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Modeling and Parameter Estimation in Biofuel Discontinuous Production by Hydrogen Forming Bacteria (HFB)

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In this work, we study the hydrogen production through a dark fermentation process by hydrogen forming bacteria consortium. In this sense, fermentation experiments are performed in a discontinuous reactor using glucose as the carbon source. Product and substrate profiles are measured in order to study the production of bio hydrogen. A kinetic model for the batch bioreactor that describes the production of all fermentation products, the growth of biomass and the consumption of substrates is developed. The model comprises fifteen differential equations and one algebraic equation. We formulate a parameter estimation problem for the bioreactor model. Fifteen input parameters are estimated taking account the experimental data obtained in the present work. The maximum hydrogen yield obtained was 2.68 mol H_2/mol glucose and the highest hydrogen production rate observed was 1.61 l H_2/ l-day. Numerical results show good agreement between experimental data and simulated profiles.

1. Introduction

Hydrogen emerges as an alternative energy carrier due to its high energy content on a mass basis, and considering that it has environmental benefits with respect to fossil fuels (Laborde and González, 2010). There are many ways to produce hydrogen, such as electrolysis, steam methane reforming and methods based on renewable energy sources as hydrogen production from waste gasification (Ahmed *et al*., 2012). Biological hydrogen production, using microorganisms, has increasing interest in the renewable fuels panorama representing promising processes that offer the possibility to obtain H_2 from a wide variety of low-price renewable feedstock (Ruggeri *et al*., 2009). There are a variety of microorganisms able to produce hydrogen. However, to make the process attractive from a technological and economical point of view, the use of bacterial consortia obtain from natural environments is required (Hawkes *et al*., 2002).
Dark fermentation is one of the biological processes for hydrogen production, in which the fermentation is carried out by hydrogen forming bacteria (HFB) consortia. The fermentation products include hydrogen, carbon dioxide and volatile fatty acids. Studies of bio hydrogen production from consortia of hydrogen forming bacteria are focusing on the kinetics of production of this biofuel from different substrates and on the effect of environmental factors such as pH, temperature, substrate concentration, nutrient availability, among others, that may affect production (Nath *et al*., 2011). However the physiological and physicochemical conditions for the optimal production of bio hydrogen require further investigation. In this sense, mathematical models validated by experimental data are becoming a powerful tool that allows analysis, prediction and improving of biofuels production (Di Maggio *et al.,* 2010).
In this work, we study the production of H_2 by dark fermentation using a bacterial consortium of hydrogen forming bacteria through fermentation experiments, as well as the formulation of a mathematical model that describes the system behaviour. Laboratory tests to produce biological hydrogen are carried out in a batch reactor, using glucose as a substrate. The anaerobic microflora used in this study as inoculum was obtained from a digester of municipal waste water treatment plant. The biomass produced at each

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sampling time is calculated and glucose and fermentation products concentrations are quantified through analytical methods. We propose a nonlinear dynamic model to describe bio hydrogen production and others fermentation products, as well as glucose consumption. We formulate a parameter estimation problem subject to the differential algebraic system, within a dynamic parameter optimization framework in gPROMS (PSEnterprise, 2012). Numerical results show a good fit between model predictions and experimental data during the entire time horizon, for both substrate and products.

2. Materials and methods

2.1 Experimental assays

The dark fermentation is carried out in a stirred batch reactor (Minifors HT, Switzerland, volume: 2L), a bacterial consortium obtained from the sewage sludge of an anaerobic digester of a wastewater treatment plant from Turin (SMAT, Turin, Italy) was used as inoculum. Fermentations conditions have been determined based on previous work in the group (Ruggeri *et al*., 2009, 2010). In the medium, the initial glucose concentration is 61 ± 2 g/L, and the macro and micro nutrients composition (unit mg/L) were: $NaHCO_3$ 1250, NH_4Cl 2500; KH_2PO_4 250; K_2HPO_4 250; $CaCl_2$ 500; $NiSO_4$ 32; $MgSO_4{\cdot}7H_2O$ 320; $FeCl_3$ 20; $Na_2BO_4{\cdot}H_2O$ 7.2; $Na_2MoO_4{\cdot}2H_2O$ 14.4; $CoCl_2{\cdot}6H_20$ 21; $MnCl_2{\cdot}4H_2O$ 30; yeast extract 50, and initial C/N was 30. The experiments were conducted at 35 ±1 °C by triplicate and mean values were used for adjust the model. The anaerobic atmosphere in the reactor is achieved by the injection of nitrogen at the beginning of the fermentation. Culture variables such as, temperature, pH, stirring speed and redox potential were monitored and controlled during the entire time horizon of the experiment. Temperature was kept at 35 ± 0.1 ºC, pH was maintained at 5.2 ± 0.1 by the addition of a NaOH solution and the stirring speed was kept at 100 rpm.

