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GROWTH, CO_2 CONSUMPTION, AND H_2 PRODUCTION OF *ANABAENA VARIABILIS* ATCC 29413-U UNDER DIFFERENT IRRADIANCES AND CO_2 CONCENTRATIONS

Halil Berberoglu, Natasha Barra, Laurent Pilon*
Department of Mechanical and Aerospace Engineering
University of California
Los Angeles, California 90095
Email: pilon@seas.ucla.edu

Jenny Jay
Department of Civil and Environmental Engineering
University of California
Los Angeles, California 90095

ABSTRACT

Hydrogen production by cultivation of cyanobacteria in photobioreactors offers a clean and renewable alternative to thermochemical or electrolytic hydrogen production technologies with the added advantage of CO_2 mitigation. The objective of this study is to experimentally investigate the CO_2 consumption, growth, and H_2 production of the cyanobacteria Anabaena variabilis ATCC 29413-U under atmosphere containing argon and CO_2. Parameters investigated are irradiance and initial CO_2 mole fraction in the gas phase.

The CO_2 consumption half-time, defined as the time at which the CO_2 concentration in the gas phase decreases to half of its initial value, appears to be an appropriate time scale for modeling cyanobacterial CO_2 consumption, growth, and H_2 production. The half-time depends on both the initial CO_2 mole fraction and the irradiance. Also, two regimes of growth have been identified depending on irradiance. Below 5,000 lux, the irradiance and the initial CO_2 mole fraction have a coupled effect on cyanobacterial growth. Above 5,000 lux, growth depends only on the initial CO_2 mole fraction. Furthermore, the optimum initial CO_2 mole fraction around 0.05 has been identified for maximum growth and CO_2 consumption rates. The growth and CO_2 consumption were not inhibited by irradiance up to about 16,000 lux.

Finally, the proposed empirical models can be used in conjunction with mass transfer and light transfer models to design and optimize the operating conditions of a photobioreactor for maximum hydrogen production and/or CO_2 consumption.

NOMENCLATURE

C volumetric mass concentration, kg/m^3
G total irradiance in the spectral range from 400 to 700 nm, lux
OD optical density
t time, h
$t_{1/2}$ half-time, h
X cyanobacteria concentration, kg dry cell/m^3
$X_{avg,\Delta t}$ average cyanobacteria concentration in the time interval Δt, kg dry cell/m^3
x mole fraction
Y_{X/CO_2} biomass yield based on CO_2, kg dry cell/kg CO_2
α exponential constant
β slope of half-time versus initial CO_2 mole fraction in the gas phase, h
$\mu_{\Delta t}$ specific growth rate in the time interval Δt, 1/h
μ_{avg} average specific growth rate, 1/h
ψ_{CO_2} average specific CO_2 uptake rate, kg CO_2/kg dry cell/h

Subscript

CO_2 refers to carbon dioxide
g refers to gas phase
H_2 refers to hydrogen
i refers to a gas species
L refers to liquid phase
max refers to the maximum amount of a gas species produced by the cyanobacteria
O_2 refers to oxygen

*Address all correspondence to this author.

o refers to initial state

1 INTRODUCTION

Increased amounts of greenhouse gas emissions as well as the exhaustion of cheap fossil fuel resources are calling for clean and renewable energy sources. Hydrogen, to be used in fuel cells, is considered to be an attractive alternative fuel since water vapor is the only byproduct from its reaction with oxygen. Hydrogen production by cultivation of cyanobacteria in photobioreactors offers a clean and renewable alternative to thermochemical or electrolytic hydrogen production technologies with the added advantage of CO_2 mitigation. However, current photobiological hydrogen production technologies suffer from (i) insufficient carbon dioxide transfer from the gas phase to the microorganisms [1, 2], (ii) insufficient light transfer within the photobioreactor [3–5], and (iii) inhibition of hydrogen production due to excessive irradiance and/or oxygen concentration [3, 5]. In order to overcome these limitations and to render the photobioreactor technology suitable for simultaneous production of hydrogen fuel and mitigation of CO_2 at an industrial scale, it is necessary to understand and model the coupling of light transfer, microbial kinetics, and mass transfer of carbon dioxide, oxygen, and hydrogen. Based on such a model a novel photobioreactor configuration can be designed, optimized, and scaled-up. The objective of this paper is to experimentally investigate the CO_2 consumption, growth, H_2, and O_2 production of the cyanobacteria *Anabaena variabilis* ATCC 29413-U in atmosphere containing argon and CO_2 as functions of irradiance and initial CO_2 mole fraction in the gas phase.

During photobiological hydrogen production, light from the sun is absorbed by microorganisms such as algae, cyanobacteria or photosynthetic bacteria to produce hydrogen [6]. The reader is referred to Refs. [3, 5–8] for detailed reviews of photobiological hydrogen production. In brief, algae and cyanobacteria utilize water as their electron source [7]. The electrons generated from water splitting are used to produce hydrogen by either the enzymes hydrogenase or nitrogenase. The reaction catalyzed by hydrogenase is reversible and hydrogenase starts converting oxygen and hydrogen into water once the total pressure in the environment starts increasing [7]. On the other hand, the reaction catalyzed by nitrogenase is irreversible and can be sustained in ambient pressures as high as 50 atmospheres [7]. In particular, the cyanobacterium *Anabaena variabilis* ATCC 29413 produces hydrogen mainly using nitrogenase [5]. Moreover, *A.variabilis* is listed among the potential candidates for hydrogen production whose genome sequence has been completed [9]. Therefore, *A.variabilis* is chosen as the microorganism of interest in the present study.

