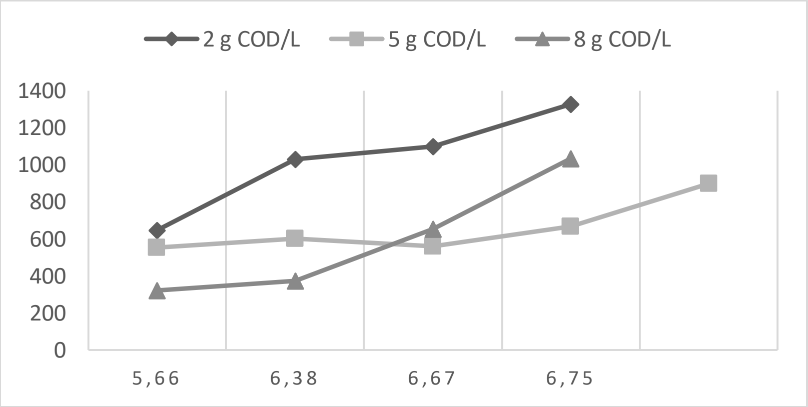


Fig. 5 pH vs. alkalinity ratio

On average, the percentage of COD removal in the tests carried out was 37.08%, with a maximum value of 64% for mixture 2; with a load of 2 gCOD /l and the lowest percentage was for mixture 10; with a concentration of 8 gCOD / l. This is consistent with that reported by other authors, because lower loads favor the removal of COD, as there is a more significant interaction between the inoculum and the substrate present in each reactor [36]. Likewise, other authors, such as Ghimire, state that a higher load has higher hydrogen production, but there is a lower percentage of COD removal [38].

3.4. Statistical analysis

3.4.1. Box-Behnken

The Box-Benhken matrix of experiments described in the methodology was assessed by applying second-order polynomials, taking as variables those presented in the design, all of them related to each other; which allowed generating a model for the mixtures of substrates that enables the maximization of the BHP, see equation 1, where the substrate concentration is represented by [ ] symbol. The equation was the result of a simulation performed through the software STATGRAPHICS that generates a prediction of the BHP.

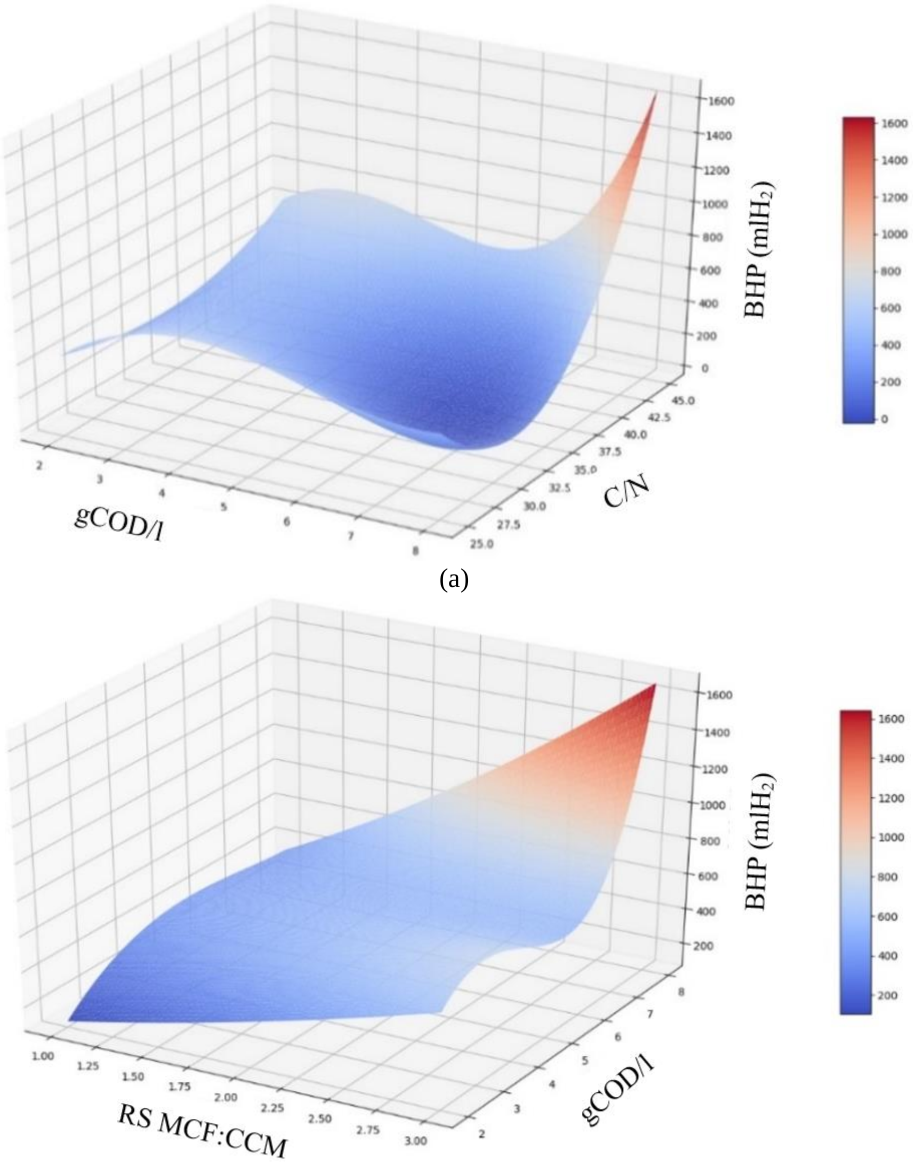
$$\begin{aligned} ml\, H_2 = {} & 374{,}5 - 8{,}48229 * RS\, CFM{:}CCM + 143{,}185 * [\,] + 11{,}5052 * C/N + 17{,}6281 * RS\, CFM{:}CCM^2 \\ & - 137{,}371 * RS\, CFM{:}CCM * [\,] + 66{,}4063 * RS\, CFM{:}CCM * C/N - 68{,}5073 * [\,]^2 \\ & + 88{,}2917 * [\,] * C/N + 202{,}716 * C/N^2 \end{aligned} \tag{1}$$