The gas produced during the fermentation was analyzed by gas chromatography (Varian, CP 4900) equipped with thermal conductivity detector (TCD) and two columns of 10 m and argon gas was used as a carrier gas; pH and RedOx potential (ROP) were measured with pH meter (Infors, AG Switzerland) and Pt4805-DXK-S8/120 electrode (Mettler Toledo, Switzerland) respectively. Liquid samples were taken with a daily frequency to analyze the glucose consumption and the production of ethanol, 1-butanol, butyl butyrate, butane-2,3-dione, ethyl butyrate and acetic, propionic, formic, hexanoic, isobutyric and butyric acids. Glucose concentration was quantified by the use of an enzymatic kit (Biopharm-Roche), on the other hand others fermentation products were quantified by gas chromatography (Model 6580, Agilent Inc. USA).

To calculate the biomass concentration a mass balance in carbon element was performed at each sampling time. The elemental biomass composition of the bacterial consortium, $CH_{1.79}O_{0.39}N_{0.24}$, was calculated considering 70 % of the species belonging to *Clostridium* generus and the remaining 30 % to *Escherichia* and *Klebsiella,* among others (Shuler and Kargi, 2001).

2.2 Bioreactor model

In this work, we develop a kinetic model for a discontinuous bioreactor which is able to describe the production of hydrogen and volatile fatty acids as fermentation products through dark fermentation by hydrogen forming bacteria consortium.

Mass balances for biomass, substrates and products can be represented in a general form assuming that the reactor is perfectly mixed and the volume is constant, by Eq. (1)

$$\frac{dC_j}{dt} = r_j \tag{1}$$

where C_j is the concentration of the component j and r_j is the reaction rate of production or consumption of component j.

For the case of biomass the growth rate can be described by Eq. (2) as follows

$$r_x = \mu X \tag{2}$$

where μ is the specific growth rate and X is the biomass concentration. For the hydrogen forming bacteria consortium used in this work the specific growth rate is represented by the Contois equation (Contois, 1959) (Eq. (3)).

$$\mu = \frac{\mu_{max} S}{K_S X + S} \tag{3}$$

where μ_{max} is the maximum growth rate, S is the concentration of limiting substrate and K_s is the half saturation constant. The mass balance for biomass is represented by Eq. (4) considering the depletion of biomass by cell mortality.

$$\frac{dX}{dt} = \mu X - \beta X \tag{4}$$

The reaction rates for product formation and substrate consumption can be related to biomass growth reaction using the respective yields ($Y_{X/Cj}$).
We consider glucose as limiting substrate and formic and propionic acid as non limiting substrates, in this way, mass balances for glucose, formic acid and propionic acid can be described by Eq. (5), (6) and (7) respectively, considering cellular maintenance.

$$\frac{dS}{dt} = -Y_{X/S}\mu X - m_1 X \tag{5}$$

$$\frac{dAFo}{dt} = -Y_{X/AFo}\mu X \tag{6}$$

$$\frac{dAPr}{dt} = -Y_{X/APr}\mu X - m_2 X \tag{7}$$

Fermentation products are classified into three groups: products associated to biomass growth, which are produced in the exponential phase of biomass growth; products not associated to biomass growth, that are produced in the stationary phase of cellular growth and products associated to mixed biomass growth, which are formed in both the exponential and the stationary phase of growth.
Hydrogen, carbon dioxide and butyl butyrate (BBu) are identified as products associated to biomass growth; their mass balance is represented by Eq. (8).