The cyanobacterium *A.variabilis* is a photosynthetic prokaryote which uses CO_2 as its carbon source and sunlight as its energy source. Figure 1 shows a micrograph of a filament of *A.variabilis* composed of vegetative cells and heterocysts. It utilizes light energy in the spectral range from 400 to 700 nm, known as the photosynthetically active radiation (PAR) to produce biomass (i.e., multiply), oxygen, and hydrogen. As part of its nitrogen fixation metabolism, it generates hydrogen as a byproduct [5]. In the absence of molecular nitrogen, nitrogenase enzyme catalyzes the production of hydrogen by the reduction of protons [5]. However, functioning of nitrogenase, both for fixing nitrogen and/or producing hydrogen, is inhibited by excessive dissolved oxygen concentration in the growth medium [10]. *A.variabilis* has evolved to contain these enzymes in special cells called heterocysts [5] that can protect the functioning of nitrogenase enzyme up to dissolved O_2 concentrations of 300 μM [11].

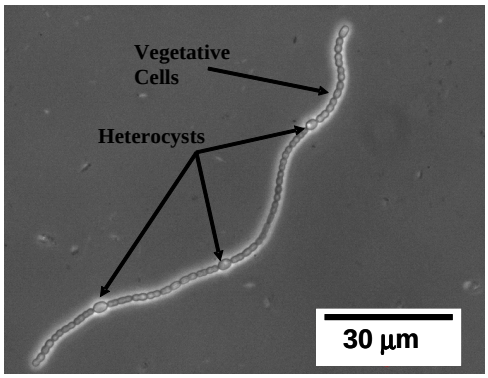

Figure 1. A micrograph showing the vegetative cells and heterocysts of *Anabaena variabilis*.

A.variabilis and its mutants have been studied extensively as hydrogen producers [8, 11–15]. Yoon *et al.* [12] studied the effect of irradiance and repeated CO_2 injections on the growth rate, CO_2 consumption, and H_2 production of *A.variabilis* ATCC 29413. The authors suggested a two stage reactor alternating between (i) the first stage designed for maximum CO_2 uptake and rapid cell growth with nitrates present in the medium, and (ii) the second stage designed for maximum H_2 production. In the first stage, for irradiance between 3,000 and 4,000 lux, increasing the CO_2 mole fraction above 0.08 in the gas phase decreased the specific cyanobacterial CO_2 consumption rate. In addition, for CO_2 mole fractions above 0.20, *A.variabilis* did not consume any CO_2 and subsequently growth was inhibited. This was attributed to the low pH induced by high concentrations of dissolved CO_2 in the growth medium. Moreover, repeated injections of CO_2 every 2 to 3 days in the head-space during the first stage resulted in an increase in specific H_2 production rate during the second stage. In the second stage, the medium was depleted of nitrates, the head-space was purged with argon and irradiance was increased to 10,000 to 12,000 lux to yield maximum specific hydrogen pro-

duction rate of about 83 mmol/kg dry cell/h (units converted at NTP with pressure of 101325 Pa and temperature of 293.15 K). More recently, Yoon *et al.* [16] doubled the specific hydrogen production rate of *A.variabilis* ATCC 29413 up to 165 mmol/kg dry cell/h in a three stage photobioreactor where they increased the irradiance between the first and the last stages for achieving high cyanobacteria concentration.

Markov *et al.* [17] also used *A.variabilis* (Kutzing) in a two stage photobioreactor to produce hydrogen at rates between 830 to 8300 mmol/kg dry cell/h (units converted at NTP with pressure of 101325 Pa and temperature of 293.15 K) over a period of 5 months. The authors immobilized the cyanobacteria on hollow fibers and successfully used partial vacuum (200 to 700 torr) instead of argon atmosphere during the hydrogen production stage to reduce cost. In a more recent study, Markov *et al.* [18] demonstrated that increasing irradiance during the first stage increased the hydrogen production rate in the subsequent second stage.

As opposed to using pure argon or partial vacuum, Tsygankov *et al.* [11] studied the hydrogen production by *Anabaena variabilis* ATCC 29413 and by its mutant PK84 in atmospheres containing different CO_2 and O_2 concentrations. The authors reported that the presence of CO_2 in the gas phase inhibited the hydrogen production rate of the wild strain due to inhibition by photosynthetically evolved oxygen. At increased oxygen concentrations, the wild strain consumed back the produced hydrogen along with the oxygen in a reaction catalyzed by the enzyme named uptake hydrogenase. At the dissolved oxygen concentration of 315 μM in the medium, hydrogen production rate of the wild strain was only 7% of the control experiment with zero dissolved oxygen concentration in the medium. On the other hand, the mutant PK 84 lacking the hydrogen uptake metabolism showed 75% of the hydrogen production rate of the control experiment. In addition, Happe *et al.* [15] demonstrated that genetically modified *A.variabilis* lacking uptake hydrogenase enzyme produced four times more hydrogen than the wild strain under similar conditions. Moreover, Tsygankov *et al.* [19] compared the potential and actual rates of hydrogen production by *Anabaena variabilis* ATCC 29413 and its mutant PK84 cultivated in a helical photobioreactor. They grew both strains under the same conditions with continuous sparging with a gas mixture containing 98% air and 2% CO_2 by volume. The authors reported that for both strains the highest rates of hydrogen production were observed during the exponential growth phase. Moreover, they observed that the wild strain produced hydrogen only in argon atmosphere whereas the uptake hydrogenase deficient mutant was able to produce hydrogen in the presence of both oxygen and nitrogen. Even when placed in argon environment containing 2% CO_2 by volume, the wild strain did not produce hydrogen. Finally, Tsygankov *et al.* [20] used *A.variabilis* PK-84 in an outdoor single stage tubular photobioreactor to produce hydrogen under continuous sparging with 98% air and 2% CO_2 mixture at a flow rate of 500 ml/min. The culture produced hydrogen continuously for up to 40 days under aerobic conditions without injection of fresh inoculum.

