An S-system Parameter Sensitivity Analysis of Biohydrogen Production by the Microalga *Chlamydomonas reinhardtii*

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Last modified: 1/4/2019 3:17 PM

Abstract

Producing biohydrogen on a commercial scale will likely require the genetic re-engineering of natural hydrogen-producing organisms. Kinetic modeling of hydrogen-producing metabolic pathways can cost-effectively help to characterize systemic (e.g., mass/energy/charge conservation) constraints in these organisms. In vitro kinetic studies suggest that the activity of the hydrogenases in several photolytic biohydrogen producers (PBPs) could be increased to as much as four times their nominal in vivo rate. It is much less clear, however, whether the in vitro activity maximum could be realized in vivo. Here I use an S-system photosynthesis-based PBP (PS-PBP) simulator to survey the sensitivity of C. reinhardtii to variation in system parameters. The analysis strongly suggests that the H_2 production efficiency of the alga cannot be increased by more than a factor of two through single-enzyme genetic modifications.

Keywords: biohydrogen, S-system, metabolic modeling

1.0 Introduction

Kinetic modeling of hydrogen-producing metabolic pathways can cost-effectively help to characterize systemic (e.g., mass/energy conservation) sensitivities in photolytic biohydrogen producers, even if all the details of hydrogen-gas producing metabolic pathways are not known. Among the more promising candidates for hydrogen-production optimization are photolytic biohydrogen producers (PBPs) such as the microalga *Chlamydomonas reinhardtii* ([7], [8]). It is generally held that the hydrogen-producing pathways in many PBPs incorporate segments of the PS-I and PS-II photosynthetic pathways ([6],[13]), and electrons from the anaerobic degradation of starch, to help accumulate the electron free energy required to allow a hydrogenase to convert protons to H_2 ([14]). *In vitro* kinetic studies suggest that the activity of hydrogenases \isolated from several PBPs could be increased to as much as four times their nominal *in vivo* rate ([1]). Here I use *bioh2gen* ([15]), an S-system ([2], [11]) PS-PBP kinetics simulator, to argue that within the context of the model, the H_2 production efficiency of *C. reinhardtii* cannot be increased by more than a factor of two through single-enzyme genetic modifications.

2.0 S-systems

An S-system ([11],[12]) is a power-law-oriented, finite-difference system of ordinary differential equations (SODE) each of whose dependent variables X_i is described by a kinetic equation of the form

$$dX_i/dt = \alpha_i \prod_j X_j^{gi\_j} - \beta_i \prod_j X_j^{hi\_j}$$

Eq. 2.1

where

- the left-hand side of Eq. 2.1 is the first derivative of X_i with respect to time
- i , j = 1, 2, 3, ..., N
- $\{X_i\}$ is the set of real-valued dependent variables of the system
- for any given X_i, only those independent and dependent variables X_j that have an action on X_i are included as factors in the products on the right-hand-side (RHS) of Eq. 2.1. The factors in the first term on the RHS of Eq. 2.1 correspond to just those entities that increase or inhibit the production of X_i; the factors in the second term of the RHS of Eq. 2.1 correspond to just those entities that contribute to, or inhibit, the consumption of X_i.
- $\alpha_i , \beta_i > 0$
- gi_j, hi_j are real-valued

There is a natural mapping from a biochemical map, K, to equations that have the form of Eq. 2.1. In particular, let $K = <\{X_k\}, E>$, $E \in \{X_k\} \otimes \{X_k\}$, k = 1, 2, …, N, be a directed graph in which each distinct $X_i \in \{X_k\}$ corresponds to a distinct variable (e.g., the concentration of a distinct chemical species in the map), and $w \in E$ if and only if $w = (X_m, X_n)$ is a directed edge in K , $m \neq n = 1, 2, ..., N$.

α_i and β_i are called *generalized rate constants* (or just rate constants) for X_i, and *gi_j* and *hi_j* are called the *generalized kinetic orders* (or just kinetic orders) for X_i, on analogy with standard chemical kinetic theory. The subexpression i_j indicates the action of X_j on X_i.

An S-system has several desirable features, including the fact that it is fully characterized by its rate constants and kinetic orders, allowing us to comprehensively survey the system's (logarithmic gain) sensitivity to its parameters. Any SODE can be *recast* ([10],[11]) as an S-system without loss of accuracy or precision; the recasting, however, is not in general unique. In addition to biochemical systems, S-systems have been successfully used to model epidemics, forest diversification, and world dynamics.

Performing (rate-constant, and kinetic-order) parameter logarithmic gain (sensitivity) surveys on S-systems is straightforward ([11], Chapter 7); performing parameter gain surveys on a SODE which is not an S-system, in general, is ill-defined.

