

Evaluation and simultaneous optimization of bio-hydrogen production using 3^2 factorial design and the desirability function[☆]

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Available online 30 January 2007

Abstract

Various mixtures incorporating a simulated organic fraction of municipal solid wastes and blood from a poultry slaughterhouse were used as substrate in a dark fermentation process for the production of hydrogen. The individual and interactive effects of hydraulic retention time (HRT), solid content in the feed (%TS) and proportion of residues (%Blood) on bio-hydrogen production were studied in this work. A central composite design and response surface methodology were employed to determine the optimum conditions for the hydrogen production process. Experimental results were approximated to a second-order model with the principal effects of the three factors considered being statistically significant ($P < 0.05$). The production of hydrogen obtained from the experimental point at conditions close to best operability was $0.97 \text{ L Lr}^{-1} \text{ day}^{-1}$. Moreover, a desirability function was employed in order to optimize the process when a second, methanogenic, phase is coupled with it. In this last case, the optimum conditions lead to a reduction in the production of hydrogen when the optimization process involves the maximization of intermediary products.

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Keywords: Dark fermentation; Hydrogen; Waste treatment; Factorial design; Optimization; Desirability function

1. Introduction

Hydrogen has a higher gravimetric energy density than any other known fuel and is compatible with electrochemical and combustion processes for energy conversion without producing the carbon-based emissions that contribute to environmental pollution and climate change [1]. There are several methods of producing this clean fuel. Among them, biological techniques are a promising option. When combined with the treatment of wastes, they are able to solve two problems: the reduction of pollution from uncontrolled degradation of waste and the generation of a clean alternative fuel [2].

Hydrogen production through dark fermentation has advantages over other processes because of its ability to produce hydrogen continuously from a number of renewable feed-stocks

without any input of external energy [3]. The major criteria for the selection of waste materials to be used in bio-hydrogen production are the availability, cost, carbohydrate content and biodegradability. Major waste materials, which can be used for hydrogen gas production, can be agricultural and food industry wastes, carbohydrate-rich industrial wastewaters and waste sludge from wastewater treatment plants [4].

Among agricultural wastes used as substrate for the production of hydrogen are wheat straw [5] and rice slurry [6]. Successful results have also been obtained from the use of food-processing wastewater [7–9] and the organic fraction of municipal solid wastes [2,10–15] as substrates, under mesophilic and thermophilic conditions. Furthermore, waste sludge has proved to be a suitable substrate for obtaining hydrogen by the use of dark fermentation [11,16]. A complete treatment of the waste is obtained by the coupling of a methanogenic phase in a two-phase configuration for the simultaneous production of hydrogen and methane allowing the stabilization of the biosolid [2,15,17].

Some of the major drawbacks of the dark fermentation process are the need for pre-treatment to obtain an H_2 -producing inoculum, and the continuous addition of alkalinity to maintain pH in the desired range, since a low pH can inhibit hydrogen-producing microorganisms. The pH value should be above 4.0,

Abbreviations: $\sum \text{Ac}$, acids concentration; Alk, volume of alkaline added; COD, chemical oxygen demand; HRT, hydraulic retention time; NH_4^+ , ammonium concentration; PH_2 , hydrogen production; RSM, response surface methodology; TS, total solids; VFA, volatile fatty acids; VS, volatile solids

[☆] This paper presented at the 2nd National Congress on Fuel Cells, CONAP-PICE 2006.

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with an optimum between 4.7 and 5.7 [18]. The production of hydrogen through continuous fermentation utilizing a non-sterile substrate with readily available mixed microflora would be commercially desirable [19]. Sewage sludge, compost and soil have been used to provide seed cultures for hydrogen-producing microflora [20–26], with the application of different pre-treatments, such as heat shock [6,8,26–28] and acidification [7,28,29], to harvest spore-forming anaerobic bacteria. However, rather than using batch studies and pure cultures, continuous operation with enriched mixed microflora maximizes reactor productivity, requires no energy for sterilization and can use technology adapted from the anaerobic digestion processes already well established on an industrial scale [30].

The aim of the experimental work being reported here was the production of hydrogen by biological means. The co-fermentation of food waste with residual blood from a poultry slaughterhouse was studied using mixed microflora obtained from an active mesophilic digestion system. Response surface methodology and a desirability function were applied to set the optimum operating conditions for maximum hydrogen production and maximum acidification when the process is coupled to a second methanogenic phase.

2. Empirical models: factorial design and the desirability function

There are many biological areas, such as acidogenic microbial processes, where basic knowledge of the phenomenon is insufficient to build a mechanistic model. In this case, empirical models and statistical analysis play an extremely important role in elucidating basic mechanisms in complex situations and thus providing better process control [9]. Response surface methodology (RSM) is a collection of statistical techniques useful for designing and characterizing the relationship between a response and a set of factors or variables of interest to the researcher and determining the optimum conditions for the desired response. The data obtained from an experiment are used to draw inferences about the process under investigation [31].