Most previous studies using *A.variabilis* have used a two stage photobioreactor with relatively limited ranges of CO_2 concentrations and light irradiance. In addition, to the best of our knowledge, there has been no reported systematic study of a photobiological process mitigating CO_2 and producing hydrogen under the same conditions. The objectives of this work are (i) to systematically study the simultaneous growth, CO_2 consumption, and H_2 production of *A.variabilis* under a wide range of irradiances and initial CO_2 mole fractions in the gas phase, (ii) to develop a method for interpreting the experimental data, and (iii) to provide recommendations on the optimum irradiance and the gas phase CO_2 mole fraction in the photobioreactor for achieving rapid growth, high CO_2 uptake, and H_2 production rates.

2 Materials and Methods

A cyanobacterial suspension is prepared and the initial microorganism concentration is adjusted to 0.02 kg dry cell/m^3 as confirmed by monitoring the optical density (OD). Then, 60 mL of the prepared suspension is dispensed in 160 mL serum vials. The vials are sealed with butyl rubber septa, crimped, and flushed through the septa with industrial grade argon, sterilized with 0.2 μm pore size syringe filter, for 10 minutes. The initial CO_2 mole fraction in the head-space, denoted by $x_{CO_2,g,o}$, is adjusted to 0.03, 0.04, 0.08, 0.15, and 0.20. This is achieved first by adjusting the gage pressure in the vials to -7.09, -10.13, -20.27, -30.40, and -40.53 kPa, respectively. Then, 7, 10, 20, 30, and 40 mL of industrial grade CO_2 were injected into the vials, respectively, through a 0.2 μm pore size syringe filter. Finally, the vials were shaken until the pressure stabilized and the head-spaces were sampled to measure the initial CO_2 mole fractions. Each vial is prepared in duplicates. The vials are placed horizontally on an orbital shaker (model ZD-9556 by Madell Technology Group, USA) and stirred continuously at 115 rpm throughout the duration of the experiments. Continuous illumination is provided from the top of the orbital shaker. The irradiance, defined in this work as the total radiant flux of visible light from 400 to 700 nm incident from all directions on a unit surface area, ranges from 1,120 to 16,100 lux.

Throughout the experiments CO_2, H_2, and O_2 concentrations in the head-space as well as the cyanobacteria concentration and pH in the liquid phase were continually monitored. In addition, the temperature and pressure of the vials are measured in order to convert the molar fractions of gas species into volumetric mass concentrations. The irradiance incident on individual vials is recorded. Details of the experimental setup and procedures are given in the following sections.

Cyanobacteria Culture and Concentration Measurements

Anabaena variabilis ATCC 29413-U^{TM} was purchased from the American Type Culture Collection (ATCC) and received in freeze dried form. The culture was activated with 10 mL of sterilized milli-Q water. The culture is then, transferred weekly and cultivated in ATCC medium 616 with air-CO_2 mixture in the head-space with an initial mole fraction of CO_2 of 0.05. One liter of ATCC medium 616 contains 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 6.0 *mg* citric acid, 6.0 *mg* ferric ammonium citrate, 0.02 g Na_2CO_3, 1.0 *mg* EDTA, and 1.0 *mL* of trace metal mix A5. One liter of trace metal mix A5 contains 2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.39 g $Na_2MoO_4 \cdot 2H_2O$, 0.079 g $CuSO_4 \cdot 5H_2O$, 49.4 *mg* $Co(NO_3)_3 \cdot 6H_2O$. The pH of the medium is adjusted to be 7.3 by adding 1M HCl and/or 1M NaOH. Then, 20 mL of HEPES buffer solution at pH 7.3 is added to one liter of medium. Finally, the medium is autoclaved at 121^oC for 40 minutes.

The cyanobacteria concentration, denoted by X and expressed in kg dry cell/m^3, is determined by sampling 1 mL of bacteria suspension from the vials and measuring the optical density (OD). A calibration curve is created by measuring both the dry cell weight of a cyanobacteria suspension and the corresponding OD. First, the OD of the cyanobacteria is measured in disposable polystyrene cuvettes with light path of 10 mm at 683 nm using a UV-Vis spectrophotometer (Cary-3E by Varian, USA). Then, the bacteria suspension is filtered through mixed cellulose filter membranes with 0.45 μm pore size (HAWP-04700 by Millipore, USA) and dried at 85^oC over night. The dried filters are weighed immediately after being taken out of the oven on a precision balance (model AT261 by Delta Range Factory, USA) with a precision of 0.01 mg. The calibration curve for OD is generated by using 14 different bacteria concentrations ranging from 0.04 to 0.32 kg dry cell/m^3. The relation between OD and bacteria concentration is linear for the OD range from 0 to 1.2 and one unit of OD corresponds to 0.274 kg dry cell/m^3.

Temperature, Pressure, and pH

The temperature of the vials is measured with a thermocouple (Dual Thermometer, Fisher Scientific, USA). The heat from the high intensity fluorescent bulbs is removed by convective cooling using a fan to maintain a steady-state temperature of 24 ± 1^oC throughout all experiments. The head-space pressure is monitored with a digital gage pressure sensor (model PX26-005GV by Omega Heater Company, USA) connected to a digital meter (model DP25B-S by Omega Heater Company, USA). Finally, the pH of the medium is measured with a digital pH probe (model Basic AB Plus, Fisher Scientific, USA).

Lighting and Light Analysis

The irradiance incident on the vials, denoted by G, is varied by changing the number of fluorescent light bulbs (Ecologic by Sylvania, USA and Fluorex by Lights of America, USA).The irradiance incident on the vials is measured with both a light meter (Fisherbrand Tracable Meter by Fisher Scientific, USA) and a quantum sensor (LI-COR, Model LI-190SL, LI-COR Inc., USA). Irradiance on each vial is measured individually. Due to experimental difficulties in achieving the exact same irradiance for all vials, five different irradiance ranges are explored namely, 1120-1265 lux, 1680-2430 lux, 3950-4600 lux, 7000-8700 lux, and 14,700-16,100 lux.