3.0 A network model of hydrogen production in PS-PBPs

I will call bioH_2 producers that exploit portions of the PSII or PSI pathways "photosynthetic" PBPs (PS-PBPs). The schematized PS-PBP model used in the present study is shown in Figure 1 and is similar to [3], [4], [5], [9] and [14]. It represents a consensus working hypothesis held by the biohydrogen research community about the high-level metabolics of hydrogen production in PS-PBPs ([7]).

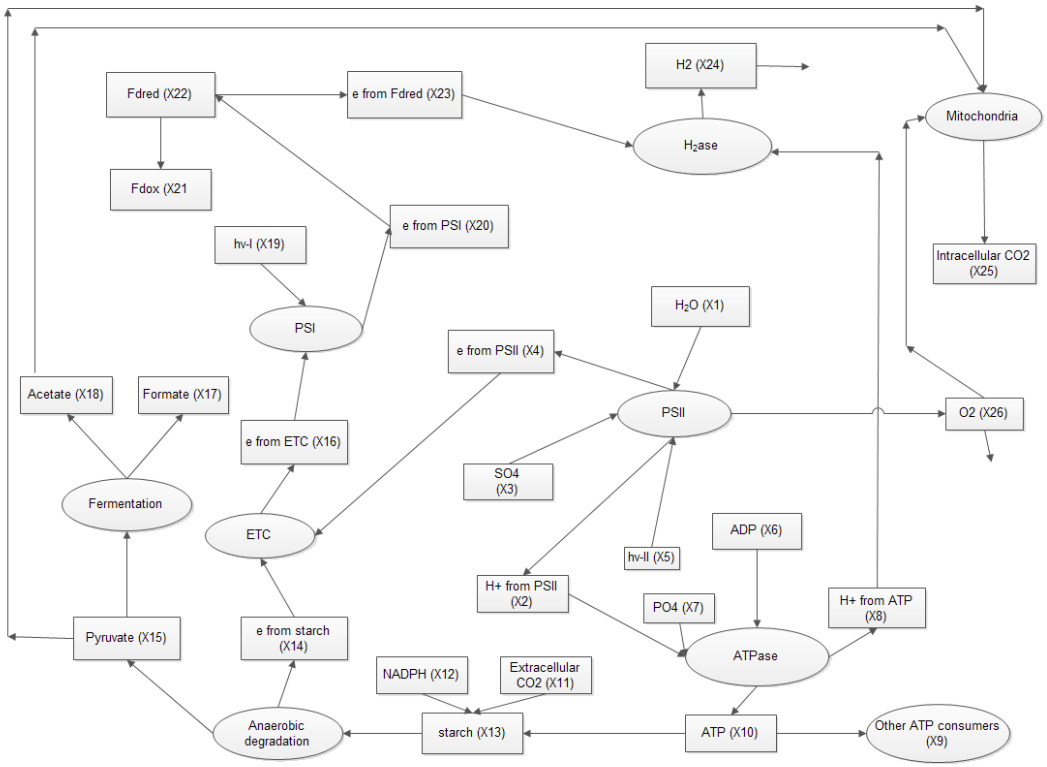

Figure 1. Schematized hydrogen producing metabolic network for PS-PBPs. Rectangles represent sources or sinks of physical quantities of interest (such as mass, concentration, or photon count) named in those rectangles, ellipses represent transforms (which may be complexes of reactions not individually modeled here), and an arrow from an ellipse to a rectangle means that the transform named in the ellipse affects the quantity/concentration of the chemical species named in the rectangle. Legend: PSI = photosynthesis stage I; PSII = photosynthesis stage II; SO4 = sulfate; hv-I = photons incident to PSI; hv-II = photons incident to photosynthesis PSII; ADP = adenosine diphosphate; ATP = adenosine triphosphate; PO4 = inorganic phosphate; O2 = oxygen gas; ATPase = adenosine triphosphatase; e from starch = electrons from anaerobic starch degradation; H2ase = hydrogenase; ETC = electron transport chain; e from PSII = electrons from PSII; e from PSI = electrons from PSI; Fdred = ferredoxin, reduced; Fdox = ferredoxin, oxidized; H2 = hydrogen gas; H+ from PSII = protons from PSII; H+ from ATP = protons from ATPase. Not all interactions exist in all PS-PBP species.

In sulfur-deprived *C. reinhardtii*, oxygen gas production under the experimental conditions of [7] (1-L, 6 x 10^6 cell/mL preparation) is about 1 mmol/h after beginning of sulfur deprivation, and spontaneously ceases ~10 h thereafter. 30 - 50 h after beginning of sulfur deprivation, the algae begins releasing hydrogen at a rate of ~0.17 millimole H_2/h (1-L, 6 x 10^6 cell/mL preparation) after beginning of sulfur deprivation. ~100 h after beginning of sulfur deprivation, hydrogen production ceases. These trajectories provide strong constraints on any model of bioH_2 production by *C. reinhardtii*.

