Solar Photobiochemistry: Simultaneous Photoproduction of Hydrogen and Oxygen in a Confined Bioreactor

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Simultaneous photoevolution of hydrogen and oxygen by an aqueous suspension of the unicellular green alga *Chlamydomonas reinhardtii* was measured in a confined bioreactor. The headspace above the liquid was about three times the volume of the liquid. The objective of the experiments was to determine the extent to which Henry's Law partitioning between the liquid and gas phases of the reactor volume can be used to minimize the antagonistic effect of photosynthetically produced oxygen on the evolution of molecular hydrogen by photosynthesis. The results indicate, to within upper limits, that light-driven oxygen and hydrogen produced by microalgal water oxidation in the aqueous phase can partition favorably into the gas phase such that photosynthetically produced oxygen does not inhibit the hydrogenase enzyme. Continual cycles of light-driven hydrogen and oxygen production, with intervals of added gas-phase carbon dioxide for rejuvenating the algae, were performed for about 60 days on the same culture. Absolute yields of hydrogen and oxygen plus computed stoichiometric ratios are reported. The average stoichiometric ratio of hydrogen to oxygen was 2.8, indicating that reducing equivalents for molecular hydrogen were derived from endogenous reductants, most likely starch, as well as water. Additional experiments on the effect of illumination and dwell time on hydrogen and oxygen yields are presented, as are "before" and "after" photomicrographs of the algae, illustrating the effects of prolonged anaerobiosis on the cells. A mathematical model of gas formation in the liquid phase and its equilibrium with the headspace above the liquid is presented. An estimate of the mass transfer coefficient on movement of oxygen from the liquid phase into the gas phase yielded a time constant of less than 2 s. This characteristic time is much shorter than the time scale over which the accumulated yields of hydrogen and oxygen are measured. The data suggest that Henry's Law partitioning may be a rational approach to addressing the issue of oxygen sensitivity of algal hydrogen production, especially when combined with *C. reinhardtii* mutants that are selected for improved oxygen tolerance.

Introduction

Photosynthesis is the conversion of electromagnetic energy into stored chemical energy. According to the standard Z-scheme model of photosynthesis, two light reactions, Photosystems I and II, cooperate in serial light-activated electron transport oxidation−reduction processes.[1] The source of electrons is water, which is photooxidized to produce atmospheric oxygen, whereas atmospheric carbon dioxide is normally the electron acceptor, whose reduction products form the basis for plant storage and structural materials. The basic equation of photosynthesis is usually written as $H_2O + CO_2 + \text{light} \rightarrow O_2 + (CH_2O)$. Although the equation is relatively simple, multiple cooperative interfacial and intramembrane reactions are required to achieve this reaction. Under CO_2-free anaerobic conditions, a variant of this reaction, $H_2O + \text{light} \rightarrow H_2 + {}^1/_2O_2$, can occur for a select group of unicellular algae. This reaction is known as the biophotolysis of water.[2]

As discovered by Gaffron and Rubin,[3] certain algae such as *Chlamydomonas reinhardtii* are capable of synthesizing a hydrogenase enzyme that can catalyze the evolution of molecular hydrogen by proton reduction, $2H^+ + 2e^- \rightarrow H_2$. However, hydrogenase activity and hydrogen production are not part of normal algal photosynthesis since the enzyme is synthesized only in the absence of oxygen and, once synthesized, is inactivated by molecular oxygen. Moreover, except for transient hydrogen bursts, atmospheric carbon dioxide must be excluded since, even in the presence of active hydrogenase, it is the preferred electron acceptor.[4] Hydrogenase and hydrogen evolution are believed to be evolutionary relics that developed in Earth's primordial anaerobic atmosphere. While hydrogen evolution represents a loss of reducing equivalents, the ability to couple proton translocation with electron transport could provide a competitive survival advantage for microorganisms possessing this pathway.[5]

From the point of view of renewable fuels and chemical feedstock production, light-activated simultaneous photoproduction of hydrogen and oxygen is of primary interest. The pioneering experiments in this field were performed by Spruit,[6] who developed a novel two-electrode polarographic technique for the simultaneous measurement of hydrogen and oxygen transients by the green alga *Chlorella*. Subsequently, using a continuous flow system that actively removed photosynthetically produced oxygen, sustained simultaneous photoproduction of