Since the relation between the response (y) and the independent variables (x_i) is unknown, the first approximation step is to fit a low-order polynomial to the response. If the response is non-linear, then a higher-order polynomial approximation will be tried. Once a second-order polynomial has been fitted to the response, it is necessary to determine whether a maximum, a minimum or a saddle point has been found in the experimental region selected. This is achieved by calculating the eigenvalues of the matrix formed by second-order coefficient of the quadratic model. If all the eigenvalues are positive, then a minimum point has been found. In contrast, when all the eigenvalues are negative, a maximum point has been found and the optimization procedure for the selected response now depends on determining this stationary point. The maximum is found by solving the equation system obtained from the partial derivative of the response with respect to each independent variable.

The equation for the second-order model for a three-factorial design can be written as:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \quad (1)$$

This second order approximation can be written in a matrix form as:

$$\hat{Y} = \beta_0 + x'b + x'Bx \quad (2)$$

where vector b is formed by the coefficients of the linear part (principal effects) and matrix B is formed by the coefficients corresponding to interactions and pure quadratic terms [32].

The maximum is calculated by solving the system given by:

$$\frac{\partial \hat{Y}}{\partial x} = b + 2Bx = 0 \quad (3)$$

The stationary point is given by:

$$x_s = -\left(\frac{1}{2}\right) B^{-1} b \quad (4)$$

The signs of the eigenvalues (λ_i) of matrix B will determine whether the stationary point is a maximum, a minimum or a saddle point.

When a saddle point has been found, and due to operational constraints translation of the experimental region is not possible, optimization technique is now devoted to determining the point of best operability. This point is calculated by a ridge analysis, which means a search for the best point on a given radius R from the centre of the design. Using Lagrange analysis, the stationary point is given by [33]:

$$(B - \mu I)x = -\frac{b}{2} \quad (5)$$

Where I is the identity matrix and μ is the Lagrange multiplier. An iterative way of solving this equation is to assume a value for μ greater than that of the maximum value obtained from the eigenvalues of matrix B . The new point is calculated, and then the radius R from this point to the centre of the three-factorial experimental design is worked out from the equation:

$$Ri = (x_1^2 + x_2^2 + x_3^2)^{1/2} \quad (6)$$

This procedure is repeated until the best point sited within the experimental region is found. This point is usually located on the sphere of maximum radius inside the experimental region [33].

Finally, a desirability function was used for optimizing the process. The application of a desirability function combines all the responses into one measurement [34] and gives the possibility of predicting the optimum levels for the independent variable. The combination of the responses into one desirability function requires the calculation of each individual desirability function [35]. Responses were maximized as follows:

$$d_i = \frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}} \quad (7)$$

And minimized as follows:

$$d_i = \frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}} \quad (8)$$

The overall desirability values were calculated from the individual values by using the following equation for n responses:

$$D = (d_1 \times d_2 \cdots d_n)^{1/n} \quad (9)$$

In this study a 3^2 central composite design was used to determine the effect of the solid concentration of the substrate, the proportion of waste blood in the feed and the hydraulic retention over the responses selected.

3. Materials and methods

3.1. Experimental materials and microbial culture

The experimental set-up was based on previous work [2]. The seed inoculum was obtained from a three-litre laboratory-scale reactor digesting slaughterhouse waste from a poultry-processing plant. This system had been working under mesophilic conditions (34 °C) for 6 months with a hydraulic retention time (HRT) of 36 d and an average methane content in the biogas produced of 65%. The total solid and volatile solid concentrations of the inoculum were 19.52 ± 0.47 and $10.92 \pm 0.33 \text{ g L}^{-1}$, respectively (from three replicates). No pre-treatment was applied to the seed sludge since this was considered impractical for large-scale applications.

The food waste was simulated by using the components described in Gómez et al. [2]. The total solid and volatile solid concentrations of the food waste thus prepared were 120.40 ± 0.32 and $110.77 \pm 0.25 \text{ g L}^{-1}$, respectively (from three replicates). The blood waste was obtained from a poultry-processing plant and was sterilized at 70 °C for 60 min, as required by Regulation (EC) no. 1774/2002 [36], before use in digestion systems. The total solid and volatile solid concentrations of the treated blood were 101.31 ± 0.22 and $92.20 \pm 0.18 \text{ g L}^{-1}$, respectively (from three replicates). The mixtures for co-fermentation were prepared according to the values set in the experimental design for the variables termed percentage of blood (%Blood) and total solid concentration (%TS) in the feed.

The factors selected to evaluate the behaviour of the responses were the percentage of blood in the feed (x_1), the total solid concentration in the feed (x_2) and the HRT (x_3). Table 1 presents the levels selected for each factor and Fig. 1 represents the experimental design boundary. The different formulations of the factorial design consisted of all possible combinations of all factors at all levels and these were used in a fully randomized order.