Gas Analysis

The gas analysis is carried out by sampling 500 μL of head-space volume of the vials every 24 hours. The concentrations of CO_2 and H_2 in the head-space are measured with a gas chromatographer (HP-5890, Hewlett Packard) equipped with a packed column (Carboxen-1000 by Supelco, USA) and a thermal conductivity detector (TCD). The gas chromatographer output is processed with an integrator (HP-3395, Hewlett Packard, USA). Throughout the gas analysis, the injector and detector temperatures are maintained at 120^oC. During the H_2 analysis argon is used as the carrier gas and the oven temperature is maintained at 35^oC. The retention times for H_2 is found to be 2.1 minutes. On the other hand, during the CO_2 analysis, Helium is used as the carrier gas and the oven temperature is maintained at 255^oC. The retention time for CO_2 is then 4.9 minutes. Calibration curves for the TCD response are prepared at seven different known gas concentrations from 16×10^{-6} to 3.2×10^{-3} kg/m^3 for H_2 and from 3.96×10^{-3} to 352×10^{-3} kg/m^3 for CO_2. All calibration curves are linear within these ranges of gas concentrations. During the experiments, peak heights are recorded and correlated to the corresponding gas amounts using the respective calibration curves.

3 Results and Analysis

In brief, the initial CO_2 mole fraction in the head-space, $x_{CO_2,g,o}$, varies from 0.03 to 0.20 while the irradiance G varies from 1,120 to 16,100 lux. Pressure, temperature, and pH are maintained at 1 ± 0.1 atm., 24 ± 1^oC, and 7.3 ± 0.5, respectively. In order to develop semi-empirical models for CO_2 consumption, growth, and H_2 production by *A.variabilis* ATCC 29413 using the experimental data, the following assumptions are made:

1. The concentration of gases in each phase and the concentration of cyanobacteria in the liquid phase are uniform within each vial and all cyanobacteria in the vial are subject to the same irradiance, owing to the vigorous stirring provided by the orbital shaker.
2. Metabolic reactions of the cyanobacteria are not mass transfer limited due to the small volume of the cyanobacteria sus-

pension and the continuous shaking.

3. The changes in the concentrations of gas species in the head-space are solely due to cyanobacterial activity. Indeed, there are no sources or sinks for the gas species other than the cyanobacteria in the vials and the gas species in the liquid and gas phases are in quasi-equilibrium at all times.
4. The only parameters affecting the bacterial growth and product formation are the CO_2 concentration and the irradiance G. The supply of other nutrients such as minerals and nitrates are assumed to be unlimited in the growth medium.
5. The death phase of microorganisms is not considered since this phase is not of interest for hydrogen production.

First, the experimental data was fitted with (i) the Monod model [21] using the CO_2 concentration in the liquid phase and/or the incident irradiance G as well as (ii) the modified Andrew's model [22, 23] accounting for CO_2 inhibition using the statistical procedure proposed by Smith *et al.* [24]. It was found that the values of the empirical constants were not consistent from one experiment to another. Therefore, new models for CO_2 consumption and bacterial growth, as well as hydrogen and oxygen productions are presented in this study.

CO_2 Consumption

Figure 2(a) shows the evolution of the CO_2 molar fraction $x_{CO_2,g}$ in the head-space as a function of time t, normalized with the initial CO_2 mole fraction $x_{CO_2,g,o}$ for different combinations of irradiance G and $x_{CO_2,g,o}$. It indicates that $x_{CO_2,g}$ decreases monotonically with increasing time.

First, the half-time, denoted by $t_{1/2}$, is defined as the time required for the CO_2 mole fraction in the gas phase to decrease to half of its initial value. Normalizing the time by the half-time and plotting the dimensionless variables $x_{CO_2,g}/x_{CO_2,g,o}$ versus $t/t_{1/2}$, collapses all the data points to a single line as shown in Figure 2(b). Performing a linear regression analysis of the data yields,

$$\frac{x_{CO_2,g}}{x_{CO_2,g,o}} = 1 - 0.5\left(\frac{t}{t_{1/2}}\right)^{1.2} \qquad (1)$$

with a correlation coefficient $R^2 = 0.94$. Equation (1) also indicates that $x_{CO_2,g}$ vanishes at time $t = 1.8t_{1/2}$.

Moreover, the half-time $t_{1/2}$ is a function of both the initial CO_2 mole fraction and the irradiance G. Figure 3(a) indicates that $t_{1/2}$ increases linearly with $x_{CO_2,g,o}$ for a given G, i.e., $t_{1/2} = \beta(G)x_{CO_2,g,o}$, where the slope $\beta(G)$ is expressed in hours and plotted in Figure 3(b). Two regimes can be identified: in the first regime, $\beta(G)$ decreases linearly with G according to $\beta(G) = 1900 - 0.3G$; in the second regime, $\beta(G)$ does not vary appreciably with G and has the approximate value of 350 hours.

