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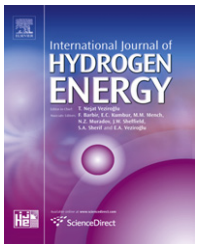

Modeling dark fermentation for biohydrogen production: ADM1-based model vs. Gompertz model

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ABSTRACT

Biohydrogen production by dark fermentation in batch reactors was modeled using the Gompertz equation and a model based on Anaerobic Digestion Model (ADM1). The ADM1 framework, which has been well accepted for modeling methane production by anaerobic digestion, was modified in this study for modeling hydrogen production. Experimental hydrogen production data from eight reactor configurations varying in pressure conditions, temperature, type and concentration of substrate, inocula source, and stirring conditions were used to evaluate the predictive abilities of the two modeling approaches. Although the quality of fit between the measured and fitted hydrogen evolution by the Gompertz equation was high in all the eight reactor configurations with r^2 ~0.98, each configuration required a different set of model parameters, negating its utility as a general approach to predict hydrogen evolution. On the other hand, the ADM1-based model (ADM1BM) with predefined parameters was able to predict COD, cumulative hydrogen production, as well as volatile fatty acids production, albeit at a slightly lower quality of fit. Agreement between the experimental temporal hydrogen evolution data and the ADM1BM predictions was statistically significant with $r^2 > 0.91$ and p-value <1E-04. Sensitivity analysis of the validated model revealed that hydrogen production was sensitive to only six parameters in the ADM1BM.

1. Introduction

Foreseeable depletion of limited fossil fuels, increased emissions of greenhouse gases, impacts on climate, and threatened global energy security are driving world-wide R&D efforts towards a renewable energy-based infrastructure [1,2,3]. Fermentation technology is receiving revived recognition as a sustainable and economically viable technology to produce a variety of energy carriers such as methane, ethanol, butanol, and hydrogen from inexpensive and renewable biomass and waste materials. Among these, hydrogen has been identified as having the highest potential as an energy carrier because it has a higher energy density and can be converted to electricity more efficiently. Among the biological methods of producing hydrogen – *biohydrogen*, dark fermentation has some advantages over the photosynthetic and photolytic bioprocesses because of lower net energy input, higher rate, and moderate yields.

In spite of over-emphasized disadvantage of moderate hydrogen yield from dark fermentation (of 4 mol of H_2/mole of

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glucose), scientists continue to optimize fermentative biohydrogen production from biomass feedstock to improve the rates and net energy yield [4]. Economics of fermentative biohydrogen process could be improved if its effluents can be fed to downstream processes such as photofermentation or microbial electrolysis cells to produce additional hydrogen; methane fermentation to produce methane; or microbial fuel cells to produce electricity [4,5,6].

1.1. *Modeling biohydrogen production by dark fermentation*

In the past, laboratory scale experiments have been conducted to identify the effects of individual process variables such as type and concentration of substrate and fatty acids, headspace pressure release methods, hydrogen partial pressure, pH, stirring and temperature on the product spectrum of dark fermentation [7]. In some instances, combined effects of two variables such as pH and substrate concentration [8]; temperature and pressure release [9], on biohydrogen production have also been discerned. However, it is difficult to conduct exploratory studies to discern synergistic effects of multiple variables ($n > 3$) on fermentative hydrogen production. Complexity of these studies is further compounded due to the syntrophic existence of multiple bacterial species when mixed cultures are used. Mechanistic mathematical models that can simulate the science behind biohydrogen processes can be beneficial in identifying the optimum combination of process variables to maximize hydrogen yields. Kinetic constants developed from such modeling studies can be validated with experimental data for use in design and development of the biohydrogen process. Models that can predict the product mix can be useful in designing downstream processing of the effluents.

1.2. *Gompertz equation in biohydrogen studies*

Previous biohydrogen researchers have used Gompertz equation to describe hydrogen evolution by dark fermentation. In this empirical approach, three model parameters – lag time, H_2 production potential, and H_2 production rate are adjusted to fit the Gompertz equation to experimentally measured hydrogen evolution data. Even though this curve-fitting approach yields high correlation coefficients between the observed and fitted hydrogen evolution data (Cited in [10]), the three model parameters determined by curve-fitting are restricted to specific experimental conditions and cannot be used in a predictive mode. Due to this empirical nature, utility of the Gompertz equation is severely limited as it cannot account for any of the relevant process variables such as substrate concentrations, temperature, pH, substrate-types etc. for predictive purposes. In certain studies, Gompertz equation has been modified to accommodate typical kinetics of substrate degradation, biomass growth, and hydrogen production [11,12].

Some biohydrogen studies have utilized the conventional kinetic expressions such as Monod's equation and Luedeking Piret's equation [10]. However, rigorous and multiple simulations, followed by a series of validations may be required to establish generality of such equations and the associated parameters. Further, comprehensiveness of such models is accomplished only when they can be readily integrated with other complex bioprocesses i.e. hydrolysis, acidogenesis, and H_2 production from complex and particulate organic substrates. Towards this end, it would be advantageous to develop/adapt a generic modeling framework, with well-defined nomenclature, and readily available kinetic parameters and constants to predict cumulative hydrogen production under varying combinations of multiple substrates, bacterial strains, and processes.

1.3. *Anaerobic digestion model I (ADM1) in biohydrogen studies*

The Anaerobic Digestion Model I (ADM1) is a mechanistic model that has open structure, common nomenclature integrating biokinetics with association-dissociation; gas–liquid transfer; cellular processes involving hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Previous researchers have successfully used the ADM1 model for describing methane production from mixed culture fermentation of domestic, industrial wastewater as well as solid wastes [2]. It is therefore prudent to adapt the ADM1 framework to predict hydrogen and volatile fatty acid formation by dark fermentation, by excluding the final step of methanogenesis. Peiris et al. [13] had demonstrated the utility of ADM1 model in biohydrogen studies by simulating the effect of carbohydrate-protein ratio on dynamic production of protons, biomass, fatty acid and hydrogen. Rodriguez et al. [14,15,16,17] have applied ADM1 model in biohydrogen studies as an energetic and metabolic network based modeling approach and alternative to multiple biomass models and in mechanistic description of product formation through a variable stoichiometry approach.

The objective of this study was to evaluate the suitability of ADM1-based model (ADM1BM) vs. Gompertz equation in predicting hydrogen production in eight dark fermentation systems varying in temperature, inocula, stirring, pH control, pressure release, or liquid-headspace volume.

2. Methods

2.1. *Culture conditions*

The following three different culture sources reported in our previous studies were evaluated in this study: LpH inocula [18], heat-treated compost [9], and raw compost [19] (Table 1). Two different substrates (glucose and sucrose) were evaluated at two different concentrations. Stock solutions were added to compost extracted media [9] to obtain final COD concentration (5–10 g COD/L) in the test reactors. The test reactors (250 mL capacity bottles; Wheaton Scientific) had a headspace volume of either 75 or 100 mL. Tests were conducted at the designated test temperatures (22 °C, 25 °C, or 37 °C) in batch mode, either with continuous agitation at 160 rpm or without any agitation. The reactors were coded based on the headspace pressure release method – continuous pressure release (CPR) or intermittent pressure release (IPR). Details of the experimental design are presented elsewhere [9,18,19,20] and summarized in Table 1.

Table 1 – Experimental conditions in eight biohydrogen systems.