The S-system equations used in this study are shown in Figure 2.

```
// protons from PSII
X2' = a2 X1^g2_1 X3^g2_3 X5^g2_5  -  b2 X10^h2_8 X2^h2_2 X5^h2_5

// e from PSII
X4' = a4 X1^g4_1 X3^g4_3 X5^g4_5 -  b4 X16^h4_16  X4^h4_4

//  protons from ATPase
X8' = a8 X6^g8_6 X7^g8_7 X2^g8_2 -  b8 X8^h8_8 X24^h8_24

// other ATP consumers
X9' = a9 X10^g9_10 -  b9 X9^h9_9

// ATP
X10' = a10 X2^g10_2 X7^g10_7 X6^g10_6 -  b10 X13^h10_13 X9^h10_9 X10^h10_10

// starch
X13' = a13 X12^g13_12 X11^g13_11 X10^g13_10  -  b13 X14^h13_14 X15^h13_15 X13^h13_13

// e from starch
X14' = a14 X13^g14_13 -  b14  X16^h14_16 X14^h14_14

// pyruvate
X15' = a15 X13^g15_13 -  b15 X25^h15_25 X18^h15_18 X17^h15_17 X15^h15_15

// e from ETC
X16' = a16 X14^g16_14 X4^g16_4 -  b16 X20^h16_20 X16^h16_16

// formate
X17' = a17 X15^g17_15  -  b17 X17^h17_17

// acetate
X18'  =  a18 X15^g18_15 -  b18 X15^h18_25 X18^h18_18

// e from PSI
X20' = a20 X16^g20_16  -  b20 X22^h20_22 X20^h20_20

// Fdox
X21' = a21 X22^g21_22 -  b21 X21^h21_21

// Fdred
// X22' = a22 X20^g22_20 -  b22 X21^g22_21 X23^g22_23 X22^h22_22

// e from Fdred
X23' =  a23 X22^g23_22 -  b23 X24^h23_24 X23^h23_23

// H2 gas
X24'  =  a24 X23^g24_23 X8^g24_8 -  b24 X24^h24_24

// Intracellular CO2
X25' = a25 X15^g25_15 X18^g25_18 X26^g25_26 -  b25 X25^h25_25
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// oxygen
X26' =  a26 X1^g26_1 X3^g26_3 X5^g26_5 -  b26 X26^h26_26 X25^h26_25 X5^h26_5
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Figure 2. S-system equations for the dependent variables used in this study. "^" is exponentiation. ">>" means "expression continuation". "'" means "first derivative with respect to time". Note that the equation for X2' has light as a *consumption* factor because activity *decreases* as light intensity increases above an optimal value.

Table 1 shows the values of the independent variables of the system. By fiat, these values remain constant during the simulation.

Table 1. Values of the independent variables of the system.

Independent variable	Value (relative units)
X1 (water)	1
X3 (SO4)	0.3
X5 (hv-II)	2.363
X6 (ADP)	100
X7 (PO4)	100
X11 (Extracellular CO2)	3e-3
X12 (NADPH)	1e-6
X19 (hv-I)	2.363

Much of the system in Figure 1 is based on PSII and PSI kinetics. Based on PSII/PSI kinetic data in [16], all generalized rate constants were set to 0.1, except a2 (= 3e-4), b2 (= 1e-4), a4 (=0.01), a24 (=1e-4), b24 (= 0.001), a26 (=10), and b26 (=1000); these exceptions were based on *in vitro* experimental values obtained in [7]. All generalized kinetic orders were set to 1.

bioh2gen and the model used in [14] differ in a few ways. First, following the conventions in [11] for modeling metabolic systems in the absence of gene-circuit dynamics, no enzyme is an explicit variable of *bioh2gen* ; several enzymes are variables in [14]. Second, *bioh2gen* employs more rate constants derived from experiment than does the model used in [14]. Third, all the kinetic orders in *bioh2gen* were set to 1; two kinetic orders were set to 2 in [14]. Fourth, *bioh2gen* study models the photon inputs to each of PSII and PSI individually; the model in [14] represents only the photon inputs to PSII.

