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Analysis of hydrogen production by anaerobic fermentation from urban organic waste

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Abstract
The production of hydrogen by anaerobic fermentation of urban organic waste from fruits and vegetables was studied. Ten tests were carried out under an incomplete factorial experiment design with four factors and three levels. The factors were: acidification pH, acidification time, operational pH, and organic load. The response variables were: maximum hydrogen content (%) and maximum hydrogen production (L/day). The results were fitted to a quadratic polynomial model. In the case of the maximum hydrogen content, an R^2 and an $R^2_{adjusted}$ of 0.9987 and 0.988 were obtained, respectively. For the maximum production, an R^2 and an $R^2_{adjusted}$ of 0.9815 and 0.833 were obtained, respectively. Three mathematical techniques were used to obtain the maximum values in the response variables. Fuzzy logic showed the best fit between the experimental values and estimated values. In addition, it predicts a maximum hydrogen content of 58.5% and a maximum production of 63.4 L/day.

Keywords: Hydrogen, anaerobic fermentation, mathematical model, organic waste, batch culture.

Análisis de la producción de hidrógeno por fermentación anaerobia de residuos orgánicos urbanos

Resumen
Se estudió la producción de hidrógeno por fermentación anaerobia de residuos orgánicos urbanos de frutas y verduras. Se realizaron 10 ensayos usando un diseño experimental factorial incompleto con cuatro factores y tres niveles. Los factores fueron pH de acidificación, tiempo de acidificación, pH de operación y carga orgánica. Las variables respuesta fueron contenido de hidrógeno máximo (%) y producción máxima de hidrógeno (L/día). Los resultados se ajustaron a un modelo polinomial cuadrático, para el contenido de hidrógeno se obtuvo un R^2 y un $R^2_{ajustado}$ de 0.99 y 0.98 respectivamente. En la producción máxima de hidrógeno, se obtuvo un R^2 y un $R^2_{ajustado}$ de 0.98 y 0.83 respectivamente. Tres técnicas matemáticas fueron usadas para obtener los valores máximos, siendo el modelo con lógica difusa el que presentó mejor ajuste entre los valores experimentales y los estimados, además pronostica un contenido de hidrógeno de 58.8% y una producción máxima de 63.4 L/día.

Palabras clave: Hidrógeno; fermentación anaerobia; modelo matemático; residuos orgánicos; cultivo discontinuo.

1. Introduction

The development of hydrogen technology has been limited due to the low availability of hydrogen. Although hydrogen is a highly abundant element in nature, it is not found isolated. Its traditional production is done with costly chemical processes (reforming hydrocarbons) or with processes with a negative energetic balance, such as electrolysis [1].

In recent years, it has been demonstrated that it is possible to generate hydrogen through the anaerobic fermentation of organic waste (biohydrogen). The process is characterized as being complex, dynamic and highly dependent on multiple factors, including the type of substrate, temperature, pH, nutrient content, agitation, water retention time, bacterial population, and bioreactor, among others [2]. However, it can be said that high yields in the production and in the composition of biohydrogen (between 50% and 60% hydrogen) are achieved when substrates that are rich in sugars are used under thermophilic conditions (temperatures between 45 and 70ºC) and a pH of around 6.0 [3].

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Studies have been carried out with the goal of identifying the combined effects of two variables; pH and substrate content and pressure and temperature. However, few studies have analyzed the synergistic effect of multiple variables when the substrates are residues and the culture is mixed [4]. Perhaps the most used model to describe the evolution of biohydrogen is Gompertz's model; this empirical approximation is based on three parameters: the *lag* phase time, potential production of H_2, and H_2 production rate. Experimental information related to these three parameters must first be obtained in order to adjust the model; this allows a high correlation between the observed data and the adjustments to be obtained. The model should not be used for predictions because it does not take into account high incidence variables in the process, such as temperature, pH, and substrate type and content [5-8].