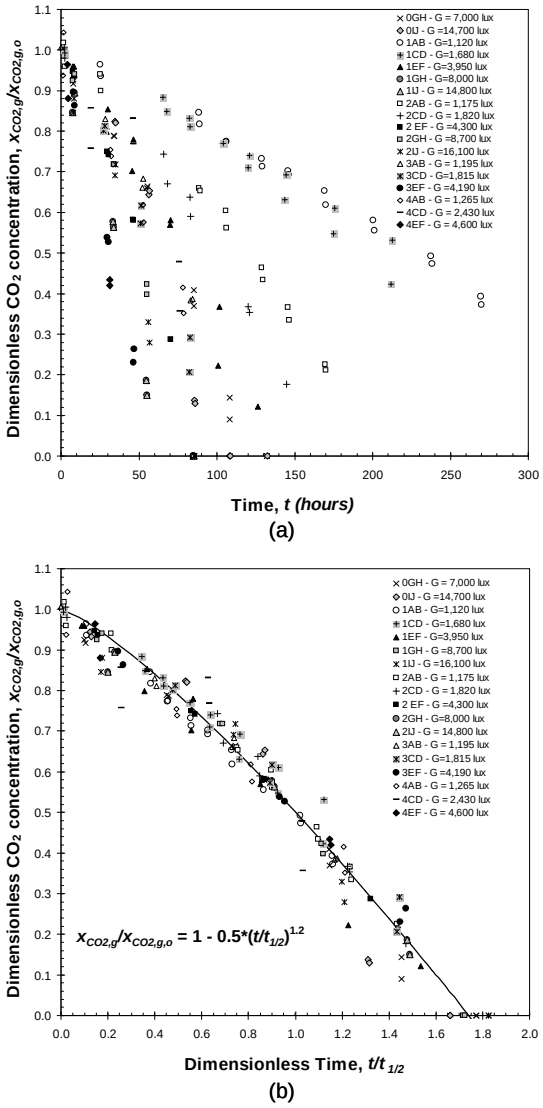

Figure 2. (a) Normalized CO_2 consumption data versus time, (b) Normalized CO_2 consumption data versus dimensionless time.

Figure 3(b) indicates that transition between the two regimes occurs around $G = 5,000$ lux. Therefore, the half-time $t_{1/2}$ can be expressed as,

$$\begin{aligned} t_{1/2} &= (1900 - 0.3G)x_{CO_2,g,o} \quad \text{for} \quad G \leq 5,000 \quad \text{lux} \\ t_{1/2} &= 350x_{CO_2,g,o} \quad \text{for} \quad G > 5,000 \quad \text{lux} \end{aligned} \qquad (2)$$

Thus, for $G \leq 5,000$ lux, increasing the irradiance enhances the CO_2 consumption rate of the cyanobacteria. However, beyond 5,000 lux the irradiance has no appreciable effect on $t_{1/2}$ which depends only on $x_{CO_2,g}$. Alternatively, the relationship between

β and G can be approximated with an exponential decay function as $\beta(G) = 350 + 1300\exp(9x10^{-8}G^2)$.

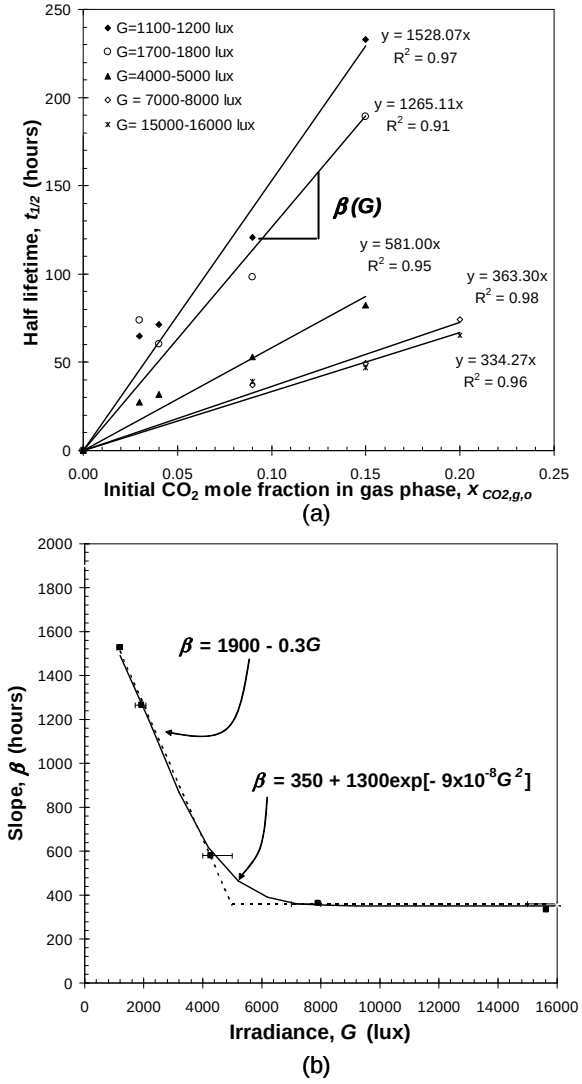

Figure 3. (a) Half-time as a function of $x_{CO_2,g,i}$, (b) Slope β as a function of irradiance G.

$t_{1/2}$ collapses the growth curves for different irradiances onto a single line as shown in Figure 4(b). Therefore, the half-time $t_{1/2}$ correctly captures the time scale of the biological processes for CO_2 consumption and bacterial growth.

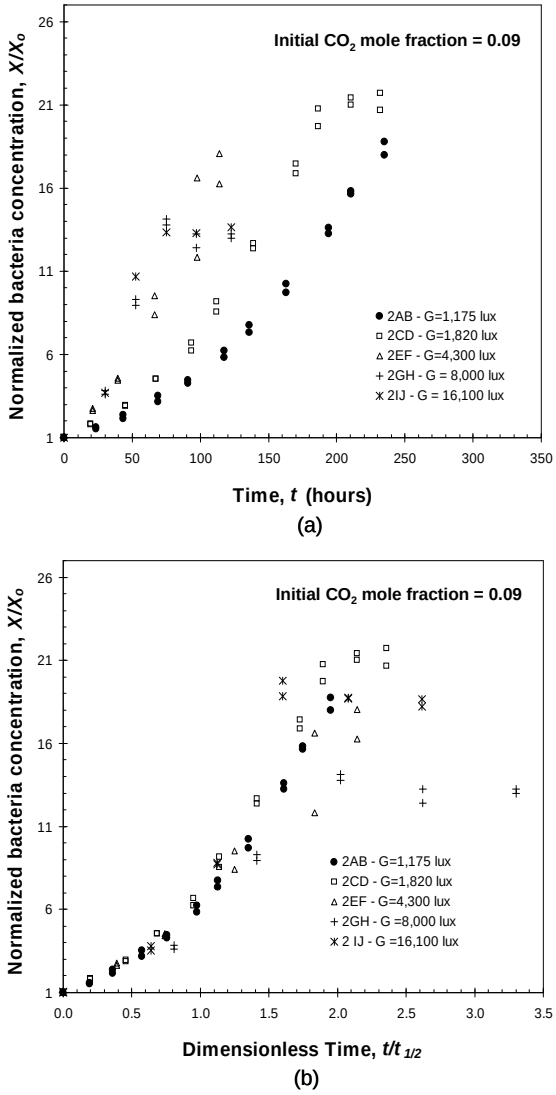

Figure 4. Normalized cyanobacteria concentrations for $x_{CO_2,g,o} = 0.09$ at all irradiances as a function of (a) time, and (b) dimensionless time