$$\frac{dP}{dt} = Y_{X/P}\mu X \tag{8}$$

In the group of products not associated to biomass growth, butane-2,3-dione (Bu_{23}), hexanoic acid (AHe), ethyl butyrate (BEt), 1-butanol (Bu_1) are included. In this case the mass balance is described by Eq. (9).

$$\frac{dP}{dt} = cX \tag{9}$$

Where c is a coefficient not associated to biomass growth.
Finally, the products associated to mixed biomass growth are acetic acid (AAc), butyric acid (ABu), isobutyric acid (IBu) and ethanol (Et). Mass balances for these products are represented by Eq. (10).

$$\frac{dP}{dt} = Y_{X/P}\mu X + cX \tag{10}$$

The complete model includes by fifteen differential equations, one algebraic equation and contains twenty three parameters.

2.3 Parameter estimation problem

The parameter estimation problem has been formulated in g-PROMS (PSEnterprise, 2012) as a Maximum Likelihood parameter estimation problem with constant variance, as follows:

$$\varphi = \frac{N}{2} ln(2\pi) + \frac{1}{2} \min_{p} \sum_{i=1}^{NM} \sum_{j=1}^{NT} \left[ln(\sigma_{ij}^2) + \frac{(C_{ij}^M - C_{ij})^2}{\sigma_{ij}^2} \right]$$

$$\begin{aligned} &s.t. \\ &\quad DAE\ model\ for\ the\ bioreactor \\ &\quad C_i(0) = C_i^0 \\ &\quad p^L \le p \le p^U \end{aligned} \tag{11}$$

where the summation in the objective function is over *NM* measured state variables and *NT* experimental data for each measured variable; σ_{ij} is the variance of the *j*th measurement of variable *i*, which is determined by the measured variable's variance model whose elements correspond to variances of the measured variables. Vector p corresponds to estimated parameters.
The concentration of biomass is calculated through a mass balance on carbon element, considering that the carbon present in biomass is, at each time, the initial carbon present in the inoculum plus the difference between the carbon present in glucose and fermentation products (Eq. (12)).

$$X^t(gC) = X^0(gC) + \left[\left(S^t(gC) - S^0(gC) \right) - \sum_i \left(P_i^t(gC) - P_i^0(gC) \right) \right] \tag{12}$$

where S is the glucose concentration, Pi represents the concentration of each fermentation product, X is the concentration of biomass and the superscripts t and 0 refer to each sampling time and initial time, respectively.
The model performance was evaluated quantitatively by calculating the mean error (ME) and the relative error (RE) based on the average values of the main differential variables.

3. Results and discussion

The parameter estimation problem is formulated in g-PROMS (PSEnterprise, 2012) as a maximum likelihood problem. Fifteen parameters of the model were selected to be estimated. Table 1 shows the optimal and nominal values for each estimated parameter and their upper and lower bounds. Figure 1 shows the profiles obtained after parameter estimation for the main variables of the model compared to experimental data during 340 hours of fermentation: biomass, glucose, hydrogen, acetic acid, butyric acid, carbon dioxide and ethanol.

Table 1: Optimal and initial values, upper and lower bounds for each estimated parameter