The conventional kinetic equations of Monod and of Luedeking and Piret have been used to study the production of biohydrogen. However, multiple rigorous simulations followed by a series of validations may be required to establish the generality of the equations and associated parameters. Additionally, a complete understanding of those models will be achieved when they can be integrated with other complex bioprocesses, such as hydrolysis and acetogenesis [9]. Other authors have used kinetic models that are highly successful in the prediction of methane generation (ADM1, open structure mechanistic model, Anaerobic Digestion Model1) and adjusted them to describe the production of biohydrogen. The ADM1 was used to predict the production of hydrogen, the chemical demand of oxygen and the formation of fatty acids, taking into account temperature, inoculum, agitation, pH control and pressure. The authors achieved a suitable prediction for the production of hydrogen with an adjustment of $R^2 = 0.91$; however, for the chemical demand of oxygen, acetate, butyrate and propionate, the adjustments were inferior ($R^2 = 0.88$; $R^2 = 0.76$; $R^2 = 0.75$; and $R^2 = 0.71$, respectively) [10].

Other models are based on experimental designs where optimal values can be found through the gradient method or through the superposition of response surfaces [11-13]. These models provide an adapted approximation for the optimization of biohydrogen production when variables such as pH, glucose content, iron sulfate content, hydraulic retention time and inoculum rate have been analyzed [14,15]. However, many factors can affect optimal conditions, especially when mixed cultures are used; therefore, these conventional techniques can be laborious and time consuming and do not always ensure the determination of optimal conditions. These methods allow for the maximum possible coverage of cases and detect the key parameters in multivariate systems rather than provide optimal values [15].

Nonconventional mathematical techniques such as genetic algorithms (GA) are being used for modeling and optimizing non-linear multivariate bioprocesses [16]. Some hybrid models that use Artificial Neural Networks (ANNs) and GAs have been developed to optimize the production of hydrogen, using the following variables: initial pH, temperature, mixed substrate, content and age of the inoculum. The results indicate that after optimization, it has been possible to increase the hydrogen yields up to 16% [17]. In general, it has been noted that, in models where an ANN is considered the objective function of a GA, a high optimization of hydrogen production has been achieved; higher than the one achieved by conventional techniques, including the response surface methodology [18,19].

Alternatively, heuristic models based on fuzzy logic have been used to predict the production rate of biogas and methane by anaerobic fermentation with notable results. A model with fuzzy logic was developed to predict the production of biogas and methane using five variables: organic load rate, chemical demand of oxygen, removal rate, alkalinity, and effluent and influent pH [20]. The model based on fuzzy logic demonstrated a high predictive capacity when compared with the non-linear regression model. The adjustment with the fuzzy logic model had $R^2 = 0.985$, and it had $R^2 = 0.893$ with the exponential model. The fuzzy logic model did not require the definition of the complex reactions or their biochemical and mathematical equations; thus a complex system with high non-linear structure such as anaerobic digestion could be easily modeled with suitable precision. In the present study, the generation of hydrogen by anaerobic fermentation from urban organic waste was studied and two stochastic techniques and one heuristic technique were used to analyze the percentage of hydrogen from gas and the daily production of hydrogen.

2. Materials and methods

2.1. Location

This study was carried out at the Laboratorio de Mecanización Agrícola of the Universidad Nacional de Colombia, Medellín campus, located at 1,488 m.a.s.l., at the coordinates of 6°13′55″N and 75°34′05″W, with an average annual temperature of 24°C, relative humidity of 88% and average annual precipitation of 1,571 mm.

2.2. Raw material

The raw material consisted of a mixture of organic waste compounds principally from lettuce leaves, cabbage, orange, lime, papayuela, mango, guava, cucumber, onion, garlic, pepper and tomato, which were blended in a processor (0.5 Hp, 1,730 rpm, Siemens). The waste was obtained from the Central Mayorista de Antioquia.