The reactors used for the hydrogen-production process were Erlenmeyer flasks with a working volume of 100 mL. The reactors were kept submerged in a water bath at 34 °C and were provided with magnetic stirrers. The oxidation reduction potential (ORP) was set at $-250 \pm 20 \text{ mV}$. This potential was achieved by allowing the reactors to have contact with the atmosphere while stirring, until the required value was attained. The pH level

Table 1
Factorial 3^2 : factors and their levels

| Run | x_1 :%Blood | x_2 : %TS | x_3 : HRT |
|-----|---------------|-------------|-------------|
| 1 | 7 | 4 | 1.5 |
| 2 | 7 | 4 | 3 |
| 3 | 7 | 8 | 1.5 |
| 4 | 7 | 8 | 3 |
| 5 | 27 | 4 | 1.5 |
| 6 | 27 | 4 | 3 |
| 7 | 27 | 8 | 1.5 |
| 8 | 27 | 8 | 3 |
| 9 | 17 | 6 | 2.25 |
| 10 | 17 | 6 | 3.5 |
| 11 | 17 | 6 | 1 |
| 12 | 17 | 9 | 2.25 |
| 13 | 17 | 3 | 2.25 |
| 14 | 0 | 6 | 2.25 |
| 15 | 34 | 6 | 2.25 |

was controlled manually, being kept in the range 5.0–6.0 by the addition of an alkaline solution during the feeding process and by initializing the pH at 6.0. The alkaline solution was prepared by mixing in a proportion of 1:1:1 (weight) NaHCO_3 , K_2HPO_4 and Na_2HPO_4 . The solution thus prepared had a total solid concentration of 60 g L^{-1} . The reactors were run on semi-continuous basis for 20 d, being fed twice a day.

3.2. Analytical techniques

The ultimate and proximate analyses for substrates were carried out on dried samples according to standard ASTM procedures. Total nitrogen was determined by the Kjeldahl method [37]. Organic matter was determined in accordance with the Walkey–Black method [37]. The organic carbon content was calculated from the organic matter value, using a correlation factor of 1.72.

During the fermentation process the following parameters were monitored: pH, total solids (TS), volatile solids (VS), ammonium concentration, chemical oxygen demand (COD), daily gas production, gas composition and the concentration

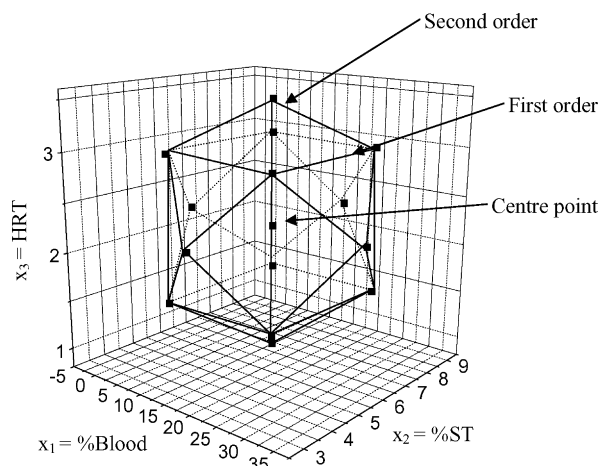


Fig. 1. Experimental design boundary of orthogonal design for quadratic models.

of volatile fatty acids (VFA). Ammonium concentration, TS, VS and pH were determined in accordance with standard methods [38]. The values for TS concentration in the reactors were corrected to eliminate the effect of solids added to control pH. Daily gas production was measured using a reversible liquid displacement device with a wet-tip counter. All gas production data reported were normalized to a standard temperature (0 °C) and pressure (760 mm of Hg). COD of the supernatant was measured using a Photometer multi-parameter Series C99 from Hanna Instruments after centrifugation of the sample at $3500 \times g$ and digestion in the presence of dichromate at 150 °C for 2 h using a Hanna C9800 reactor.

Biogas composition was analyzed using a gas chromatograph (Varian CP 3800 GC) equipped with a thermal conductivity detector. A 4-m-long column packed with HayeSep Q 80/100 followed by a 1-m-long molecular sieve column was used to separate CH₄, CO₂, N₂, H₂ and O₂. The carrier gas was argon and the columns were operated at 331 kPa and a temperature of 50 °C. Volatile fatty acids were analyzed using a gas chromatograph (Varian CP 3800 GC) equipped with a capillary column (from Supelco) and a flame ionization detector. The carrier gas was helium and the temperature of the injector was 250 °C. The temperature of the oven was set at 150 °C for 3 min and thereafter increased to 180 °C.

Multiple regression analysis for the data sets collected was performed using Origin 6.1 software. The optimization process and ridge analysis were carried out using Matlab R12.

4. Results

The chemical characterization of the substrates used as feed in the fermentation study is presented in Table 2. All fermentation systems were initially fed with food waste to accelerate acidification of the inoculum. By day 3 the pH reached a value of 5.2 and from that day on, the systems were fed with the relevant substrates. Table 3 presents a matrix for the experiments and the results obtained for the responses in the hydrogen production process: the volume of alkaline (Alk) solution added

Table 2

Chemical characterization of the substrates used in this study

| (%) ^a | Food waste | Blood waste |
|------------------|------------|-------------|
| Volatiles | 80.2 | 76.5 |
| Ash | 2.5 | 6.4 |
| C | 45.1 | 49.5 |
| H | 6.4 | 6.5 |
| N | 1.4 | 14.6 |
| S | 0.2 | 1.06 |
| O | 46.9 | 28.3 |
| Organic carbon | 42.7 | 32.3 |
| Organic matter | 73.41 | 55.6 |
| Total nitrogen | 1.33 | 11.5 |
| C/N | 32.1 | 2.81 |

^a The percentages are based on dry weight.

for pH regulation, the total concentrations of acetic, propionic, butyric and caproic acids ($\sum \text{Ac}$), the ammonium concentration (NH₄⁺) and the chemical oxygen demand of the supernatant (CODs).