	IPRB5	IPRB10	IPR22	CPR22	CPR37	IPR37	IPR22-UC	IPR22-LpH
Reactor	Buffered	Buffered	Unbuffered	Unbuffered	Unbuffered	Unbuffered	Unbuffered	Unbuffered
Conditions	Stirred	Stirred	Stirred	Stirred	Stirred	Stirred	Unstirred	Unstirred
Substrate	Glucose	Glucose	Sucrose	Sucrose	Sucrose	Sucrose	Sucrose	Sucrose
g COD/L	5	10	10	10	10	10	10	10
Inocula	HC	HC	HC	HC	HC	HC	RC	LpH
T (°C)	25	25	22	22	37	37	22	22
PR	IPR	IPR	IPR	CPR	CPR	IPR	IPR	IPR
Volume (mL)	175	175	175	175	175	175	150	150
Stirring (rpm)	160	160	160	160	160	160	None	None

Notes: HC, heat-treated compost; RC, raw compost; LpH, inocula from low pH systems: PR: pressure release; IPR: intermittent PR, CPR: continuous PR.

2.2. Bioreactor configurations

Four unbuffered reactors (IPR22, CPR22, IPR37, and CPR37) were inoculated with heat-treated compost and tested under the two pressure release conditions (IPR and CPR) and temperatures (22 °C and 37 °C) [9]. Previously demonstrated cultures capable of hydrogen production under low pH range of 3.3–4.3 constituted reactor IPR22-LpH [18]. Another reactor, coded IPR22-UC, was inoculated with unconditioned compost and operated without any buffers or stirring [19]. Two additional reactors, IPRB5 (with 5 g COD/L glucose) and IPRB10 (with 10 g COD/L glucose) were buffered with 50 mM MES (pH = 7), inoculated with heat-treated compost and tested under stirred conditions (160 rpm) [20]. Control reactors were set up to verify the absence of biotic, abiotic and background H_2 production from compost.

2.3. Analytical

Liquid and gas samples were drawn with gas tight syringes and analyzed following procedures described elsewhere [9,18]. Reducing sugars were measured spectrophotometrically (Hach, wavelength = 575 nm) using di-nitro salicylic acid (DNS) assay. pH was measured using Cole-Palmer pH electrode probe. Volatile fatty acids in the liquid phase and composition of the headspace gases (hydrogen, oxygen, nitrogen, and methane) were measured using gas chromatograph following the methods described previously [9]. Hydrogen production was calculated from headspace measurements and the total volume of biogas produced at each time interval, and reported at STP. Hydrogen gas composition between sampling time was assumed to follow a linear change in concentration over the sampling interval [9,18].

3. Model development

3.1. Gompertz equation

Procedure suggested to minimize the sum of square error to the correlation coefficients ratio (SSE/R^2) was used to fit the Gompertz equation by adjusting the three Gompertz parameters: hydrogen production, (H_{max}, mL), H_2 production rate, (R, mL/h), and the lag phase, (λ, h). This curve-fitting exercise was carried out using Microsoft Excel software [8].

$$H(t) = H_{max}\cdot\exp\left\{-\exp\left[\frac{R}{H_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

3.2. ADM1-based model (ADMBM)

In our experiments, the end-products of dark fermentation of sugar substrates (S_1) were butyrate (S_2), propionate (S_3), acetate (S_4), hydrogen (S_5) and biomass (X_1). Lactate typically observed during higher organic loading [3] was not detected in this study. Ethanol production was also insignificant (<5% of total COD) and was not considered in our model. Based on these results, the schematic shown in Fig. S1 (Supplementary) was adapted to generate ADM1BM. The following equations were used to describe the intermediate steps.

$$C_6H_{12}O_6 \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (3)$$

$$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2H_2O + 2CO_2 \quad (4)$$

Product formation from the monosaccharide fermentation was obtained by following the constant stoichiometry approach described in ADM1 [2]. The relative split, via which dark fermentation proceeds according to Eqs. (2)–(4), was experimentally determined to establish individual yield values for butyrate (f_{1-2}), propionate (f_{1-3}), acetate (f_{1-4}), hydrogen (f_{1-5}), and biomass (Y_1). Assimilation of organic acids is thermodynamically unfavorable ($\Delta G^0 > 48$ kJ/mol) in the absence of external energy input [21] and was therefore not considered in this model. All the state variables (S_1 (COD input), S_2 (butyrate), S_3 (propionate), S_4 (acetate), S_5 (hydrogen) and X_1 (acidogenic biomass)) are expressed as g COD/L. Definitions for the other variables were adapted from ADM1 model. Substrate uptake rate assuming substrate-limited conditions is described by Monod's equation:

$$\left\{\frac{dS_1}{dt}\right\}_U = -k_1\left(\frac{S_1}{K_{S1} + S_1}\right)X_1 \quad (5)$$

where, k_1, g COD/g biomass-day is the specific uptake rate and K_{S1}, g COD/L is the half saturation constant.

It has been reported that substrate uptake rate can be inhibited at higher proton levels: i) low pH promotes diffusion of undissociated acids into the cell membrane where it dissociates in the cell to release a proton which has

implications on higher cellular maintenance energy requirements ii) proton uptake decreases the availability of coenzyme A and phosphate pools to cause subsequent reduction in glucose flux through glycolysis [22]. Substrate uptake rate in Eq. (5) has, therefore, been modified to include pH inhibition.

$$\left\{\frac{dS_1}{dt}\right\}_U = -k_1\left(\frac{S_1}{K_{S1}+S_1}\right)X_1 I_{pH} \quad (6)$$

where, the pH inhibition term, I_{pH}, inhibits the substrate uptake rate and hence the hydrogen production at both the lower and the upper pH values according to the model suggested in ADM1 [2]:

$$I_{pH} = \frac{1+2\times 10^{0.5(pH_{LL}-pH_{UL})}}{1+10^{(pH-pH_{UL})}+10^{(pH_{LL}-pH)}} \quad (7)$$

Biomass growth is dependent on the biomass yield (Y_1), COD uptake rate, and cell death rate due to lysis and disintegration ($k_{d,1}$) [2].

$$\frac{dX_1}{dt} = Y_1\left(\frac{dS_1}{dt}\right)_U - k_{d,1}X_1 \quad (8)$$

Formation of butyrate (S_2), propionate (S_3), and acetate (S_4) are described by the following equations as suggested in ADM1 [2]:

$$\frac{dS_2}{dt} = (1-Y_1)f_{1\text{-}2}\left(\frac{dS_1}{dt}\right)_U \quad (9)$$

$$\frac{dS_3}{dt} = (1-Y_1)f_{1\text{-}3}\left(\frac{dS_1}{dt}\right)_U \quad (10)$$

$$\frac{dS_4}{dt} = (1-Y_1)f_{1\text{-}4}\left(\frac{dS_1}{dt}\right)_U \quad (11)$$

Finally, hydrogen production is obtained from Eq. (12) [2]

$$\frac{dS_5}{dt} = (1-Y_1)f_{1\text{-}5}\left(\frac{dS_1}{dt}\right)_U + (1-Y_2)f_{2\text{-}5}\left(\frac{dS_2}{dt}\right) + (1-Y_3)f_{3\text{-}5}\left(\frac{dS_3}{dt}\right) + (1-Y_4)f_{4\text{-}5}\left(\frac{dS_4}{dt}\right) \quad (12)$$

where, $f_{2\text{–}5}$, $f_{3\text{–}5}$, and $f_{4\text{–}5}$ are set to zero.

As methane production was not observed in any of the reactors due to the heat treatment technique or operating conditions [9,18,19,20], the initial concentration of methanogens was set to zero.