2.3. Materials

For the tests, three cylindrical bioreactors of 2,000 L were used; these were operated in batch culture mode. The gas production was recorded with a gas meter (Metrex G2.5, precision of 0.040m³/h and maximum pressure of 40 kPa or 5.8 PSIG). The pH measurements were carried out daily with a digital pH-meter (Hanna Instruments) with a precision of ±0.2 (at a temperature of 20°C).

Table 1.
Operational ranges of the factors in the tests.

Factor	Level 1	Level 2	Level 3
Organic Load (OL, mg /L)	8000	15000	30000
Acidification Time (Ta, days)	5	10	>15
Acidification pH (pHa)	3.5-4.5	4.6-5.5	>5.6
Operational pH (pHo)	4.5-5.0	5.1-5.5	5.6-6.0

Source: Authors.

2.4. Methods

Ten tests were carried out under an incomplete factorial design with four factors and three levels. The factors were: acidification pH (pHa), acidification time (Ta), operational pH (pHo) and organic load (OL). The response variables were: maximum hydrogen production (PH_2, L/day) and maximum hydrogen content (CH_2, %). The acidification pH corresponded to the pH of the substrate at the beginning of the test, which permitted the elimination of the methanogenic bacteria (hydrogen consumers). The acidification time represented the days in which the test remained with an acidification pH; when this was completed, agricultural lime was added (CaO 54%) until the operational pH that corresponded to each test was reached. Finally, the organic load associated with the chemical demand of oxygen (mg/L) was measured at the beginning of the tests and its magnitude was a function of the decomposition degree of the available waste.

The operation volume of bioreactors was 1,400 L and each test had an average of 550 ± 20 kg of substrate blended with water at a proportion of 1:2 [21]; this represented 70% of the bioreactors' volume. The digestion time was variable according to the acidification time and all tests were performed without stirring. In tests, an inoculum was not used; the microorganisms were native from the waste used. In Table 1, the ranges of each factor are presented.

2.4.1. Physicochemical Analysis

The physicochemical analyses of COD (Chemical Oxygen Demand) followed the standardized methods of APHA in the 19 edition of 1995 (standard method 5220-C). To determine the composition of the generated gas, daily samples were taken using one (1) liter Tedlar bags. The samples were analyzed with a Perkin Elmer chromatograph, equipped with a thermal conductivity detector (TCD) and two columns connected in series (CP-5A and Moliseve CP Porabond Q). Oven temperatures and detector temperatures of 60 and 253°C, respectively, were used. The analysis of the gas included the quantification of CO_2, O_2, H_2, CO, CH_4 and N_2 [22].

2.4.2. Process Modeling

Response surface and non-linear regression model. Two nonlinear models were constructed by means of nonlinear regression and adjusted by least squares. For this the NonLinearModel.fit option of Matlab 2012 was used. A pure quadratic model was employed, which included independent, linear and quadratic terms. In order to optimize the models, the *rstool* tool for the multidimensional statistical analysis of the response surface was used.

Genetic algorithm and non-linear regression model. The aptitude function that was optimized with the genetic algorithm was the non-linear model obtained with the *NonLinearModel.fit* option of Matlab 2012. The individuals were composed of four genes, each one corresponding to a factor, codified in real values. The genetic algorithm was applied with the *optimtool* option of Matlab 2012. The genetics operators were: mutation, cross and replication by elite (4 individuals).

Heuristic model with fuzzy logic. A model was created using fuzzy logic, which was integrated with four input variables corresponding to the four factors of the experiment and two output variables associated with the response variables. Both the input variables and the output variables were represented by five trapezoidal fuzzy sets linguistically labeled very low, low, medium, high, and very high. The employed fuzzy inference system was Mandani and the operator was the minimum (method And). The method for transforming the fuzzy results was centroid. The model was implemented with the *Fuzzy Logic* tool of Matlab 2012.