From this point onwards in the text, when reference is made to the fermentation systems, they will be denoted by the corresponding levels of variables $x_1/x_2/x_3$. Table 4 presents the methane concentration in biogas for the last day of experimentation which corresponds to the highest values recorded for the low solid concentration systems ($x_2 = 3\%$ and $x_2 = 4\%$). It also presents the average solid concentration in the reactors together with the concentration of individual acids. Systems which were incapable of reaching stable operation were assigned a value of zero for hydrogen production in the response surface analysis.

The response for hydrogen production approximated to a second-order polynomial, the results being shown in Table 5. Fig. 2 gives a three-dimensional graph of the quadratic model. The residual plots for the model and data sets showing no patterns or trends are presented in Fig. 3.

The response surface is characterized by the sign of the eigenvalues obtained using factor codification. The eigenvalues found for the response were $\lambda_1 = -18.8$, $\lambda_2 = 8.0$, $\lambda_3 = 5.0$.

Table 3

Factorial 3²: matrix of the experiments and results for the measured responses and composite desirability function

| Run | H ₂ (%) | PH ₂ (mL d ⁻¹) | Alk (mL d ⁻¹) | $\sum \text{Ac}$ (mg L ⁻¹) | NH ₄ ⁺ (mg L ⁻¹) | CODs (mg L ⁻¹) | <i>D</i> |
|-----|--------------------|---------------------------------------|---------------------------|--|--|----------------------------|----------|
| 1 | 26 | 56.8 | 5.2 | 9769 | 131 | 47160 | 0.000 |
| 2 | 23.5 | 28.7 | 3.0 | 12072 | 272 | 39315 | 0.310 |
| 3 | 15.7 | 54.3 | 7.0 | 10937 | 255 | 58875 | 0.422 |
| 4 | 4 | 7.4 | 2.9 | 15967 | 341 | 72880 | 0.396 |
| 5 | 25 | 48.7 | 3.3 | 10371 | 252 | 35910 | 0.281 |
| 6 | 0 | 0 | 2.3 | 10188 | 461 | 48120 | 0.000 |
| 7 | 24.7 | 84.3 | 4.1 | 14038 | 532 | 53508 | 0.700 |
| 8 | 23.4 | 39.2 | 2.0 | 14292 | 605 | 61060 | 0.648 |
| 9 | 23.6 | 45.5 | 2.7 | 12610 | 361 | 44460 | 0.442 |
| 10 | 24.7 | 33 | 2.0 | 12126 | 407 | 56145 | 0.476 |
| 11 | 24 | 97 | 7.9 | 7687 | 349 | 50745 | 0.317 |
| 12 | 4.8 | 16.7 | 4.6 | 14056 | 634 | 71925 | 0.565 |
| 13 | 0 | 0 | 2.6 | 7169 | 257 | 27660 | 0.000 |
| 14 | 22 | 67 | 4.6 | 14331 | 226 | 55500 | 0.474 |
| 15 | 27.2 | 58 | 2.7 | 9523 | 780 | 52560 | 0.545 |

Table 4
Methane content in biogas (on day 20 of the experimentation), acid concentration and solid content for bio-hydrogen producing systems

| Run | CH ₄ (%) | Propionic acid (mg L ⁻¹) | Acetic acid (mg L ⁻¹) | Butyric acid (mg L ⁻¹) | Caproic acid (mg L ⁻¹) | TS reactor (g L ⁻¹) | VS reactor (g L ⁻¹) | VS removal (%) |
|-----|---------------------|--------------------------------------|-----------------------------------|------------------------------------|------------------------------------|---------------------------------|---------------------------------|----------------|
| 1 | 0.22 | 1317 | 3866 | 4248 | 302 | 30.3 | 20.7 | 43.5 |
| 2 | 3.5 | 375 | 5634 | 2677 | 3386 | 34.4 | 20.0 | 45.4 |
| 3 | 0 | 173 | 8132 | 1947 | 685 | 54.8 | 30.1 | 58.9 |
| 4 | 0 | 211 | 8080 | 2927 | 4749 | 45.5 | 30.4 | 58.5 |
| 5 | 3.4 | 224 | 6047 | 2087 | 1986 | 30.5 | 21.3 | 41.2 |
| 6 | 24.3 | 367 | 5672 | 2564 | 1585 | 30.1 | 19.9 | 45.0 |
| 7 | 0 | 132 | 7683 | 2160 | 4063 | 52.9 | 35.5 | 51.0 |
| 8 | 3.6 | 318 | 6362 | 3916 | 3697 | 46.7 | 31.2 | 56.9 |
| 9 | 0.06 | 1029 | 6547 | 3007 | 1999 | 37.5 | 22 | 59.7 |
| 10 | 6.3 | 1291 | 5742 | 2638 | 2455 | 31.3 | 18.9 | 65.4 |
| 11 | 0.19 | 55 | 6318 | 1315 | 0 | 46.5 | 30.3 | 44.5 |
| 12 | 2.2 | 159 | 6329 | 4610 | 2957 | 64.1 | 43.2 | 47.3 |
| 13 | 38.2 | 661 | 3372 | 2498 | 638 | 25.3 | 12.8 | 24.3 |
| 14 | 0 | 111 | 7704 | 3708 | 2808 | 45.9 | 24.7 | 55.3 |
| 15 | 2.2 | 175 | 4446 | 1839 | 3063 | 44.2 | 28.3 | 47.7 |