Cyanobacterial Growth

Figure 4(a) shows the normalized concentration of *A.variabilis*, X/X_o, versus time for all irradiances and for $x_{CO_2,g,o} = 0.09$. The initial cyanobacteria concentration X_o is equal to 0.02 kg dry cell/m^3 in all cases. It establishes that for a given $x_{CO_2,g,o}$, increasing the irradiance increases the growth rate of *A.variabilis*. Here also, scaling the time with the half-time

In addition, the cyanobacterial growth is exponential and the cyanobacteria concentration $X(t)$ at time t can be expressed as,

$$\frac{X(t)}{X_o} = \exp\left(\frac{\alpha}{t_{1/2}}t\right) \tag{3}$$

where α is a constant depending on $x_{CO_2,g,o}$ and can be expressed as,

$$\alpha = 4x_{CO_2,g,o}^{0.35} \tag{4}$$

with a correlation coefficient R^2=0.93. Note that the evolution of $X(t)$ as a function of the irradiance G and $x_{CO_2,g,o}$ is accounted for through the half-time $t_{1/2}$ given by Equation (2).

Figure 5(a) exhibits the variation of the average specific growth rate of *A.variabilis* denoted by μ_{avg} and expressed in h^{-1}, as a function of $x_{CO_2,g,o}$ for all irradiances. The average specific growth rate is the arithmetic average of the specific growth rates, denoted by $\mu_{\Delta t}$ and determined in the time intervals Δt during the exponential growth phase of *A.variabilis* according to,

$$\mu_{\Delta t} = \frac{\Delta X}{\Delta t}\frac{1}{X_{avg,\Delta t}} \tag{5}$$

where $X_{avg,\Delta t}$ is the average cyanobacteria concentration during that time interval. The error bars indicate the standard error. Figure 5(a) establishes that an optimum $x_{CO_2,g,o}$ around 0.05 exists for maximum average specific growth rate for all irradiances. Moreover, it shows that the average specific growth rate increases with increasing irradiance up to 16,000 lux. This observation contradicts the results reported by Yoon *et al.* [12]. The authors indicated that for $x_{CO_2,g,o}$ around 0.11 the average specific growth rate decreased from 0.054 to 0.046 h^{-1} as the irradiance increased from 3,500 to 7,000 lux. In the current study, μ_{avg} increased from 0.028 to 0.038 h^{-1} for the same conditions.

Furthermore, the average specific CO_2 uptake rate, denoted by ψ_{CO_2} and expressed in kg/kg dry cell/h, is computed using the same method as that used by Yoon *et al.* [12],

$$\psi_{CO_2} = \frac{\mu_{avg}}{Y_{X/CO_2}} \tag{6}$$

where Y_{X/CO_2} is the biomass yield based on CO_2 expressed in kg dry cell/kg of CO_2. The latter is computed as the ratio of the final mass of cyanobacteria produced to the total mass of CO_2 injected into the vials. Figure 5(b) shows the variation of ψ_{CO_2} as a function of $x_{CO_2,g,o}$ for all irradiances. The average specific CO_2 uptake rate exhibits similar trends to those of the average specific growth rate with an optimum $x_{CO_2,g,o}$ around 0.05 for maximum ψ_{CO_2}. Yoon *et al.* [12] reported an average specific CO_2 uptake rate of about 0.130 kg CO_2/kg dry cell/h for $x_{CO_2,g,o}$ around 0.05 and irradiance around 4,000 lux, whereas, in the present study, it was only 0.060 kg CO_2/kg dry cell/h under the same conditions. The difference can be attributed to the fact that the experiments of the present study were conducted at 24^{o}C instead of 30^{o}C in

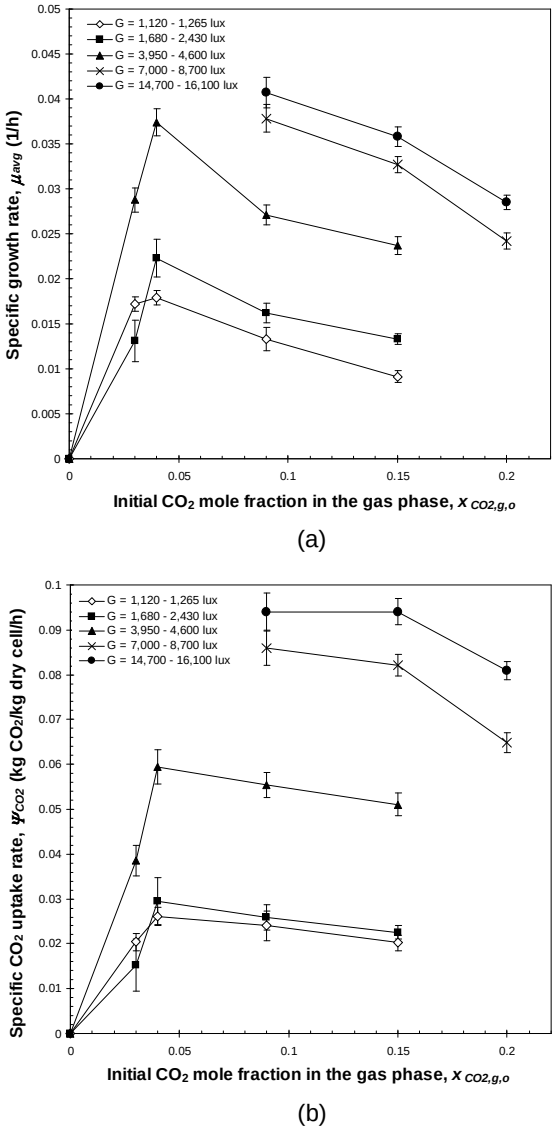

Figure 5. (a) The average specific growth rate μ_{avg}, and (b) the average specific CO_2 uptake rate ψ_{CO_2} of *A.variabilis* as functions of $x_{CO_2,g,o}$ at all irradiances.