3. Results

3.1. Hydrogen Content and Production

Table 2 presents the results obtained for maximum hydrogen content and maximum production in ten tests. The values of the factors remained in the previously defined ranges for each level. The content and production values correspond to the maximums achieved in each test. The most outstanding results were obtained for an organic load over 18,000 mg/L; a pHa between 4.1 and 4.5; a Ta between 7 and 8 days; and a pHo between 5.2 and 5.3 These operation conditions are similar to those reported by different authors when urban organic waste is used [14]. From experimental results (see table 2), the maximum hydrogen content obtained was 18% and the maximum production was 37.8 L/day. These values are considered low; however, it is important to emphasize that in all the tests, the temperature was in the mesophilic range, an inoculum was not employed, and the fermentation process was done without stirring.

Table 2.
Results for maximum hydrogen content and maximum production.

Test	OL (mg/L)	pHa	Ta (days)	pHo	H_2 (%)	H_2 (L/day)
1	8667	4.3	6	5.0	6.7	4.0
2	12000	4.2	11	4.5	0	0
3	12000	5.3	10	5.1	5.7	5.4
4	12000	5.3	8	5.6	3.5	1.7
5	18000	4.1	7	5.3	10.5	37.8
6	19133	3.7	30	6.0	17.3	14.5
7	21000	4.8	5	6.2	9.2	32.7
8	23340	4.5	8	5.2	18.0	33.6
9	23340	4.3	14	5.1	7.0	12.5
10	27140	4.2	15	5.8	8.2	14.9

Source: Authors.

Table 3.
Adjustment indicators of the models for the estimation of maximum hydrogen content and maximum production

Variable	(Root Mean Squared Error, RMSE)	R^2	$R^2_{adjusted}$
Hydrogen content	0.62	0.9987	0.988
Hydrogen production	5.77	0.9815	0.833

Source: Authors.

The results fit a second order polynomial model, both for the hydrogen content and for the production of hydrogen. The adjustment made with the NonLinearModel.fit option of Matlab facilitated obtaining equations with independent, linear, and quadratic terms; when the interaction terms between the factors were included, it was not possible to carry out the adjustment. Information related to the adjustment of the models is presented in Table 3. The obtained models were:

Model for maximum hydrogen content (CH_2)

$$CH_2 = 358.6180 - 0.0013 * OL - 150.5930 * pHa - 5.4706 * ta + 8.5685 * pHo + 8.6143 * 10^{-8} * OL^2 + 16.7956 * pHa^2 + 0.1498 * ta^2 - 2.1048 * pHo^2 \quad (1)$$

Model for maximum hydrogen production (PH_2)

$$PH_2 = 1235.3 + 0.0043 * OL - 462.4066 * pHa - 7.9847 * ta - 42.2072 * pHo - 2.2832 * 10^{-8} * OL^2 + 49.2637 * pHa^2 + 0.1339 * ta^2 + 2.5467 * pHo^2 \quad (2)$$

The model obtained for the estimation of hydrogen content presented a high fit with an $R^2_{adjusted}$ value of 0.988. This means that 98.8% of the variation in the hydrogen content was attributable to the independent variables of the study. Additionally, the RMSE value was low (0.62), which indicates a low error between the model prediction and the experimental information. In the case of hydrogen production, the adjustment was good, albeit inferior to that of the model for hydrogen content. Its $R^2_{adjusted}$ value was 0.833, which indicates that 83.3% of the variation in hydrogen production was attributable to the independent variables.

3.2. Hydrogen content optimization

The optimization with the multiple response surfaces methodology was effective with a significance level of 0.05 (alpha). Despite the use of a model with high fit (see Table 3), this tool did not produce a suitable optimization because the resulting prediction was negative in magnitude (-5.6947, an unlikely situation for the experiment) with a broad interval (± 87.0799). This is attributable to the broad interval of confidence (red lines in Fig. 1) for each of the factors, possibly associated with the variability of the experimental information. This implies weakness in the prediction. The maximum estimation with this tool was 81.4%; a value that is very unlikely to be obtained with a bioprocess like the one developed in the present study: a batch system without inoculum cells, without temperature control and without agitation [23,24].