The primary objective of this study was to evaluate the suitability of ADM1-based model (ADM1BM) vs. Gompertz equation in predicting cumulative biohydrogen production in eight different biohydrogen systems. Ion association/dissociation and gas–liquid transfer processes are not considered in this preliminary work and may be necessary to match the comprehensiveness of the ADM1 framework. Effects of undissociated fatty acid concentrations on acid accumulation and hydrogen production are not discussed here and readers are directed elsewhere [14].

4. Results and discussion

Experimental results confirmed absence of background hydrogen production in the control reactors. As demonstrated in our previous studies, heat treatment of inocula and/or the right operating conditions (unbuffered, low pH as suggested in our previous study) [9,18] can effectively suppress methane production. Hydrogen conversion efficiencies (%) determined from experimental cumulative H_2 production data and substrate consumption calculated from stoichiometry (Eq. (2)) for IPR22, IPRB10, IPR22-LpH, CPR22, CPR37, IPR22-UC, IPR37, and IPRB5 are: 53%, 42%, 43%, 40%, 29%, 36%, 21%, and 36%, respectively. Cumulative H_2 production and substrate consumption data ($n = 3$, SD < 6%) are shown in Fig. 1a and b respectively, with the sole purpose of demonstrating differences in process conditions and performances among the eight reactors (Table 1), and associated challenges in predicting hydrogen production using a calibrated model with a specific set of parameters. Fig. 1c shows pH dynamics in the six unbuffered reactors, which are fed into ADM1BM under simulation mode, implications of which are discussed in later sections.

4.1. *Gompertz equation*

The three Gompertz parameters – specific hydrogen production potential (SHHP), hydrogen production rate (Rs) and lag time (λ), for each of the eight biohydrogen reactors determined by the curve-fitting process are tabulated in Table 2. Gompertz equation-based best fit curves for cumulative H_2 production for the eight reactors are shown in Fig. 2. The overall goodness of fit between the measured hydrogen production data and those fitted with the Gompertz equation was high in IPR37 and CPR37 ($r^2 = 0.99$, $p < 1.3\text{E-}07$; and $r^2 = 0.99$, $p < 1.6\text{E-}07$ respectively; Fig. 2e, f). While Gompertz equation could fit cumulative hydrogen production in IPR22 and CPR22 reasonably well, the overall goodness of fit between the measured and fitted values was slightly lower ($r^2 = 0.93$, $p < 4.1\text{E-}18$; and $r^2 = 0.91$, $p < 5.8\text{E-}15$ respectively; Fig. 2a, d). This is likely due to mismatch between temporal and experimental data due to untypical H_2 production characteristics specific to unbuffered IPR22 and CPR22 reactors: when pH reached 4.5 self induced pH increase was observed possibly accompanied with acid reassimilation and solvent production [9].

Overall goodness of fit by the Gompertz model in the case of the buffered reactors (IPRB5 and IPRB10) at the two glucose concentrations was high ($r^2 = 0.98$, $p < 3.3\text{E-}17$; and $r^2 = 0.99$, $p < 4.7\text{E-}25$ respectively; Fig. 2b, c). Gompertz equation was able to follow the temporal trend of cumulative hydrogen production in IRP22-UC also well (Fig. 2g). The overall goodness of fit by the Gompertz model was high ($r^2 = 0.98$, $p < 6.77\text{E-}12$). A bacterial consortium capable of producing hydrogen in low pH (LpH) range of 3.3–4.3 was used in IPR22-LpH [18]. Gompertz equation was able to trace the temporal trend of cumulative H_2 production in IRP22-LpH well ($r^2 = 0.99$, $p < 6.5\text{E-}10$; Fig. 2h).

The quality of the fit by the Gompertz equation in all eight reactors is comparable to that reported in the literature (23 biohydrogen studies cited by Jianlong et al. [10]). This is as expected because the results are based on curve fitting for the specific experimental data from the eight different systems. In spite of excellent statistical evidence for goodness of fit, no single set of parameters (SHPP, R_S and λ) found from any one reactor could be generalized to fit H_2 production data from the

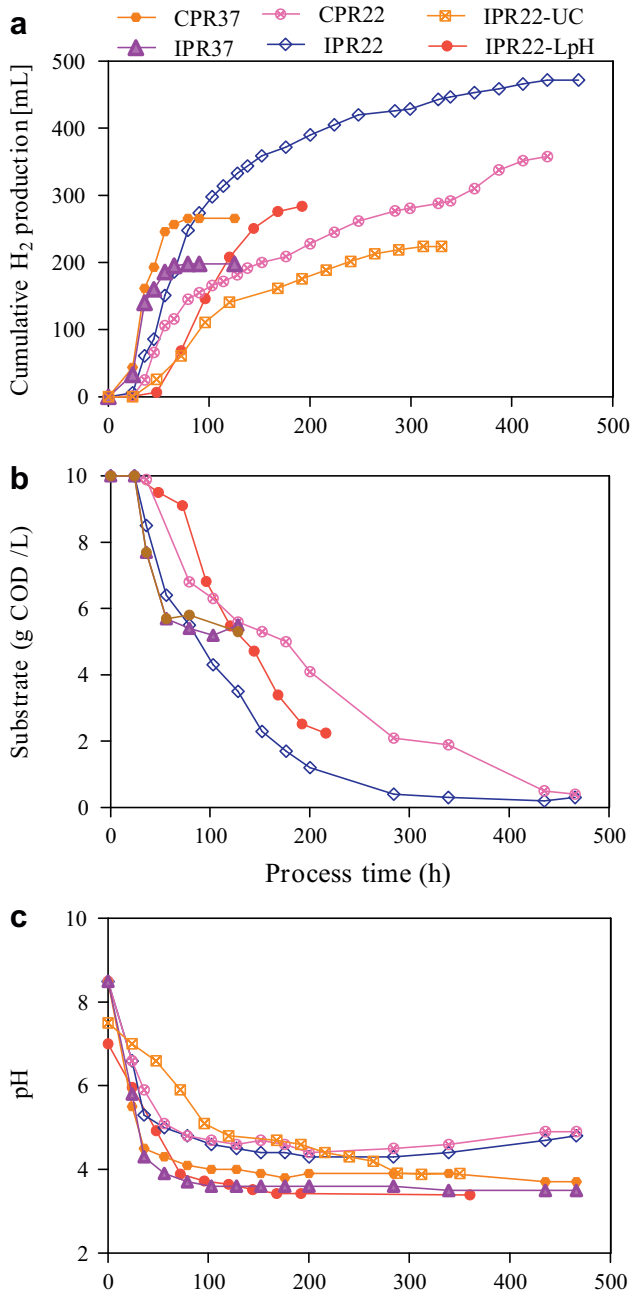

Fig. 1 – a) Cumulative hydrogen production (mL) b) COD consumption (g COD/L) and c) pH in six unbuffered batch reactors.

other reactors. Experimentally determined Gompertz parameters do not distinguish differences in process conditions across the eight reactors shown in Table 1.

4.2. *ADM1-based model (ADM1BM)*

In our approach, the variation of pH was not explicitly modeled; instead, experimentally measured, time dependent pH values were incorporated into the model. When *substrate* is not the limiting factor, *pH inhibition* switch (I_{pH}) in Eq. (6) controls H_2 production in the defined pH limits with $pH_{LL} = 5.5$ and $pH_{UL} = 6.5$ [8,23]. While the kinetic model based on pH inhibition and substrate limitation can be deemed as oversimplification of the ADM1 model, advanced models for specific biohydrogen systems should be simulated under variable stoichiometry mode [14] to explore precise and comprehensive details in the specific reactor. We adapted the simpler approach here because the major objective in this study was to evaluate suitability of ADM1BM vs. Gompertz model in predicting biohydrogen production from dissimilar bioreactors. For this purpose, the six unbuffered biohydrogen systems, IPR22, CPR22, IPR37 and CPR27, IPR22_UC, IPR22_LpH varying in inocula source, pressure release method and temperature were selected as the base case. Experimental results from IPR22 were used to calibrate our model; results from the other five were used to validate the model.