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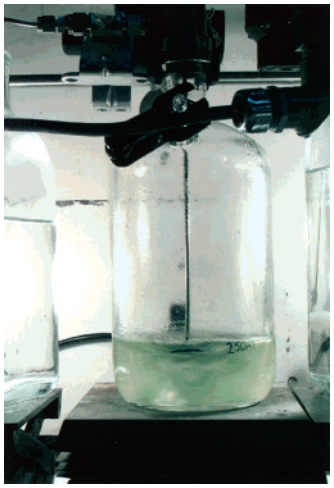

Figure 1. Illustration of the photoreactor for the simultaneous photoevolution of hydrogen and oxygen. Gases that were produced in the aqueous phase equilibrated between the headspace and liquid. Following predetermined periods of illumination, the accumulated hydrogen and oxygen were swept out of the reactor and transported downstream to gas-specific sensors for measurement.

hydrogen and oxygen was observed for 15 h.[7] Moreover, by actively removing inhibitory oxygen and working in the linear low-intensity region of the light-saturation curve, hydrogen and oxygen energy conversion efficiencies of 5−10% were measured.[8]

The data reported in this paper address the issue of oxygen sensitivity of algal hydrogen production from a simple physicochemical approach. Using Henry's Law[9] partitioning between liquid and gas phase, the local partial pressure of oxygen in the aqueous phase can be kept low enough to at least partially relieve the inhibition of hydrogen production by photosynthetically produced oxygen. In addition, an elementary model of the partitioning of oxygen from the liquid phase to the gas phase is presented. This bioreactor approach, especially in conjunction with the development of oxygen-tolerant mutants,[10] may provide a combined physical chemistry and genetic selection procedure for the production of renewable hydrogen and oxygen by light-activated microalgal water oxidation.

Materials and Methods

The Reactor. All reactions for the simultaneous photoproduction of hydrogen and oxygen were performed in a static reactor. A 1-L Pyrex vessel with a gastight glass to stainless steel tubular opening was constructed as illustrated in Figure 1. The 1-in. o.d. of the tube mated with an O-ring compression tube fitting that established a static enclosed volume. The compression tube fitting was modified so that electrically actuated solenoid ball valves, providing inlet and outlet ports, could be used to introduce a nitrogen flow into the reactor for initial removal atmospheric oxygen via a slender $^1/_8$-in. o.d. stainless steel tube that dipped into the liquid. This 3-h purge step, performed in the dark, induced the synthesis of hydrogenase. After anaerobiosis was established, the inlet and outlet valves were closed. Upon illumination, the hydrogen and oxygen that were produced in the magnetically stirred liquid phase equilibrated with the headspace volume. After a predetermined interval of illumination, the accumulated hydrogen and oxygen in the headspace were swept out of the reactor volume by the nitrogen carrier and measured with gas-specific sensors located downstream from the reactor. The inlet and outlet solenoid valves were computer controlled and operated on a 24-h cycle.

Algae and Light Sources. The alga used for the experiments was *C. reinhardtii* grown on minimal medium[11] with atmospheric carbon dioxide as the sole carbon source. This ensured that all reducing equivalents were derived from water, either via direct water oxidation or by dehydrogenation of endogenous substrates (such as starch) that were themselves the products of autotrophic photosynthesis. The algae were grown in 125-mL Erlenmeyer flasks on rotary shakers using overhead incandescent lamps. Incident light intensity, measured with a LiCor quantum flux meter. gave a value of 150 μE m^{-2} sec^{-1} in the spectral range 400−700 nm. Two symmetrically positioned incandescent lamps, filtered through water to minimize heating effects, were used for hydrogen and oxygen production studies. The incident light intensity at the surface of the reactor was 150 μE m^{-2} s^{-1}. Cycles of light-on/light-off were computer controlled. Periods of illumination and darkness, depending on the specific experiment, are further described in the results and discussion section. The experiments were done with algae containing 200 μg of chlorophyll in a liquid volume of 250 mL.