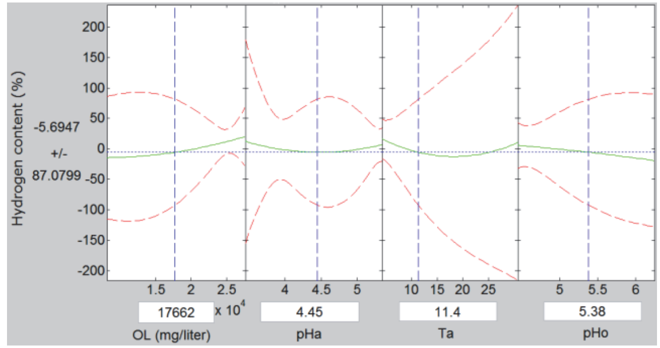

Figure 1. Optimization of the maximum hydrogen content with multiple response surfaces.
Source: Authors.

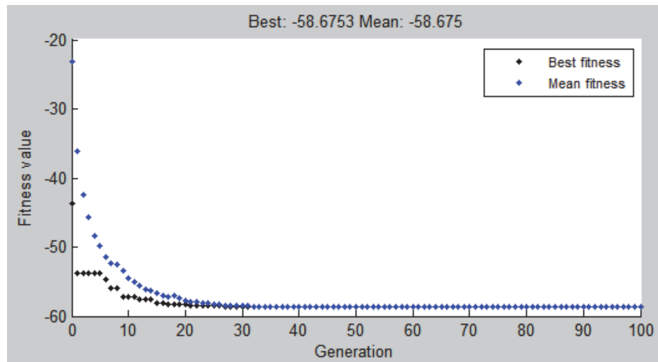

Figure 2. Optimization of the maximum hydrogen content with the genetic algorithm.
Source: Authors.

In the case of the predictions with the genetic algorithm, 100 interactions were required to obtain the results. The maximum hydrogen content was 58.7%. The initial population size was established at 40 individuals. The function employed for the creation of the initial population was obtained from a random initial population with a uniform distribution. The *shift linear* function was used for the classification of the individuals. The selection was statistically uniform. An elite population of 4 individuals was defined for the reproduction and the cross fraction was 0.6. For the mutation, the *adaptive feasible* function was employed. The cross function was *intermediate*, and the migration proceeded with a fraction of 0.2. The stopping criterion was defined by the number of generations. Fig. 2 contains the fit behavior of the genetic algorithm during optimization. Starting at generation number 40, a convergence can be seen between the mean value and the best value, presenting a high fit.

The model, based on fuzzy logic, estimated the maximum hydrogen content at 58.8%, a value similar to that achieved with the genetic algorithm. This value was obtained when the organic load was between 20,000 and 25,000 mg/L (see Fig. 3), the acidification pH was between 4.4 and 4.6, the acidification time was between 5 and 7 days, and the operational pH was 5.8. In the surfaces obtained with the fuzzy logic model (Fig. 3), the existence of various maximum values was observed; however, there

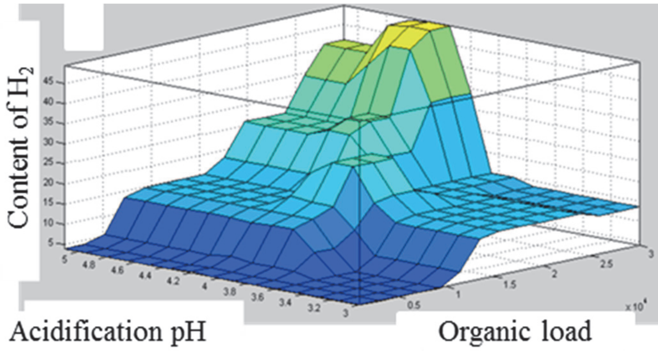

Figure 3. Estimation of maximum hydrogen content with fuzzy logic.
Source: Authors.