Sucrose was used as the substrate and heat-treated compost as the inocula in IPR22, CPR22, IPR37 and CPR37 reactors. Butyrate was the major end product in these reactors, followed by acetate and propionate. Yield values interpolated experimentally from separate batch studies were used to establish the following f-factors: butyric acid: $f_{1-2} = 0.54$; acetic acid, $f_{1-4} = 0.11$; propionic acid, $f_{1-3} = 0.03$; hydrogen, $f_{1-5} = 0.19$; and biomass, $Y_1 = 0.1$. The model Equations (5)–(12) were implemented in Extend (ImagineThat Inc.) simulation software and solved using Euler's backward numerical method ($\Delta t = 300$ s).

All the kinetic parameters used in Equations (5)–(12), except K_{S1} were adapted from Batsone et al. [2]. Values of K_{S1} in Eq. (6) were modified as μK_{S1} where the modifier 'μ' is hypothesized here as a function of reactor conditions, while the value of K_{S1} was kept the same as in ADM1. Initially, μ was considered to be a function of temperature, and was determined by curve fitting using one set of experimental hydrogen data at 22 °C and another set at 37 °C. For this initial model, identified here as the *General ADM1BM*, the following modifier values were established: $\mu = 22$ at 22 °C and $\mu = 2$ at 37 °C. This initial model was further refined by considering μ to be a function of both temperature and the pressure release method, and was established by fitting the hydrogen data from the four reactors. For this model, identified here as the *Refined ADM1BM*, the following modifier values were established: $\mu = 16$, 36, 2, and 4 for IPR22, CPR22, IPR37 and CPR37 respectively.

4.2.1. *Calibration of general ADM1BM*

Experimental COD results from IPR22 were used to calibrate the General ADM1BM. COD consumption, VFA (acetate, propionate, and butyrate) production and hydrogen evolution fitted by the calibrated model are compared against the corresponding experimental values in Figs. 3a, 4a, and 5a, respectively. Existence of fermentative bacteria in IPR22 adept in responding to inhibitory pH conditions by switching from exponential to stationary growth phase for concurrent participation in sucrose degradation, and reassimilation of volatile fatty acids was hypothesized to sustain hydrogen production under unbuffered conditions [9]. Since liquid phase analysis indicated no pH inhibition in IPR22, except for the initial short period, H_2 prediction by the model was largely dependent on substrate availability; nearly 99% of the substrate was utilized, confirmed by both experimental results and modeling results. The predicted temporal hydrogen production in IPR22 matched the experimental

Table 2 – Gompertz analysis for eight biohydrogen systems.

	IPRB5	IPRB10	IPR22	CPR22	CPR37	IPR37	IPR22-UC	IPR23-LpH
a) Gompertz parameters for proposed biohydrogen systems								
SHPP, (Ps)	33	37	47	36	27	20	22	28
Lag time, λ (h)	42	60	40	30	18	18	40	54
Rs (mL/L h)	13	15	23	9	54	46	13	25
b) Quality of fit for Gompertz model								
Data points	16	25	26	25	8	8	14	9
r^2	0.98	0.99	0.93	0.91	0.99	0.99	0.98	0.99
F	2524	2542	556	317	754	807	670	2065
P	3.E-17	5.E-25	4.E-18	6.E-15	2.E-07	1.E-07	7.E-12	7.E-10

Notes: SHPP, specific hydrogen potential (mL H_2/(g COD/L of hexose)); Rs: hydrogen production rate.

results well with $r^2 = 0.96$, $p < 0.0001$, $F = 584$ (Fig. 3a). The quality of fit for COD profile and that for VFA production were also statistically significant as shown in Figs. 4 and 5 and Table 3. The fact that the model predictions followed the temporal trend in the experimental COD profile and product formation (H_2, VFAs) suggests that the general-model formulation and the modified K_{S1} value are adequate in describing the dark fermentation process for hydrogen production.

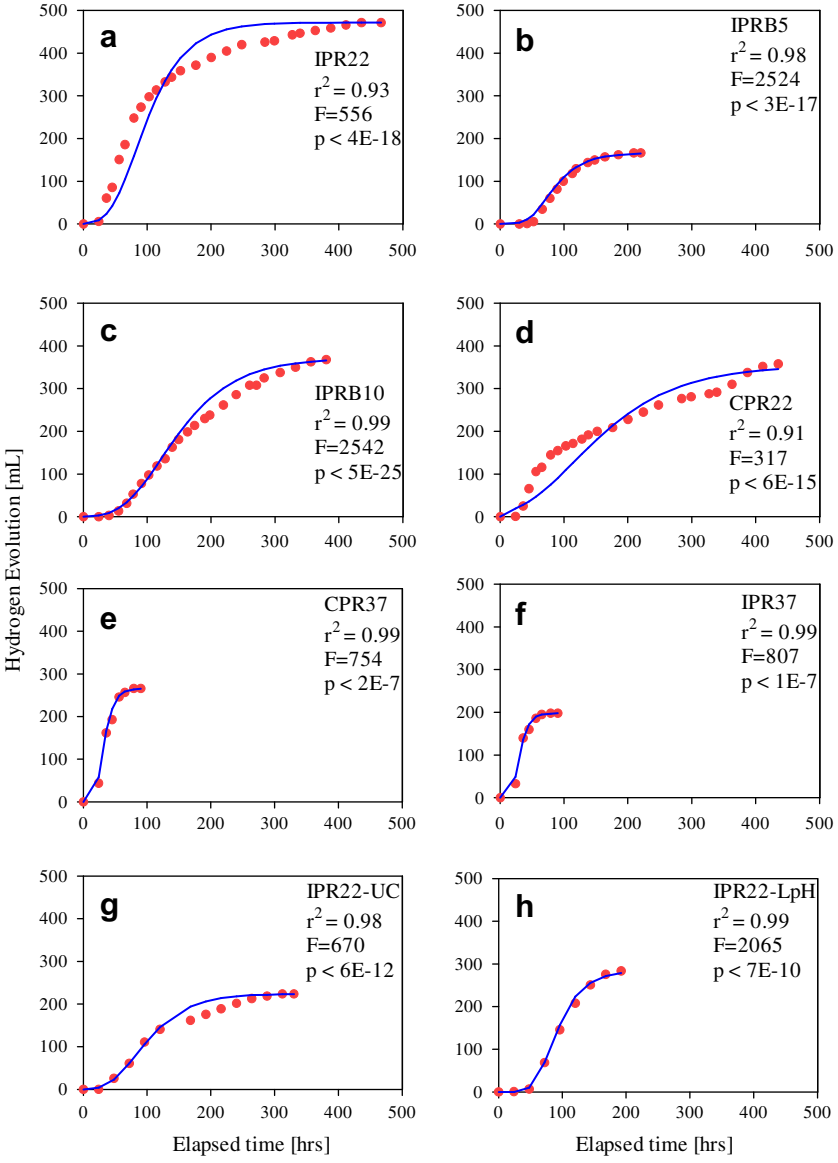

Fig. 2 – Hydrogen production (mL): experimental (▲) vs. Gompertz-Fit (–). (a) IPR22 (b) IPRB5 (c) IPRB10 (d) CPR22 (e) CPR37 (f) IPR37 (g) IPR22-UC (h) IPR22-LpH.