The H_2 and O_2 production rates of *bioh2gen* were compared to [7], and the logarithmic gains ([11], Chapter 7) of the H_2 production rates were computed as a function of the generalized rate constants and kinetic orders in the model. Gains whose absolute values were less than 1 were excluded from consideration.

4.0 Results and discussion

Figure 3 show the hydrogen and oxygen output predicted by the model described in Section 3.0. The H_2 and O_2 outputs agree well with [7].

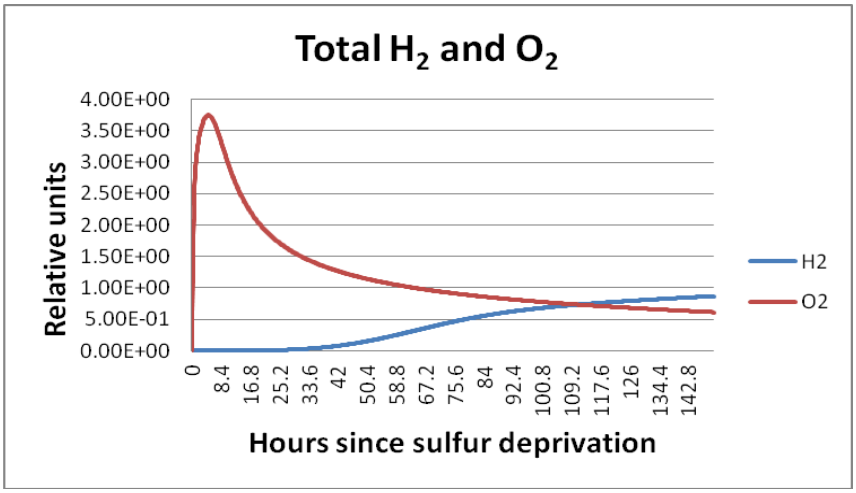

Figure 3. Total hydrogen and oxygen gas production as a function of time (units on the horizontal axis are hours after t_0). The values predicted by the model agree well with the results shown in [7].

The generalized rate constants in the model described in Section 3.0 exhibited no logarithmic gain $\geq$ 1. Figure 4 shows the logarithmic gain of H_2 gas production in the model to kinetic orders, if the sensitivity is $\geq$ 1. Changing generalized kinetic orders typically requires changing the genetics of enzymes associated with those kinetic orders. Logarithmic gains > ~10 can be opportunities for single-enzyme genetic modification; gains less than ~10 typically are not ([17]).

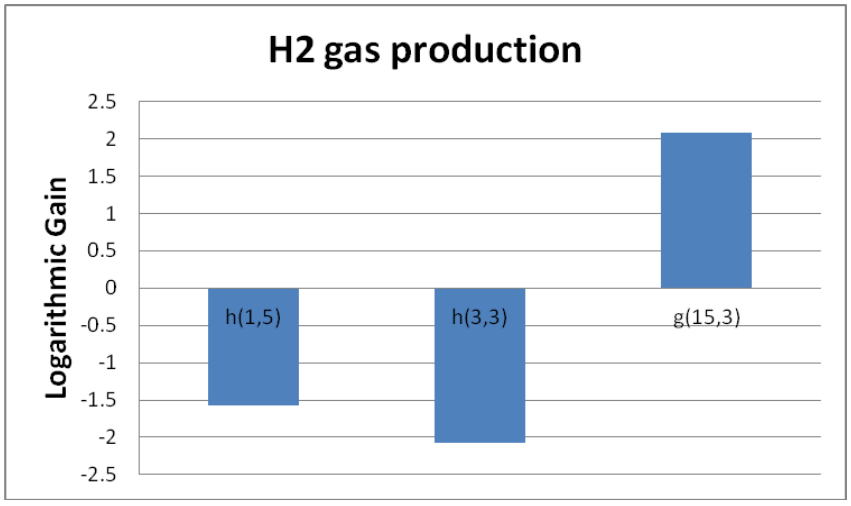

Figure 4. Logarithmic gain of H_2 production as a function of kinetic order. (The kinetic orders are shown with *PLAS* internal names. In each of the following, the PLAS internal name appears on the left-hand side of the equality; the name shown in Figure 1, on the right-hand-side: h(1,5) = h2_10; h(3,3) = h8_8; g(15,3) = g24_8. The gains suggest that ([11], p. 226) H_2 production efficiency cannot be increased by more than a factor of two through single-enzyme genetic modification.

Figure 4 strongly suggests that, within the model described in Section 3.0, the H_2 production efficiency of *C. reinhardtii* cannot be increased by more than a factor of two ([11], p. 226) through single-enzyme genetic modification. This is consistent with Figure 1: restrictions on the photolytically generated protons from PSII, and the proton turnover rate of ATPase, constrain the throughput of protons available to produce H_2. These results are consistent with the implications of [14].

5.0 Acknowledgements

This work benefited from discussions with Maria Ghirardi and Michael Seibert of the National Renewable Energy Laboratory, Anastasios Melis of the University of California/Berkeley, Anatoly Tsygankov of the Institute of Basic Biological Problems (Pushchino, Russia), Orlando Jorquera of the Federal University of Bahia, Murray Wolinsky of Los Alamos National Laboratory, and Jorge Soberón of the University of Kansas Biodiversity Institute. For any errors that remain, I am solely responsible.

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PLAS software appears is broken as of 1 January 2012. A copy of the software is available on request from the author of the present paper.

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