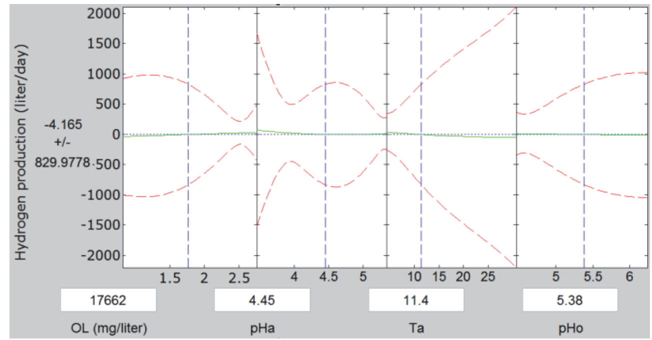

Figure 4. Optimization of maximum hydrogen production with multiple response surfaces.
Source: Authors.

Table 4.
Estimation of the maximum hydrogen content

Model	H_2 (%)	OL (mg/L)	pHa	Ta (days)	pHo
Response surface	-5.6947 ± 87.1	17662	4.5	11.4	5.4
Genetic algorithm	58.7	13350	4.5	18.3	6.2
Fuzzy logic	58.8	20000	4.5	5	5.8

Source: Authors.

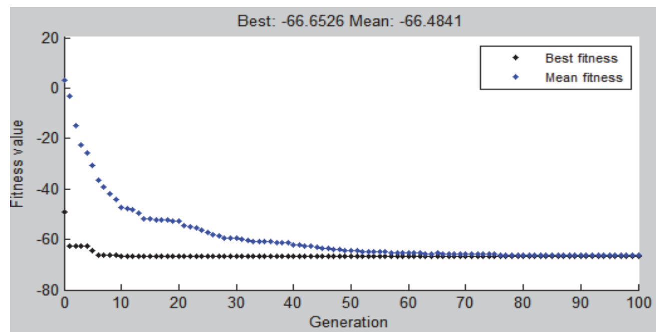

Figure 5. Optimization of maximum hydrogen production with genetic algorithm.
Source: Authors.

was only one global maximum, which corresponded to the value estimated by the model. This indicates that the model was able to respond to the dynamic and non-linear behavior of the bioprocess in a suitable manner.

Table 4 presents the optimal values obtained with the response surfaces and the genetic algorithm, as well as the estimation obtained with the fuzzy logic model. The latter two provided similar maximums for the hydrogen content. The genetic algorithm produced an estimation that required a substrate with a lower organic load as compared to that provided by the fuzzy logic model; however, the genetic algorithm estimated the acidification time at 18 days while the fuzzy logic model estimated it at 5 days. The acidification time is an adaptive stage of the substrate in which the hydrogen-consuming methanogenic bacteria are eliminated. During this time, hydrogen is not generated, so this time must be made as short as possible.

3.3. Hydrogen production optimization

Similarly to the optimization of the hydrogen content, the estimation of the maximum daily production with the multiple response surfaces methodology was overly high. The achieved prediction was a negative value (-4.165), with a very broad interval (± 829.9778) associated with the broad confidence interval (see Fig. 4, especially for the acidification time). This represents low confidence for the predictions produced by this tool. There was a high overestimation, with an estimated maximum production of 825.8 L/day, a value well beyond the maximum experimental value with organic waste in a batch culture without inoculum cells or agitation and at a mesophilic temperature [25-27].

With the genetic algorithm, the maximum value for hydrogen production was 66.7 liters/day. The size of the initial population was established at 40 individuals, and it was created from an initial randomized population with uniform distribution. The function for the classification of the individuals was *shift linear* with a maximum survival rate of 5. The selection was uniform; the reproduction was carried out maintaining an elite of 4 individuals with a cross fraction of 0.6. For the mutation, an *adaptive feasible* function was employed; the cross function was *intermediate*. The migration proceeded with a fraction of 0.2. The stopping criterion was the number of generations, which was established at 100. The convergence between the mean value and the best value was achieved at generation 70 (Fig. 5).