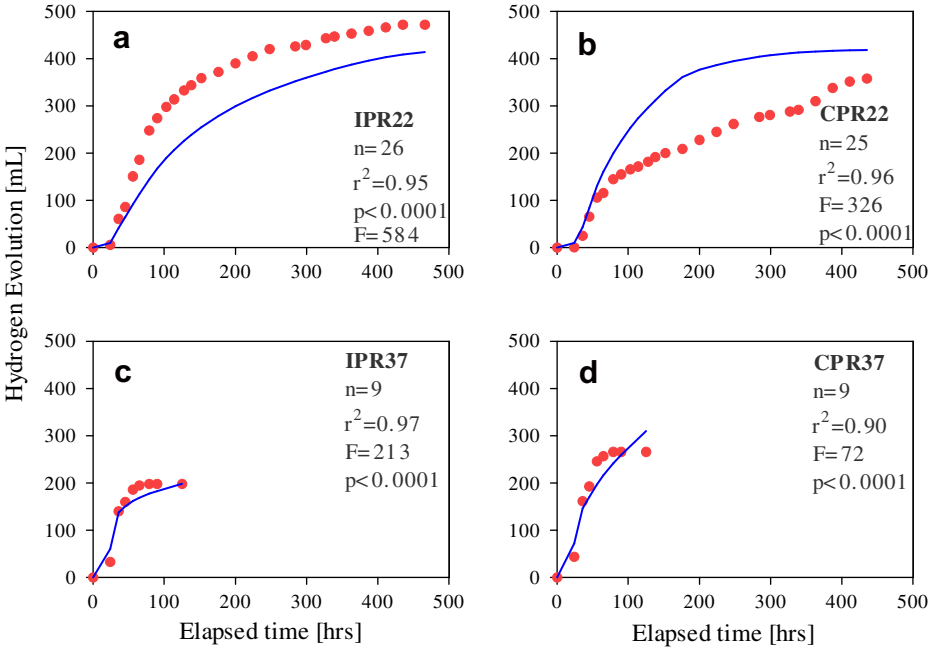

Fig. 3 – Hydrogen production (mL): experimental (•) vs. General ADM1BM-Fit (–). (a) IPR22 (b) CPR22 (c) IPR37 (d) CPR37.

4.2.2. *Validation of the general ADM1BM*

The model was first validated using the experimental data from CPR22. As in the case of IPR22, pH inhibition was not observed in this reactor, and the entire sucrose content was utilized (Fig. 4b). Though the model predicted cumulative hydrogen production reasonably well in CPR22 ($r^2 = 0.93$, $p < 0.0001$, $F = 326$), the temporal hydrogen production did not match well with experimental data (Fig. 3b). This is likely due to the untypical nature of unbuffered conditions: self induced pH increase caused by slight shift from acid-production to solvent production phase, coupled with hydrogen assimilation during endangered pH conditions (pH ~4.5 h at $t = 266$ h) [9]. Similar effects in IPR22 might have been slightly masked as this reactor was used as a base model for calibration purpose. Figs. 3b, 4b, 5b clearly show that COD consumption, VFA production and hydrogen evolution predicted by model in this case compare well against corresponding measured values with statistically significant quality of fit (Table 3).

Next, the model was validated using experimental data from IPR37, run at a slightly higher temperature (37 °C). As shown in Fig. 3c, predicted cumulative hydrogen production

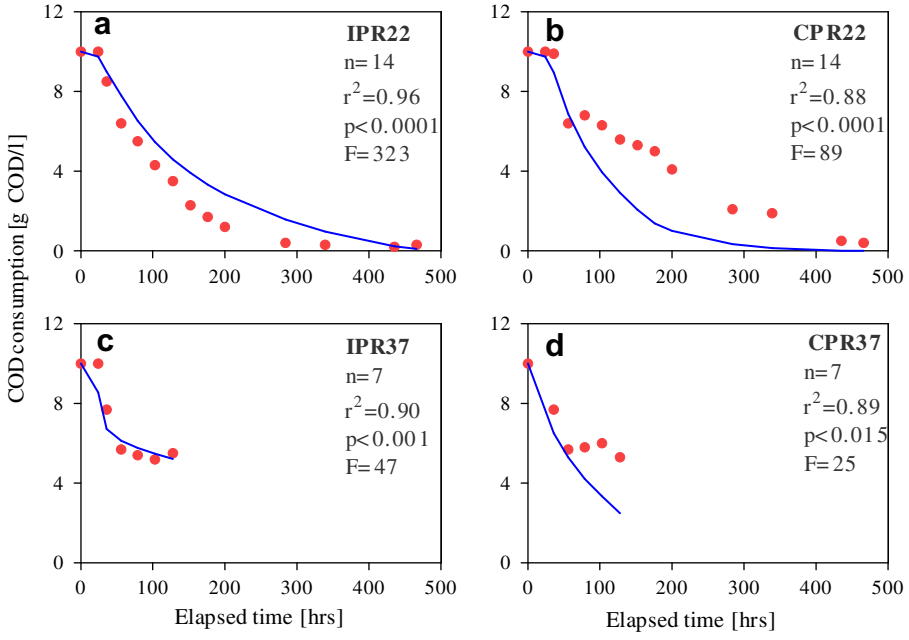

Fig. 4 – COD consumption (g COD/L): experimental (•) vs. General ADM1-Fit (–). (a) IPR22 (b) CPR22 (c) IPR37 (d) CPR37.

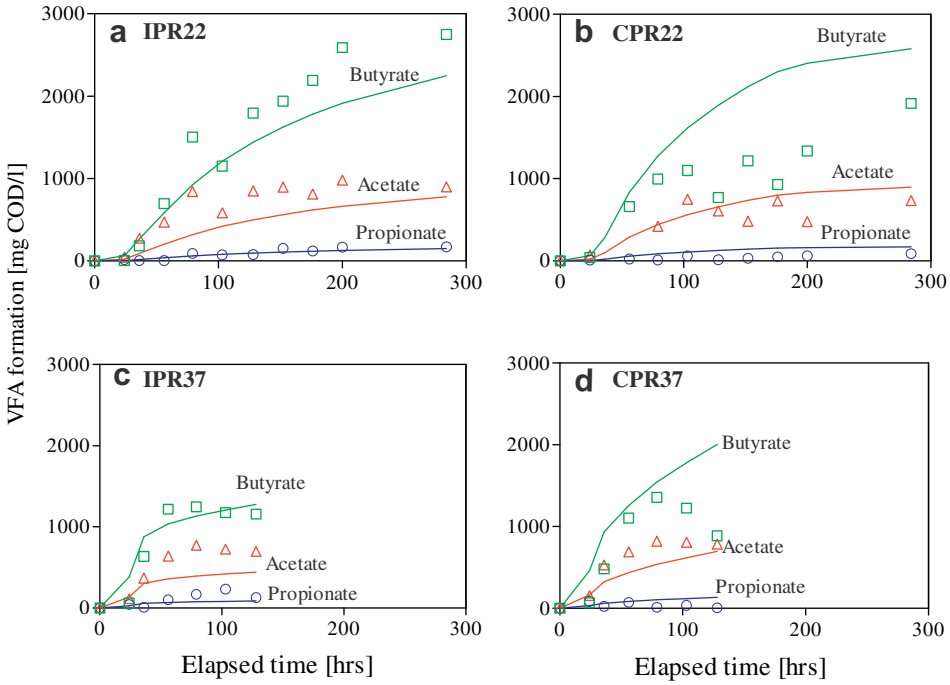

Fig. 5 – VFA formation (mg COD/L): experimental butyrate (□), acetate (△), propionate (○) vs. General ADM1BM-Fit (–) (a) IPR22 (b) CPR22 (c) IPR37 (d) CPR37.

curve followed the trend in the measured values. Within 90 h of operation of this reactor, volatile fatty acids accumulated rapidly to lower the pH to inhibitive levels, hindering substrate consumption and hydrogen production [9]. Hindered hydrogen production in IPR37 due to acidic conditions, and the lower consumption of sucrose are well predicted by this model (Fig. 4c). Both experimental and model results indicated that 50% of sucrose was still available after cessation of hydrogen production (Fig. 4c). Temporal data of predicted butyrate, acetate, and propionate production matched the measured data well (Fig. 5c). As shown in Table 3, except for propionate, overall goodness of fit between predicted and measured substrate concentration as well as that for gaseous and liquid products were statistically significant ($r^2 > 0.9$, p-value >0.001, $F > 44$).