Parameter	Initial value	Lower bound	Upper bound	Optimal value
m_1	1.00×10^{-5}	9.00×10^{-6}	2.00×10^{-2}	9.18×10^{-4}
m_2	4.53×10^{-6}	1.50×10^{-8}	5.50×10^{-5}	1.50×10^{-8}
c_1	7.43×10^{-3}	2.00×10^{-13}	8.40×10^{-3}	1.15×10^{-4}
c_2	3.58×10^{-3}	1.50×10^{-5}	4.50×10^{-2}	1.50×10^{-5}
c_3	1.63×10^{-5}	6.00×10^{-10}	2.60×10^{-4}	2.39×10^{-6}
β	1.00×10^{-3}	1.00×10^{-3}	4.30×10^{-3}	4.21×10^{-3}
$\mu_{máx}$	9.75×10^{-3}	8.70×10^{-3}	6.20×10^{-1}	6.20×10^{-1}
$Y_{x/s}$	6.00×10^{-1}	5.00×10^{-1}	2.00	1.05
$Y_{x/AFo}$	1.00×10^{-2}	9.00×10^{-3}	1.30×10^{-2}	1.30×10^{-2}
$Y_{x/Et}$	7.55×10^{-4}	1.00×10^{-4}	1.00	5.04×10^{-4}
$Y_{x/Bu23}$	1.41×10^{-4}	9.00×10^{-7}	2.40×10^{-4}	5.51×10^{-6}
$Y_{x/Bu1}$	2.82×10^{-5}	1.00×10^{-7}	3.80×10^{-2}	6.21×10^{-7}
$Y_{x/BEt}$	1.12×10^{-3}	1.00×10^{-5}	2.10×10^{-3}	8.65×10^{-5}
$Y_{x/AHe}$	1.90×10^{-2}	8.00×10^{-5}	2.80×10^{-2}	4.19×10^{-4}
$Y_{x/BBu}$	1.90×10^{-2}	1.00×10^{-4}	1.00	9.51×10^{-2}

The biomass profile, adjusted with Contois´s equation, shows a reasonable fit to the experimental data. Analyzing the behaviour of the biomass curve a none existing lag phase can be observed, followed by an exponential growth phase of microorganisms until 111 h of fermentation. At this time, microbial growth stops, with a corresponding decrease in biomass concentration, showing the death phase of the culture. The degradation profile of glucose and the production profiles of hydrogen, carbon dioxide and ethanol are in good agreement with experimental data, while the adjustments for acetic and butyric acids profiles are regular. Acetic and butyric acids are the major products during the exponential growth phase, showing the joint occurrence of mixed acid and butyric fermentations. The ME for biomass has the highest value (-4,01), while ethanol had the lowest mean error ($-2{,}73 \times 10^{-4}$). Respect to the RE, the best variables fit by the model are ethanol (0,84%), glucose (0,12%) and butyric acid (ER=2.17%); while the relative errors values for acetic acid (7,63%), carbon dioxide (8,06%), hydrogen (9,68%) and biomass (22,23%) show that the adjustment is acceptable for the most important variables of the model.
The maximum hydrogen yield obtained was 2.68 mol H_2/mol glucose. The percentage of hydrogen present in the gas product ranged between 49.5 to 55.6 % v/v. Recently, Kan (2013) reached in dark fermentation tests 0.9 mmol H_2/mmol glucose, after 72 hours of discontinuous culture using a pretreated anaerobic sludge as inoculum. Another study showed a maximum yield of 1.52 mol H_2/mol hexose on immobilized medium of *Clostridium butyricum* (Plangklang et al., 2012), while Zhao et al (2009) obtained even lower yields (0.73-0.83 mol H_2/mol glucose) using *Enterobacter aerogenes* mutants.
The highest hydrogen production rate observed was 1.61 l H_2/ l-day, reached at 64 hours of culture. This value was held until 150 hours, after that time it started decreasing, as the microorganisms entered the death phase. Other authors reported similar maximum hydrogen production rates: 1.70 l H_2 / l-day using a pure culture *Clostridium beijerinckii* (Skonieczny and Yargeau, 2009) and 1.62 l H_2 / l-day working with different substrates (Ye et al., 2012).