Yoon *et al.* [12]. It is apparent that increasing the temperature enhances the CO_2 uptake metabolism of *A.variabilis* as confirmed by Tsygankov *et al.* [19]. Finally, Figure 5 shows that changes in irradiance above 5,000 lux does not yield as significant effect on μ_{avg} and ψ_{CO_2} as changes in irradiance below 5,000 lux. Note that due to experimental difficulties in capturing fast CO_2 consumption rate with the available equipment no experiments were conducted for initial CO_2 mole fraction less than 0.08 for irradiances higher than 5,000 lux.

Hydrogen Production

Figure 6(a) shows the concentration of hydrogen measured in the head-space as a function of the dimensionless time $t/t_{1/2}$ for all runs. It indicates that maximum hydrogen concentrations are achieved at high irradiance. Moreover, the concentration of hydrogen accumulated in the head-space normalized with its maximum value $C_{H_2,g,max}$ as a function of dimensionless time $t/t_{1/2}$ at irradiance levels higher than 7,000 lux is shown in Figure 6(b). It establishes that $C_{H_2,g}/C_{H_2,g,max}$ varies exponentially with $t/t_{1/2}$ and can be expressed as,

$$\frac{C_{H_2,g}(t)}{C_{H_2,g,max}} = \exp\left[4.45\left(\frac{t}{t_{1/2}}\right) - 6.1\right] \tag{7}$$

The decrease in hydrogen concentration observed beyond the exponential growth phase can be attributed to uptake hydrogenase which consumes back the produced hydrogen [11].

Finally, $C_{H_2,g}$ reaches its maximum at dimensionless time $t/t_{1/2}$ equal to 1.37, shortly before the CO_2 concentration vanishes at $t/t_{1/2}$ equal to 1.8 and growth stops around $t/t_{1/2}$ equal to 2.0 as shown in Figure 4. Note that hydrogen is both produced and consumed by the cyanobacteria and the measured $C_{H_2,g}$ is the net hydrogen concentration in the gas phase. The fact that $C_{H_2,g}$ increases exponentially during the exponential growth phase, and decreases after growth stops implies that the produced hydrogen in the present study is a growth related product unlike the results reported by Yoon *et al.* [12].

In order to use Equation (7) to determine the evolution of hydrogen concentration, the maximum concentration $C_{H_2,g,max}$ must be expressed in terms of the initial CO_2 mole fraction $x_{CO_2,g,o}$ and irradiance G. However, it is difficult to establish a simple relationship between $C_{H_2,g,max}$ and the parameters G and $x_{CO_2,g,o}$ due to the complexity of the hydrogen metabolism of *A.variabilis*. This complexity arises because (i) the hydrogen production is a strong function of both the irradiance G and the initial CO_2 concentration [18], and (ii) the produced hydrogen is being consumed back by the microorganisms at a rate comparable to that of the production of hydrogen [11]. Tsygankov *et al.* [11] reported that presence of CO_2 in the gas phase inhibited the hydrogen production of the wild strain *A.variabilis* ATCC 29413 due to production of oxygen by photosynthesis. In contrast, the present study indicates that hydrogen production by the wild strain is possible under argon and CO_2 atmosphere albeit at a lower production rate. The maximum hydrogen production observed in our experiments was 0.3 mmol/kg dry cell/h whereas reported rates for wild *A.variabilis* strains range from 5.58 mmol/kg dry cell/h in dark fermentation [25] to 165 mmol/kg dry cell/h in a three stage photobioreactor [16], and to 720 mmol/kg dry cell/h under nutritional stress [26]. The main reason for the low hydrogen production in our experiments

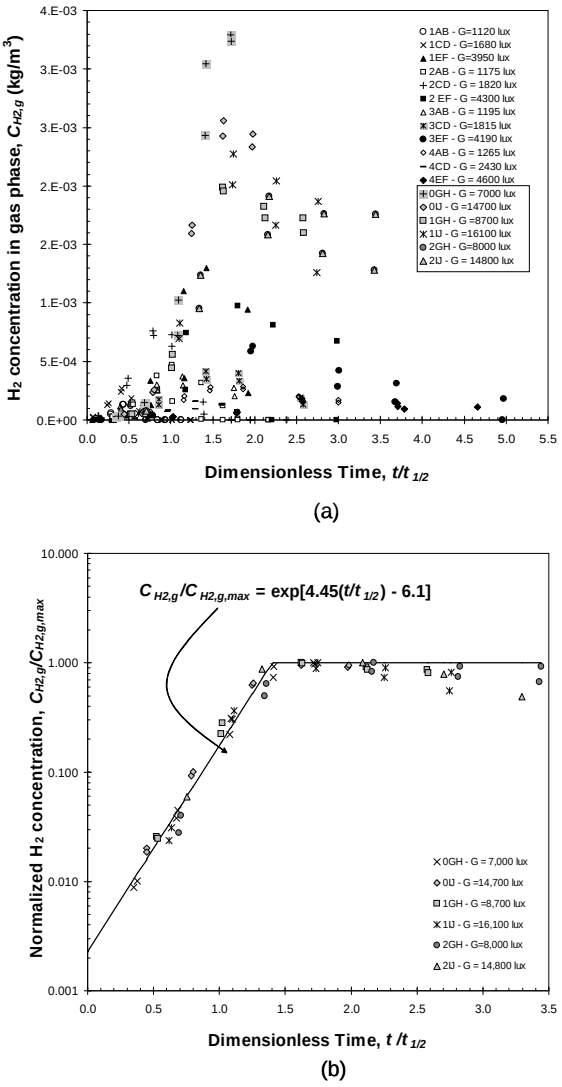

Figure 6. (a) Concentration of hydrogen accumulated in the head-space against dimensionless time. (b) Hydrogen production during high irradiance experiments normalized with the maximum concentration produced versus dimensionless time.

is attributed to the presence of nitrates and accumulation of excessive oxygen in the vials [11, 25].