The model was further validated using the experimental data from CPR37 run at 37 °C, but under continuous pressure release conditions. The model predictions of cumulative hydrogen production agreed precisely with measured values: 259.5 vs. 260 mL. Similar to IPR37, 50% of the substrate was left

Table 3 – Quality of 'General-ADM1' prediction in four bioreactors.

Bioreactor		Quality of fit for 'General-ADM1' predictions				
		COD	H_2	Acetate	Propionate	Butyrate
IPR22	n	14	26	11	11	11
	r^2	0.96	0.96	0.84	0.88	0.97
	p	0.0001	1E-04	0.0001	0.0001	0.0001
	F	323	584	48	67	272
CPR22	n	14	25	9	10	10
	r^2	0.88	0.93	0.76	0.62	0.81
	p	0.0001	1E-04	0.0021	0.0075	0.0004
	F	89	326	23	12	35
IPR37	n	7	9	7	7	7
	r^2	0.9	0.97	0.93	0.58	0.9
	p	0.001	1E-04	0.0005	0.0458	0.0001
	F	47	213	63	7	44
CPR37	n	7	9	7	7	7
	r^2	0.89	0.91	0.92	0.024	0.75
	p	0.015	1E-04	0.0007	0.74	15
	F	25	72	56	0.123	15

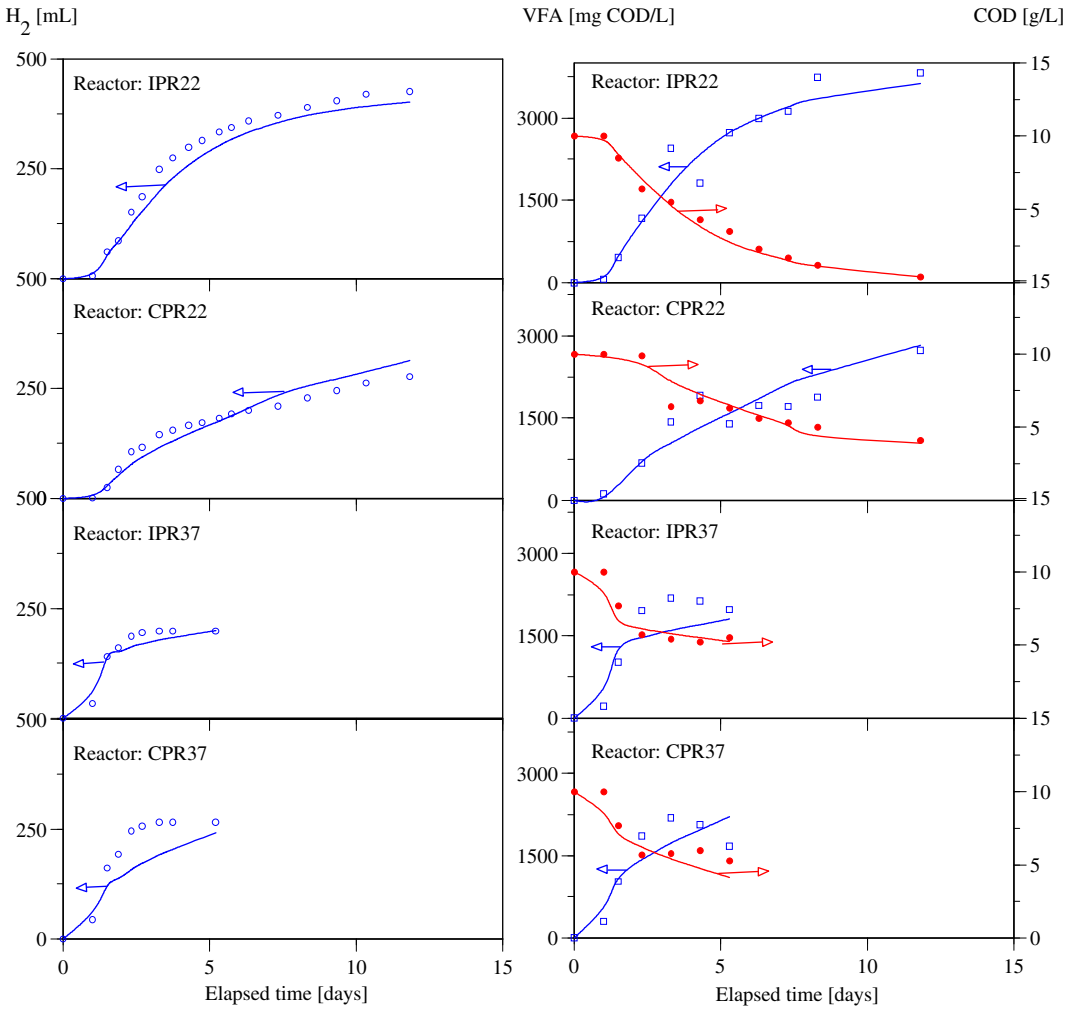

Fig. 6 – 'Refined-ADM1BM' customized to IPR22, CPR22, IPR37 and CPR37 Temporal profiles of H_2 evolution (○), COD remaining (•), and VFA production (□): curves represent predicted values; symbols represent experimental values. Goodness of fitness data shown in Table S1.

unutilized due to pH inhibition, a fact well captured by the model (Fig. 3d). The overall goodness of fit between predicted and measured values for the COD profile and gaseous and liquid products were reasonably significant except for propionate ($r^2 > 0.75$, p-value > 0.001, $F > 25$; Figs. 4d and 5d).

The model was also able to predict hydrogen evolution from two other reactors IPR22_UC and IPR22_LpH with reasonable statistical significance with $r^2 = 0.89$ and 0.96 respectively and p-value <0.001 (Data not shown here).

4.2.3. *Refined-ADM1 model*

Though the General ADM1BM was well calibrated to predict hydrogen production with reasonable statistical significance in six different biohydrogen systems, the proposed refinement showed a better quality of fit, albeit reducing its generality. Other workers have suggested similar customization too. For example, Penumathsa et al. [14] ran a series of experiments on a single continuous-biohydrogen system to develop a comprehensive set of 'training-data' for calibration purposes, and further used the same combination of calibrated model & bioreactor for prediction purposes.