Table 5
ANOVA results (*P*-values): effect of the variables on PH₂

| Factors | Coefficient | <i>P</i> |
|---|-------------|----------------------|
| Constant | 37.88 | – |
| <i>x</i> ₁ | –4.70 | 0.01342 ^a |
| <i>x</i> ₂ | 47.58 | 0.00455 ^a |
| <i>x</i> ₃ | –65.28 | 0.0339 ^a |
| <i>x</i> ₁ <i>x</i> ₂ | 0.616 | 0.00265 ^a |
| <i>x</i> ₂ <i>x</i> ₃ | –0.313 | 0.340 |
| <i>x</i> ₁ <i>x</i> ₃ | –1.27 | 0.433 |
| <i>x</i> ₁ ² | 0.0529 | 0.0794 |
| <i>x</i> ₂ ² | –4.35 | 0.00207 ^a |
| <i>x</i> ₃ ² | 11.36 | 0.0504 ^a |
| <i>R</i> ² | 0.971 | |
| <i>R</i> ² adj | 0.919 | |

Regression coefficients.

^a Statistically significant (*P* < 0.05).

The differing signs are indicative of a saddle point. Application of ridge analysis allowed determination of the point of best operability, giving as result $\mu = 12.8$ and $x_1 = 22$, $x_2 = 6.5$, $x_3 = 1$ with a predicted value of 104 mL d⁻¹. The response predicted by the model was similar to that obtained from experimental point $x_1 = 17$, $x_2 = 6$, $x_3 = 1$ with a production of hydrogen of 97 mL d⁻¹.

In order to obtain the best operating conditions for the hydrogen producing phase when the system was coupled to a two-phase configuration, the following responses were selected: \sum Ac, CODs and NH₄⁺. Table 6 presents the results from the polynomial approximation for responses Alk, CODs and NH₄⁺. The response \sum Ac could not be adjusted to a second-order polynomial ($R^2 = 0.817$, R^2 adj = 0.487, $P = 0.165$). From the desirability function (d_i) defined for each response, a single composite response was calculated. The composite desirability function (D) was calculated by simultaneous maximization of responses PH₂ (d_1), CODs (d_2), NH₄⁺ (d_3) and \sum Ac (d_4) (see Table 3). The equation found to describe the influence of the

Table 6
ANOVA results (*P*-values): effect of the variables on Alk, NH₄⁺ and CODs

| Factors | Alk | | NH ₄ ⁺ | | CODs | |
|---|-------------|----------------------|------------------------------|--------------------|-------------|--------------------|
| | Coefficient | <i>P</i> | Coefficient | <i>P</i> | Coefficient | <i>P</i> |
| Constant | 12.18 | – | –549.69 | – | 74365.52 | – |
| <i>x</i> ₁ | –0.19 | 0.019 ^a | –3.61 | 0.66 | –1108.27 | 0.16 |
| <i>x</i> ₂ | 0.64 | 0.31 | 84.70 | 0.21 | –1230.32 | 0.84 |
| <i>x</i> ₃ | –6.57 | <0.0001 ^a | 369.50 | 0.020 ^a | –32267.42 | 0.023 ^a |
| <i>x</i> ₁ <i>x</i> ₂ | –0.0070 | 0.30 | 1.45 | 0.045 ^a | –78.71 | 0.24 |
| <i>x</i> ₂ <i>x</i> ₃ | 0.049 | 0.0073 ^a | 0.95 | 0.61 | 216.52 | 0.22 |
| <i>x</i> ₁ <i>x</i> ₃ | –0.28 | 0.0027 ^a | –16.00 | 0.097 | 1541.77 | 0.085 |
| <i>x</i> ₁ ² | 0.0015 | 0.33 | 0.16 | 0.35 | 26.75 | 0.070 |
| <i>x</i> ₂ ² | 0.027 | 0.58 | –2.33 | 0.66 | 415.47 | 0.36 |
| <i>x</i> ₃ ² | 1.21 | <0.0001 ^a | –50.21 | 0.12 | 5079.74 | 0.065 |
| <i>R</i> ² | 0.745 | | 0.835 | | 0.762 | |
| <i>R</i> ² adj | 0.726 | | 0.810 | | 0.719 | |
| <i>P</i> | <0.0001 | | <0.0001 | | <0.0001 | |

Regression coefficients.

^a Statistically significant (*P* < 0.05).