Figure 7(a) shows $C_{H_2,g,max}$ as a function of both irradiance and the initial CO_2 mole fraction. It shows that within the parameter ranges explored, the optimum irradiance for maximum H_2 production was around 10,000 lux. Figure 7(b) shows $C_{H_2,g,max}$ as a function of $x_{CO_2,g,o}$ for irradiances larger than 7,000 lux for which H_2 production is the largest. It shows that $C_{H_2,g,max}$ increases with increasing $x_{CO_2,g,o}$. As a first order approximation, the relationship between $C_{H_2,g,max}$ and $x_{CO_2,g,o}$ for $G \geq$7,000 lux

can be written as,

$$C_{H_2,g,max} = 1.50 \times 10^{-2} x_{CO_2,g,o} - 3.75 \times 10^{-4} \qquad (8)$$

with a correlation coefficient $R^2 = 0.75$.

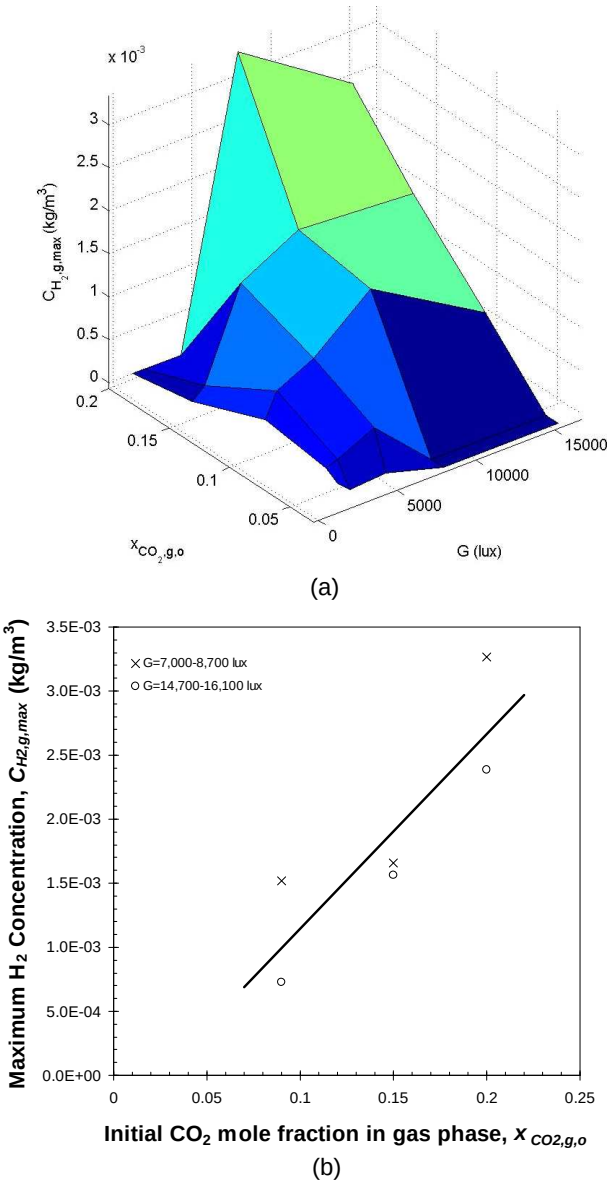

Figure 7. (a) Maximum concentration of hydrogen accumulated in the head-space as a function of the initial CO_2 mole fraction and irradiance. (b) $C_{H_2,max}$ as a function of $x_{C_{CO_2},g,o}$ for two of the highest irradiances.

4 Conclusions

A parametric experimental study has been performed to assess the CO_2 consumption, growth, and H_2 production of the cyanobacteria *Anabaena variabilis* ATCC 29413-U using irradiance and the initial CO_2 mole fraction in the head-space as parameters. The microorganisms were grown in atmosphere containing argon and CO_2, at a constant pH of 7.3 with nitrates in the medium. A new dimensionless analysis for CO_2 consumption, growth, and H_2 production is presented. It shows that the relevant time scale is the CO_2 consumption half-time, defined as the time when the CO_2 mole fraction in the gas phase decreases to half of its initial value, is expressed in terms of irradiance and the initial CO_2 mole fraction. The following conclusions can be drawn,

1. Light saturation for CO_2 consumption and growth occurs at irradiances greater than 5,000 lux.
2. Optimum irradiance for maximum H_2 production has been found to be around 10,000 lux despite the low overall H_2 production during the experiments due to nitrate and O_2 inhibitions.
3. *A.variabilis* could simultaneously consume CO_2, grow, and produce H_2.
4. For maximum specific CO_2 consumption and specific growth rates of *A.variabilis*, the optimum initial CO_2 mole fraction in the gas phase is about 0.05 for any irradiance between 1,000 and 16,000 lux.
5. Neither the CO_2 consumption nor the growth of *A.variabilis* was inhibited by irradiance up to about 16,000 lux.

Finally, the derived semi-empirical models can be used in conjunction with mass transfer and light transfer models [27] to optimize the operating conditions of a photobioreactor for maximum CO_2 consumption and rapid cyanobacteria cultivation. In addition, the analysis method presented here can be used for analyzing other photobiologically H_2 producing microorganisms.

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