In this study, General ADM1BM (presented in Figs. 3–5 and Table 3) was customized to each individual bioreactor (i.e. IPR22, CPR22, IPR37 and CPR37) by modifying K_{S1} values as described in Section 4.2. Temporal predictions of COD profile, H_2 evolution, and total VFA production by the "Refined-ADM1BM" model are shown in Fig. 6. Temporal profiles of individual VFAs derived from experimental and modeling data are provided in supplementary section (Fig. S2). With this approach, excellent match between model predictions and measured values can be seen in all four systems. Overall goodness of fit of this model summarized in Table S1 can be seen to be higher than that of the "General-Model' summarized in Table 3.

4.3. *Sensitivity analysis*

The proposed model was simulated under sensitivity analysis mode to identify the most significant parameters affecting hydrogen production. Reactor IPR22 was chosen for this purpose. For each of the model parameters, five values were selected within a range of ±5% and five simulations were run

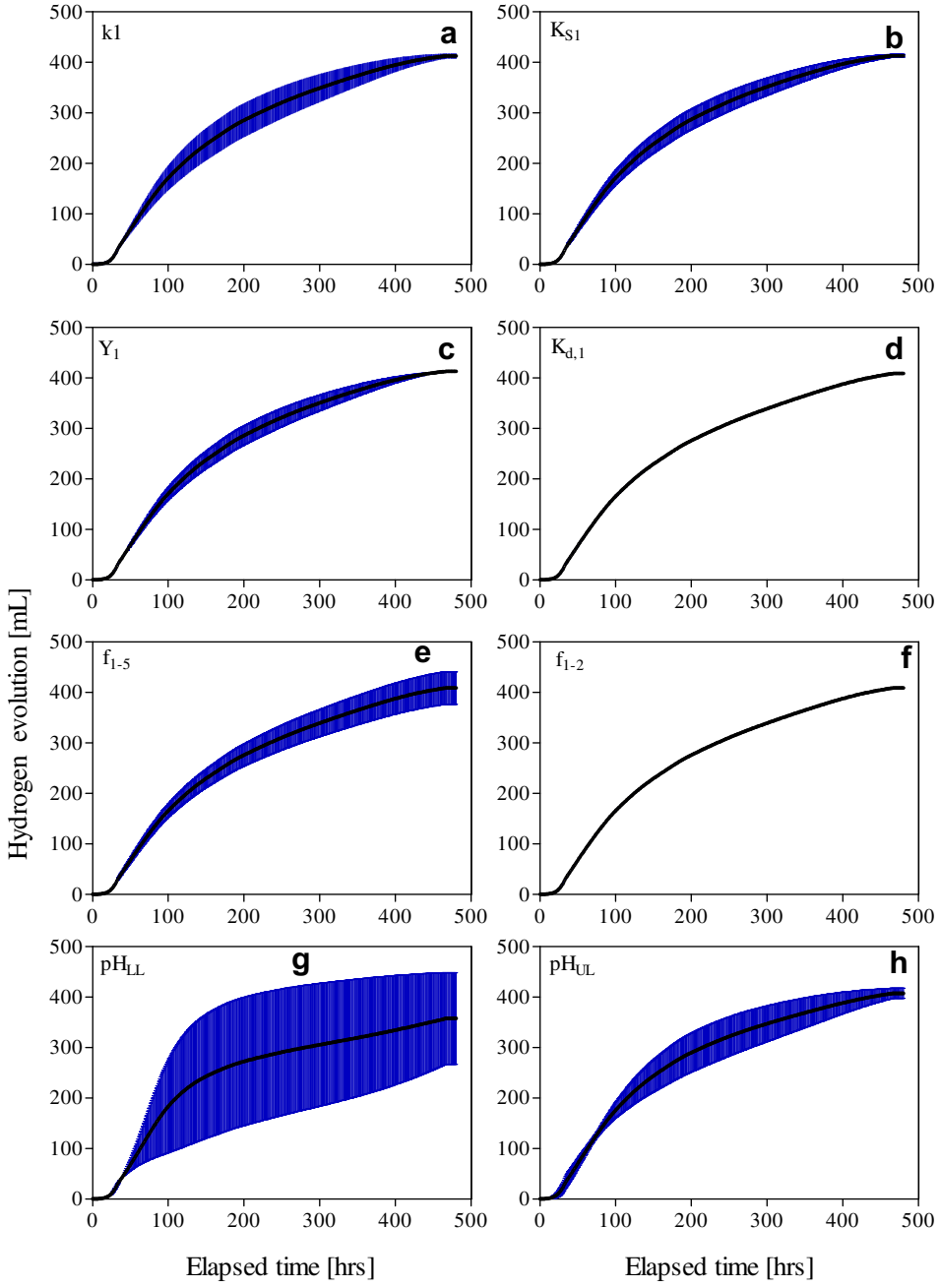

Fig. 7 – Sensitivity profiles for K_1, K_S, Y_1, $K_{d,1}$, f_{1-5}, f_{1-2}, pH_{LL}, pH_{UL}.

at each of those values keeping all the other parameters fixed at the base values established in this study. The five hydrogen production curves generated from these five simulations were combined to produce a mean profile with a spread of one standard deviation. Based on this study, only the following six parameters out of the ten studied were found to be significant: K_1, K_{S1}, Y_1, f_{1-5}, pH_{LL}, pH_{UL}.

Considering the cumulative hydrogen volume, these simulations indicate that the least significant parameters affecting cumulative hydrogen production are $K_{d,1}$, f_{1-2}, f_{1-3}, f_{1-4}: when these parameters were varied within a range of $\pm$5% from the base values, the standard deviations in cumulative hydrogen production were less than 5% of the standard deviation (413 $\pm$ 6, 413 $\pm$ 5, 413 $\pm$ 0.1, 413 $\pm$ 0.5), and showed no significant change in the temporal values (Fig. 7d, f; Data not shown for f_{1-3}, and f_{1-4}). However, the significance of f_{1-2}, f_{1-3}, f_{1-4} on biohydrogen production cannot be underestimated based on these results as it is known that domination of each acid has major impact on the hydrogen yield [22]. Significance of these three parameters in our study are masked by constant stoichiometry approach used by us as the values for f_{1-2}, f_{1-3}, f_{1-4} and f_{1-5} are based on the stoichiometric coefficients in Eqs. (2)–(4) and experimentally determined COD distribution values (Fig. S1, Fig. 5) were permanently coded into the model. Therefore, variations in f_{1-2}, f_{1-3}, f_{1-4} will affect corresponding products but not hydrogen production. For example, Fig. 7e

shows significant effect of f_{1-5} (COD distribution towards hydrogen production) on hydrogen evolution i.e. 8% variation in standard deviation (408 ± 32). Sensitivity effect of the f-factors will become prominent in variable stoichiometry ADM1 framework suggested by Rodriguez et al. [15].

Reasonable sensitivity of hydrogen evolution to K_{S1} values is exhibited in this study (Fig. 7b): 7–8% and <2% variation in standard deviation during $t = 100$–200 h and during last 100 h of the whole duration. Further, K_{S1} value of 11 g COD/L, obtained from the calibration purpose, is nearly 20 times higher than that recommended in the original ADM1 study. High value of K_{S1} may have implications on high cell turn over and maintenance energy requirements for these fermentative bacteria, and may have resulted from the selected untypical, unbuffered nature of IPR22 fermentation system characterized by low temperatures. This value was readily reduced by nearly 10 fold in our high temperature reactors (CPR37 and IPR37). In another unpublished study, when the ADM1BM was calibrated with low value of K_{S1} (0.5 g COD/L) there was less than 1% variation in the standard deviation value of the hydrogen evolution.