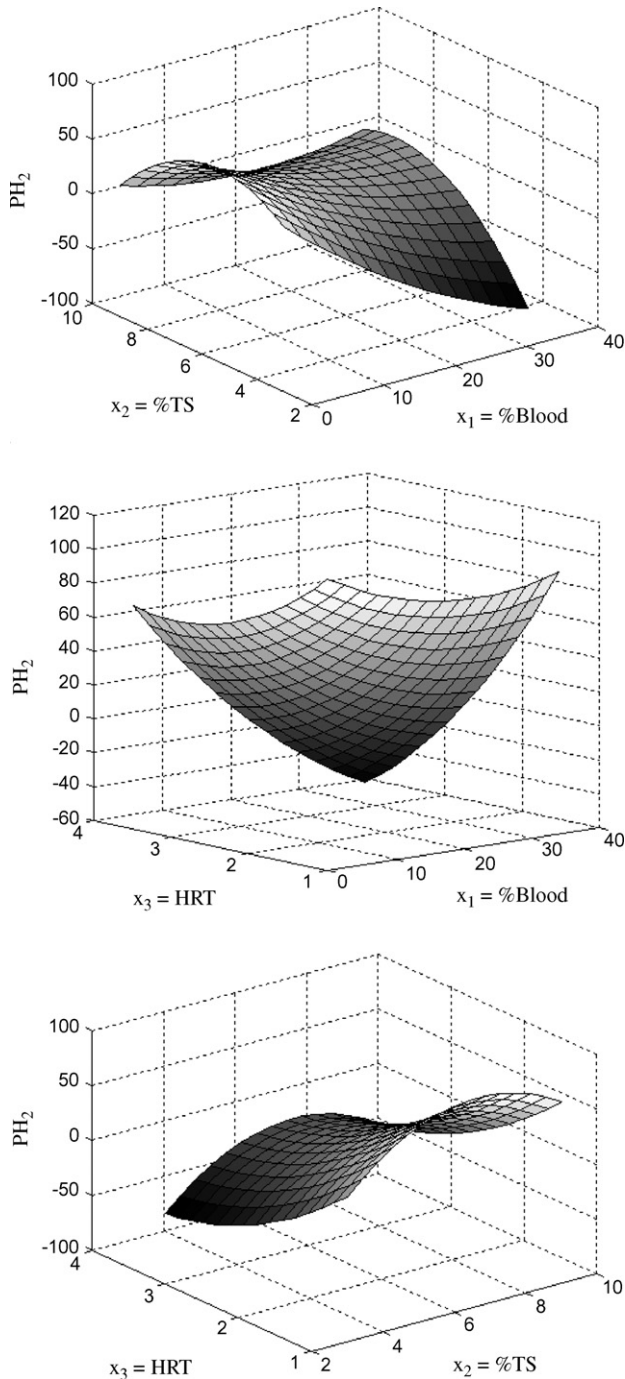


Fig. 2. Three-dimensional graphs of the quadratic model for the production of hydrogen within the orthogonal design.

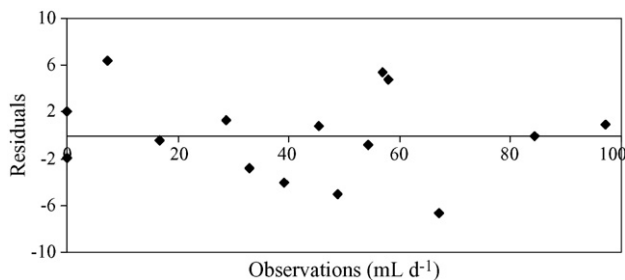


Fig. 3. Residual plots of quadratic model for hydrogen production.

Table 7

Predicted and experimental values at optimum point conditions set by the desirability function

| Response | Predicted values | Experimental values | Bias% ^a |
|--|------------------|---------------------|--------------------|
| PH ₂ (mL d ⁻¹) | 23 | 24.5 | 6.5 |
| NH ₄ ⁺ (mg L ⁻¹) | 372 | 366 | 1.6 |
| CODs (mg L ⁻¹) | 64146 | 50670 | 21 |
| ∑Ac (mg L ⁻¹) | – | 12664 | – |
| Alk (mL d ⁻¹) | 2.64 | 3.03 | 14.8 |

^a Bias was calculated using the equation: [(predicted value – experimental value)/predicted value] × 100.

various factors on overall desirability was:

$$\begin{aligned}
 D = & -1.39 + 0.0022x_1 + 0.32x_2 + 0.47x_3 + 0.0035x_1x_2 \\
 & -0.010x_2x_3 - 0.0089x_1x_3 + 0.00013x_1^2 - 0.022x_2^2 \\
 & -0.48x_3^2 \quad (R^2 = 0.918, R^2_{\text{adj}} = 0.77, P = 0.029)
 \end{aligned}
 \tag{10}$$

The adjusted polynomial allowed determination of the optimum point $x_1 = 10.3$, $x_2 = 7.5$, $x_3 = 3$. Fig. 4 presents a three-dimensional graph of the desirability function D . The adequacy of the predicted response was examined by an additional independent experiment at the suggested optimum point. The predicted value for hydrogen production calculated from the adjusted second-order polynomial was 23 mL d⁻¹; this value was close to the experimental mean (24.5 ± 5.41 mL d⁻¹) obtained from the reactor operating at optimum conditions. Table 7 presents the predicted values from the second-order models and the experimental values obtained from the reactor operating at the optimum conditions set by the desirability function.