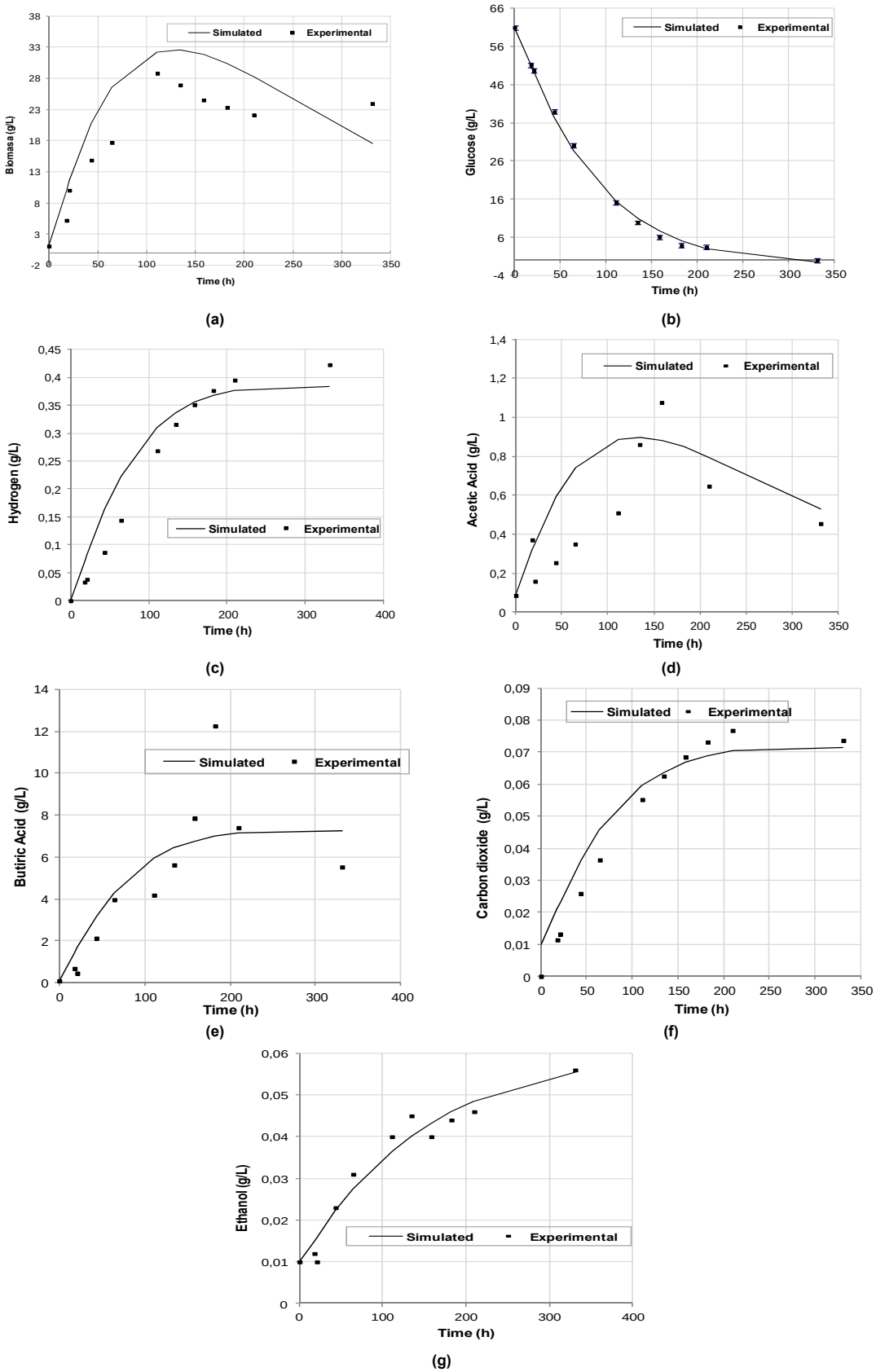

*Figure **1.** Simulated profiles and experimental data observed for concentrations (g/l) of: a-biomass, b-glucose, c-hydrogen, d-acetic acid, e-butyric acid, f-carbon dioxide and g-ethanol, during 340 hours of a dark fermentation experiment*

4. Conclusions

In this work, we have formulated a dynamic kinetic model for hydrogen production by dark fermentation, using a bacteria consortium in a batch stirred bioreactor. The model has 15 state variables and we have estimated 15 kinetic parameters that describe the behaviour of biomass, and the main substrates and products associated and not associated to growth within a 340 hours time horizon. Contois model has been used to adjust the kinetic growth of microorganisms. The hydrogen production process has been accurately adjusted with the proposed kinetic model, with advanced mathematical programming algorithms.

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