The maximum hydrogen production estimated with the fuzzy logic model was 63.4 L/day, a magnitude slightly lower than the genetic algorithm estimation. The value

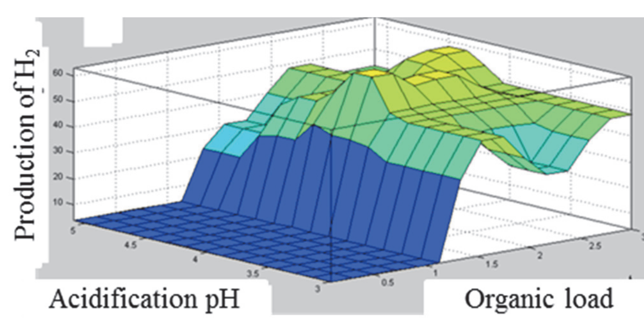

Figure 6. Estimation of the maximum hydrogen production with fuzzy logic.
Source: Authors.

Table 5.
Estimation of the maximum hydrogen production and the value of the factors.

Model	H_2 (liters/day)	OL (mg/liter)	pHa	Ta (days)	pHo
Response surface	-4.165 ± 829.9	17,662	4.5	11.4	5.4
Genetic algorithm	66.7	14,945	4.8	25	5.9
Fuzzy logic	63.4	18,000	4.5	5	5.5

Source: Authors.

estimated with fuzzy logic was obtained with an organic load between 15,000 and 18,000 mg/L (see Fig. 6), an acidification pH between 4.4 and 4.6, an acidification time between 5 and 7 days and an operational pH of 5.5. Various maximum values can be seen, but with a unique global maximum, corresponding to the estimated value. The fuzzy logic model, as with the maximum hydrogen content estimation, responded suitably to the dynamic and non-linear behavior of the bioprocess [28,29].

A summary of the results obtained for the maximum hydrogen production for the three methods is presented in Table 5. Similarly to the maximum content, the maximum value obtained with fuzzy logic was close to that of the genetic algorithm estimation, requiring a substrate with a slightly higher organic load but a much lower acidification time, changing from 25 days to 5 days. This creates an advantageous situation for the fuzzy logic model because it is desirable to start hydrogen production as soon as possible. In addition, the fuzzy logic model gave values of each factor for the hydrogen content very similar to those values obtained for production. This means that a gas with high hydrogen content and high production could be produced simultaneously; a desired situation for use in fuel cell, internal combustion engine and turbine [30-32].

4. Conclusions

The production of hydrogen was possible with the use of 2,000-liter bioreactors, without agitation, without adding inoculum cells, at ambient temperature (mesophilic, 25°C) and using a substrate of urban waste (fruit and vegetable waste). The results obtained with the four independent variables and the two dependent variables allowed for the construction of two models, one for each of the dependent variables. The results of these variables presented good fit to polynomial models of degree two. A posterior optimization of both models involved the use of three mathematical tools: multiple response surfaces, genetic algorithm, and fuzzy logic; the values obtained after optimization for both the hydrogen content and the hydrogen production were higher than the values reached in experimental tests. The fuzzy logic produced results more in accordance with the expectations; this tool allowed to increase hydrogen content 3.3 times and hydrogen production 1.7 times.

The values of independent variables should be between 18,000 and 20,000mg/L for the organic load; 4.5 for acidification pH; 5 days as the acidification time; and between 5.5 and 5.8 for operational pH. These values were found at levels reported by various authors in similar conditions of the bioprocess (waste and operation mode of reactor) [33,34].

The genetic algorithm model presented estimations that were very similar to those produced by the fuzzy logic model; however, the estimated values for the optimization of the hydrogen content and the production of the gas are associated with an acidification time, notably higher than the values obtained by the fuzzy logic model: 3.6 times higher. This represents a huge limitation because the hydrogen production would be delayed without any additional increase in hydrogen content. In the case of optimization with the multiple response surfaces, the obtained results were not in accordance with the experiment tests.

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