5. Discussion

From Table 4 it may be observed that the methane content of the biogas produced did not follow any trend but an increasing pattern was seen in reactors fed with a low solid concentration feed. For systems with a solid concentration greater than 6%, the methane content remained very stable during the whole experimentation period and the values presented in Table 4 are very close to the mean values. The high methane concentration observed for Systems 27/4/3 (24.3%) and 17/3/2.25 (38.2%) is striking. The first of these presented a maximum hydrogen content of 31% on day 6 of experimentation. However, the quality of biogas gradually decreased until no hydrogen was detected. A similar trend was observed for the second system, but in this case with a lower value recorded on the day of maximum hydrogen production, this value being 14.8% and also corresponding to day 6 of experimentation. In relation to removal of VS, the greatest reduction was obtained from system 17/6/3.5 with a value of 65.4% (Table 4). Its counterpart with the lowest value for VS removal (24.3%) was system 17/3/2.25, which also showed the lowest TS content and no production of hydrogen.

In relation to acid generation, the most striking aspect was the great accumulation of propionic acid, this not being related

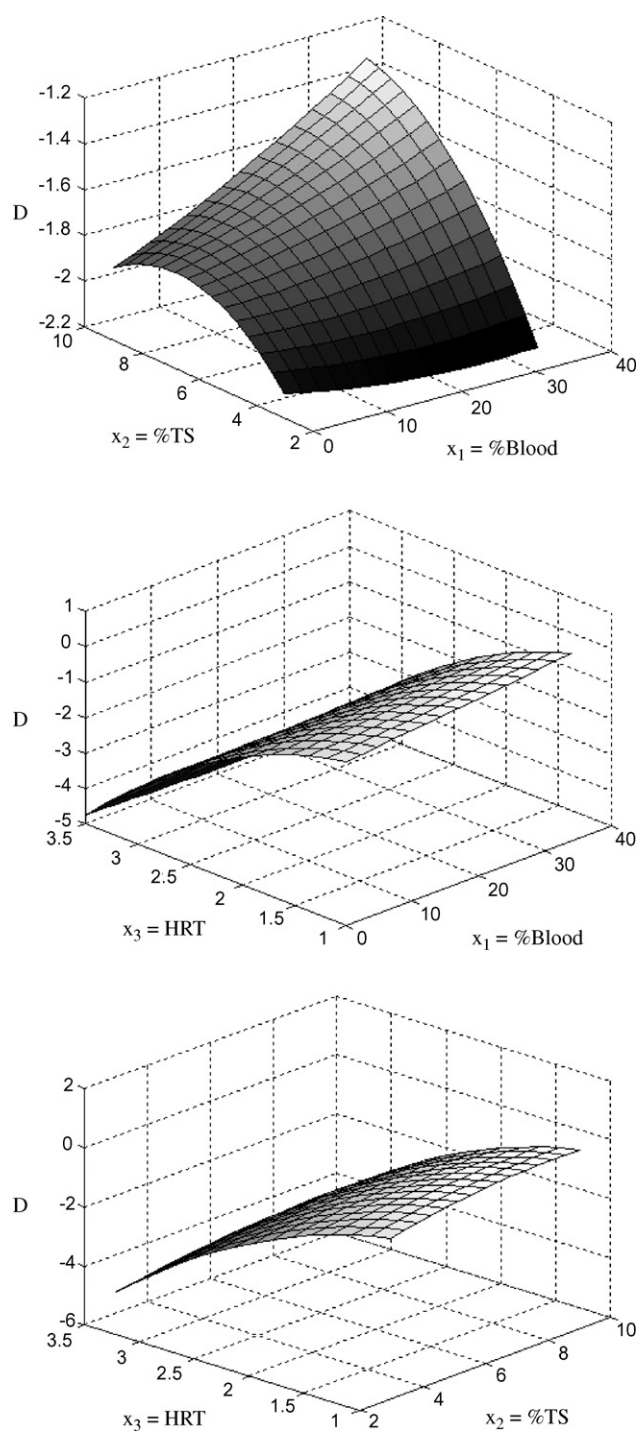


Fig. 4. Response surface plot of the composite desirability (D) when PH_2 , CODs, NH_4^+ , $\sum \text{Ac}$ are maximized simultaneously.

to the hydrogen yield obtained from the systems. System 7/4/1.5 with a short HRT (1.5 d) presented a particularly high concentration of propionic acid, with a high average hydrogen production (56.8 mL d^{-1}), this value being much greater than that reported by its counterpart with a longer HRT (system 7/4/3 with an HRT of 3 d and a hydrogen production of 28.8 mL d^{-1}). The other two systems presenting a high propionic acid concentration were 17/6/2.25 and 17/6/3.5 with a value of 1029 and 1291 mg L^{-1} ,

respectively. Despite such results, these systems were capable of attaining a stable hydrogen production (45.5 and 33 mL d^{-1}). Similar results were also reported by Youn and Shin [14] studying the fermentation of food wastes, they observed high levels of propionic acid under mesophilic conditions. Wang et al. [27] studying propionic acid accumulation in bio-hydrogen fermentation systems, concluded that bio-hydrogen generation rates are not always linked to the accumulation of propionic acid, correlating propionic acid production to facultative micro-organisms and shock-loading circumstances.