The equation that was obtained presents a correlation coefficient capable of explaining 75% of the variability associated with BHP, also, the Durbin-Watson statistic (D=1,623) shows that there is not a sub-estimation in the correlation coefficient. The model allowed the definition of an optimal combination, which has a concentration of 8gCOD/L, RS CFM:CCM of 1:3 and C/N ratio of 45, predicting a maximum production of 842,377 mL H_2; which is close to the mixture with the highest production in the design.

$$\begin{aligned} ml\ H_2 = {} & -483{,}059 - 25{,}9812x_1x_1 - 170{,}278x_0x_1 - 1{,}42628x_0x_0x_1x_1 + 40{,}1349x_0x_0x_1 \\ & - 0{,}0523742x_2x_0x_1x_1 + 30{,}5188x_2x_1 - 0{,}551239x_2x_2x_1 - 0{,}0523742x_2x_0x_1x_1 \\ & - 11{,}6596x_2x_0x_1 - 0{,}12025x_2x_0x_0x_1x_1 - 0{,}00351634x_0x_2x_2x_0x_1x_1 \\ & + 0{,}00479633x_0x_2x_2x_2x_1 + 7{,}80912^{-5}x_1x_1x_0x_2x_2x_0x_1x_1 \end{aligned} \quad (2)$$

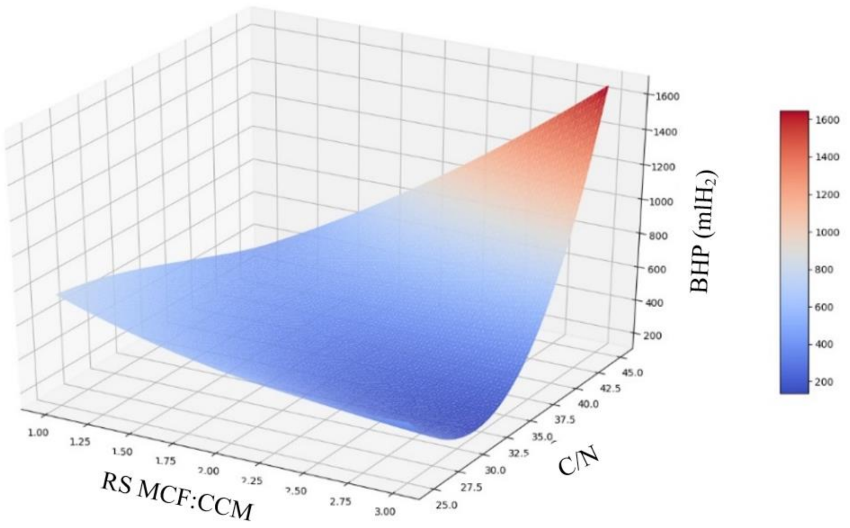
*x_0: RS CFM:CCM, x_1: substrate concentration, x_2: C/N ratio

The model equation has a correlation coefficient of 76% for the variability associated with the BHP, very similar to that obtained through the mathematical model of the Box-Behnken. Using the MARSplines regression, it was obtained that the maximum possible cumulative production is equivalent to 1669 ml H_2 with an initial concentration of 8gCOD/L, C/N close to 45 and an RS CFM: CCM of 3:1, this is consistent with pareto. Since there is no evident influence of this relationship on the potential.



(a)

(b)



(c)

Fig. 6 Surface diagram for the BHP, based on the relation of the variables (a) substrate concentration vs. C N ratio, (b) RS CFM: CCM vs. substrate concentration and (c) RS CFM:CCM vs. C/N ratio

Fig. 6 shows surface diagrams of different relations of the independent variables and their influence on the BHP dependent variable determined by MARSplines. In diagram (a) it is observed that the relation between the highest concentration and the C/N of 45 favor the production of hydrogen. Diagram (b) shows that the best RS CFM:CCM has a value of 2.80 and in (c) the values for C/N and RS CFM:CCM that was presented concerning the concentration are supported.

4. Conclusions

The maximum hydrogen production achieved was 155.33 ml H_2/d when the organic loading rate was 8 gCOD/l, the RS CFM:CCM of 2:2 and C/N ratio was 45 in the combination 12. In general, the mixtures with organic loads between 5 and 8 gCOD/l reported higher production. Regarding the C/N ratio, it was found that the best hydrogen productions are achieved with the lower and higher value (25 and 45). On behalf of RS CFM:CCM, the conclusion is that mixtures with a significant content of CCM produce more quantity of hydrogen thanks to the higher content of carbohydrates of this substrate.

The lower influence of the RS CFM: CCM variable that was presented in the Pareto chart does not directly affect the study, because, in order to scale up the project, it would be under the behavior of these residues in the country. This is because these crops are not produced throughout the year in all regions of the country.

The removal of COD of 37% allows suggesting secondary processes associated with biorefinery schemes, which allows higher removals of COD and the obtention of other value-added sub-products such as VFA. All the substrates used are susceptible to be valorized by waste to energy technologies, decreasing the associated carbon footprint.

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