The other important parameters affecting cumulative hydrogen production in increasing order of their significance are Y_1, K_1, pH_{UL}, pH_{LL} (413 ± 0.8, 413 ± 5, 407 ± 10, and 357 ± 90 mL H_2 respectively). Though 5% variation in values of K_1, Y_1, pH_{UL} varied the standard deviation of cumulative hydrogen production by less than 1%, the sensitivity of model towards these parameters is apparent due to variations in the temporal hydrogen production profiles (Fig. 7a, c, and h respectively).

Larger influence of K_1 value is due to the typical characteristics of coupled differential equations (Eqs. (5)–(12)), both cell growth and product formation dynamics are tied to substrate consumption and therefore ramping factor (K_1) in Eq. (6) exerts a major influence on the cumulative hydrogen production. Here, the selected K_1 value (30 g COD/g COD-day) is well-suited to COD rich glucose substrates and these values will be considerably low when less-preferential COD source such as domestic waste or wastewater are employed as feedstock for biohydrogen production. Similarly, biomass yield (Y_1) influences instantaneous biomass concentration, which directly influences substrate consumption rate (Eq. (6)) and corresponding relative shifts in cumulative hydrogen production (Eq. (12)). These results are slightly different from that of Batstone et al. [2] where they found that hydrolysis parameters are more significant and this disagreement is valid because ADM1 model was typically designed to model complex, particulate matter unlike readily degradable glucose substrates employed in our laboratory study.

Hydrogen evolution was more sensitive to pH_{LL} than to pH_{UL} (Fig. 7g, h). This observation on dramatic influence of low pH values on hydrogen evolution is in agreement with literature reports [8]. As mentioned in the introductory paragraphs, low extracellular pH conditions amplify the energy requirements for the transport of the undissociated acids outside the cell wall; proton uptake also decreases the availability of coenzyme A and phosphate pools to cause subsequent reduction in glucose flux through glycolysis [22]. Nearly 20–60% variation in standard deviation values of hydrogen production is apparent when pH_{LL} value is changed from the base value by 5%. Such pH variations are mitigated by using buffers and pH control, or special process conditions incorporated in our IPR22 reactor [9].

5. Conclusions

Experimental data from eight dark fermentation reactors varying in temperature, pressure release, biomass seed, and substrate were used to evaluate the predictive ability of the Gompertz equation and an ADM1-based model developed in this study. Even though the statistical quality of fit by the Gompertz equation was high, three reactor-specific parameters had to be found for each reactor to trace their hydrogen production profiles. In its generic form, Gompertz equation is also limited by its inability to predict volatile fatty acid formation and substrate consumption. The ADM1-based model was able to predict well not only the hydrogen profile ($r^2 > 0.91$), but also the COD ($r^2 > 0.88$), acetate ($r^2 > 0.76$), and butyrate ($r^2 > 0.75$). In contrast to the Gompertz equation, only one specific parameter per reactor had to be chosen in the case of the ADM1-based model. Based on the above findings, it can be concluded that the models and associated parameters adapted from ADM1 framework are appropriate for modeling fermentative hydrogen production.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ijhydene.2009.11.007.

REFERENCES

[1] Angenent LT, Wrenn BA. In: W JD, Harwood CS, Demain A, editors. Optimizing mixed-culture bioprocesses to convert wastes into biofuels; 2008. Washington.
[2] Batstone DJ, Keller J, Angelidaki I, Kalyuzhni SV, Pavlostathis SG, Rozzi A, et al. Anaerobic Digestion Model No. 1 (ADM1); 2002. Report No. 13.
[3] Sasikala K, Ramana CHV, Rao PR, Kovaks KL. Anoxygenic phototrophic bacteria: physiology and advances in hydrogen production technology. Advanced Applied Microbiology 1993;38:211–95.
[4] Prasad K, Maria S, Anne BT, Prawit K, Angelidaki I. Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresource Technology 2009; 100:2562–8.
[5] Hong Liu, Shaon C, Logan BE. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environmental Science & Technology 2005;39: 658–62.

[6] Sun M, Sheng G-P, Zhang L, Xia C-R, Mu Z-X, Liu X-W, et al. An MEC–MFC coupled system for biohydrogen production from acetate. Environmental Science & Technology 2009; 42(21):8095–100.
[7] Kapdan IK, Kargi F. Bio-hydrogen production from waste materials. Enzyme and Microbial Technology 2006;38(5): 569–82.
[8] Van Ginkel S, Sung S, Lay JJ. Biohydrogen production as a function of pH and substrate concentration. Environmental Science & Technology 2001;35(24):4726–30.
[9] Gadhamshetty V, David CJ, Nirmalakhandan N, Geoff BS, Deng S. Feasibility of biohydrogen production at low temperatures in unbuffered reactors. International Journal of Hydrogen Energy 2009;34(3):1233–43.
[10] Jianlong W, Wei W. Kinetic models for fermentative hydrogen production: a review. International Journal of Hydrogen Energy 2009;34(8):3313–23.
[11] Liang-Ming W, Chia-Jung H, Cheng S-S. A dual-substrate steady-state model for biological hydrogen production in an anaerobic hydrogen fermentation process. Biotechnology and Bioengineering 2006.
[12] Mu Y, Han-Quing, Wang G. Kinetic modeling of batch hydrogen production process by mixed anaerobic cultures. Bioresource Technology 2006;97(11):1302–7.
[13] Peiris BRH, Rathnashri PG, Johansen JE, Kuhn, Bakke R. ADM1 simulations of hydrogen production. Water Science and Technology 2006;53(8):129–37.
[14] Penumathsa BVK, Premier GC, Kyazze G, Dinsdale R, Guwy AJ, Esteves S, et al. ADM1 can be applied to continuous biohydrogen production using a variable stoichiometry approach. Water Research 2008;42(16): 4379–85.
[15] Rodriguez J, Lema JM, van Loosdrecht MCM, Kleerebezem R. Variable stoichiometry with thermodynamic control in ADM1. Water Science and Technology 2006;54(4):101–10.
[16] Rodriguez J, Lema JM, Kleerebezem R. Energy-based models for environmental biotechnology. Trends in Biotechnology 2008;26(7):266–374.
[17] Rodriguez J, Kleerebezem R, Lema JM, Van Loosdrecht MCM. Modeling product formation in anaerobic mixed culture fermentations. Biotechnology and Bioengineering 2006;93(3): 592–606.
[18] Gadhamshetty V, David CJ, Nirmalakhandan N, Geoff BS, Deng S. Dark and acidic conditions for fermentative hydrogen production. International Journal of Hydrogen Energy 2009;34(2):821–6.
[19] Gadhamshetty V, Johnson C, David, Nirmalakhandan N, Smith G, Deng S. Critical aspects of fermentative hydrogen production with mixed cultures. IWA World Water Congress and Exhibition; Vienna, Austria; Sep 7–12, 2008.
[20] Gadhamshetty V, Johnson C, David, Nirmalakhandan N, Smith G, Deng S. Intermittent pressure release for enhanced biohydrogen production. Vth International symposium on Anaerobic Digestion of Solid Wastes and Energy crops Vth ISAD-SW&EC; Hammamet, Tunisia; May 25–28, 2008.
[21] Gadhamshetty V, Anoop S, Nirmalakhandan N, Maung TM. Photofermentation of malate for biohydrogen production-A modeling approach. International Journal of Hydrogen Energy 2008;33:2138–46.
[22] Jones DT, Woods DR. Acetone–butanol fermentation revisited. Microbiological Reviews 1986;50(4):484–524.
[23] Khanal SK, Chen W-H, Li L, Sung S. Biological hydrogen production: effects of pH and intermediate products. International Journal of Hydrogen Energy 2005;29(11):1123–31.