The highest levels of acetic acid concentration were recorded for systems 7/8/1.5 and 7/8/3. Both systems showed nil production of methane and low propionic acid build-ups. However, hydrogen production was quite different, as system 7/8/1.5 showed production of 54.3 mL d^{-1} , while 7/8/3 presented a much lower value (7.4 mL d^{-1}). For all the systems studied, acetic acid was the major soluble product, with the exception of system 7/4/1.5, in which the major product was butyric acid. With respect to butyric acid levels, system 17/6/1 had the lowest concentration; this system also had the lowest level of caproic acid.

Caproic acid concentration was the only acid response that could be approximated to a second-order polynomial ($R^2 = 0.96$, $R^2_{\text{adj}} = 0.887$, $P = 0.0055$). From the model, the principal factor having a significant effect over the response was seen to be the interaction between HRT and the percentage of blood in the feed. Increases in the percentage of blood and in HRT led to greater concentrations of caproic acid in the reactors.

5.1. Optimizing hydrogen production

The response for hydrogen production approximated to a second-order polynomial. From Table 5 it may be concluded that the principal effects of the three factors were statistically significant ($P < 0.05$) for mean hydrogen production. The ANOVA results also showed that the interaction x_1x_2 had a significant influence on hydrogen production. An interaction is the failure of a factor to produce the same effect on the response at the different levels of the other factor [39].

From the results obtained it can be inferred that there is a considerable increase in the production of hydrogen when HRT is reduced. Increases in the solid content in the feed yielded a higher production of hydrogen until an inflection point was found, whereupon further increments in the TS content led to reductions in the response. With respect to the variable percentage of blood in the feed, its effect was also related to the solid content variable. With low concentrations of solids in the feed, increases in the blood content led to lower hydrogen production and in consequence a higher methane content in the biogas generated (systems 27/4/3 and 17/3/2.25). However, with higher concentrations of solids the effect was less marked.

Kim et al. [11], using RSM, found an optimum point for batch fermentation with a VS concentration of 3.0% for a waste composition of 87:13 (food waste to sewage sludge). Increasing the VS content to 5.0% led to a reduction in hydrogen production. Shin et al. [12] tested hydrogen production from food waste under batch conditions for mesophilic and thermophilic

systems, reporting an increase in hydrogen production when there were increases in the substrate VS concentration from 3 to 10 g VS L⁻¹. Similar results were reported by Youn and Shin [14] for continuously operated fermentation of food waste under thermophilic and mesophilic conditions. Chen et al. [3] testing a variety of substrates (sucrose, non-fat dried milk and food waste) and working under batch operation at mesophilic temperatures, found an increase in the hydrogen production rate on increasing the substrate concentration. On the other hand, Van Ginkel and Logan [24] reported a reduction in the hydrogen yield with increased organic loadings when using glucose as substrate under mesophilic conditions. However, they also reported an increase of biomass concentration in the reactor with the increment in organic loading.

5.2. Desirability function

The coupling of a methanogenic phase for the treatment of the effluent requires from the hydrogen-producing phase conversion of the solid substrate into soluble material. High proportions of organic acids enhance volume reduction of wastes and methane production in a following methanogenic phase [40]. Results presented in Table 6 show the second-order model obtained for the responses Alk, NH₄⁺ and CODs, indicating the HRT as significant factor. Although Alk was not included in the desirability function, it is relevant in relation to the operational costs of the process. Alkali consumption is linked to the percentage of blood in the feed. In this way, increases in the blood content reduce the volume of alkaline solution needed for pH regulation. Although the interaction between the solid content in the feed (x_2) and HRT (x_3) is shown as significant in respect of the Alk response, this effect is due to the influence of HRT, since variable x_2 is not significant as principal factor.

It is noteworthy what differences are found in the optimum point in a two-phase process when the aim is the optimization of hydrogen production as opposed to the generation of intermediary products. With regard to the latter, setting the optimum conditions in the first acid phase leads to a reduction in the production of hydrogen of 75%, based on the results yielded by the desirability function. Differences in the optimum conditions for maximum hydrogen production and for fermentation efficiency have also been reported in previous research [11,18,41].

6. Conclusions

Stable production of hydrogen was obtained from an active mesophilic inoculum when no pre-treatment was applied for the generation of hydrogen microflora. Response surface methodology (RSM) and central composite design were successfully applied to optimize the process when using as substrates mixtures involving the organic fraction of municipal solid wastes together with blood from a poultry slaughterhouse. Experimental results showed that hydraulic retention time, solid content and the percentage of blood in the feed all had individual significant influences on the production of hydrogen.

Multiple regression analysis of the results led to equations that adequately described the influence of the selected variables

on the responses under study. Optimization of the process using a desirability function when the dark fermentation phase was to be coupled with a second methanogenic phase resulted in a reduction in the production of hydrogen when the generation of intermediary products was maximized. Hydrogen production fell from 0.97 L Lr⁻¹ day⁻¹ when the optimum point was based on hydrogen maximization, to 0.25 L Lr⁻¹ day⁻¹ when maximization of soluble products was also considered.

Acknowledgement

The authors wish to thank the ENDESA corporation for giving them the NOVARE award.

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