Hydrogen and Oxygen Measurements. Hydrogen and oxygen that accumulated in the reactor headspace above the algal suspension were measured by periodic sparging of the liquid and gas phases with nitrogen using the slender stainless steel tube. The two gases were transported downstream to their respective sensors. The oxygen sensor was a Hersch electrogalvanic cell[12] comprised of lead and silver electrodes separated by a 24% KOH-impregnated filter paper membrane. Introduction of oxygen into the cell caused a redox reaction generating a current that was linearly proportional to the gas-phase concentration of oxygen. Details of construction of the cell have been reported.[13] The hydrogen sensor was a tin oxide semiconductor manufactured by the Figaro Corp. (Glenview, IL). Gas-phase hydrogen causes a change in the conductivity of the sensor, which is incorporated into one arm of a Wheatstone bridge. Hydrogen in the gas phase causes the bridge to become electrically unbalanced, whose output is then amplified. Absolute calibration of the oxygen and hydrogen sensors was achieved with an inline electrolysis cell and a Keithley model 220 programmable current source. Baselines were established in pure nitrogen. Using Faraday's law of electrochemical equivalence, calibrating step currents were generated that spanned the range of sensor responses encountered in the photosynthetic hydrogen and oxygen production stage. A schematic illustration of this measuring system has been published.[14]

Results and Discussion

Experimental. Experimental data for pulsed intervals of simultaneous photoevolution of hydrogen and oxygen for over 1400 h are presented in Figure 2. The corresponding stoichiometric ratios for the data of Figure 2 are presented in Figure 3. Each data point was obtained by illumination of the sealed reactor for 1 h, during which time hydrogen and oxygen accumulated in the headspace and liquid phase. After the 1-h period of illumination, hydrogen and oxygen in the reactor were swept out by a nitrogen carrier and transported downstream to the measuring system. The flow velocity of the carrier was 50 mL min^{-1}. Two hours was allowed between intervals of illumination to fully purge the reactor of photosynthetically produced hydrogen and oxygen. It can be seen in Figure 2 that hydrogen and oxygen yields tracked each other closely. For the first 200 h, hydrogen and oxygen increased initially and then declined. The interval of time, from about 220 to 240 h, for which no hydrogen and oxygen data are recorded, corresponded

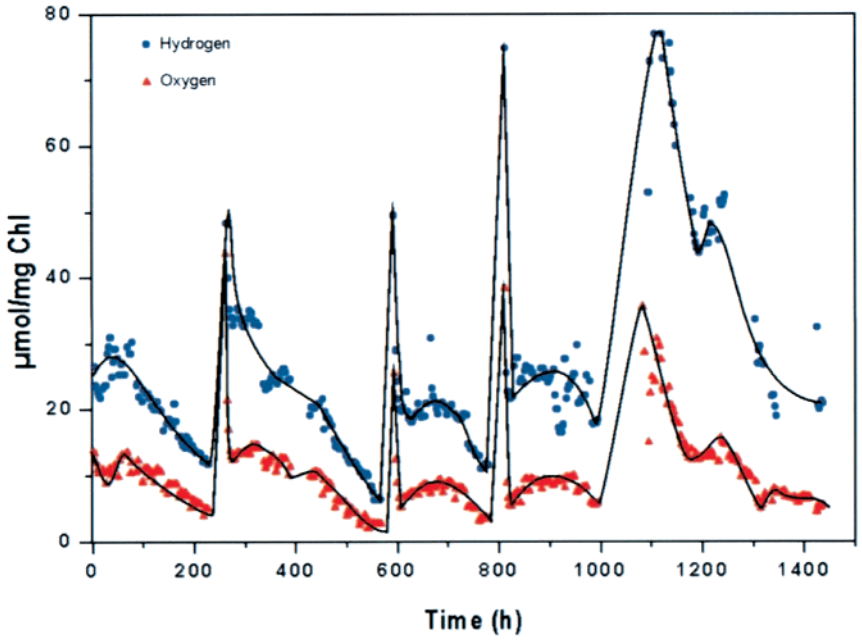

Figure 2. Pulsed intervals of the simultaneous photoevolution of hydrogen and oxygen. Each data point corresponds to 1 h of illumination, followed by a 2-h purge in darkness to fully remove all photosynthetically produced hydrogen and oxygen. The cycle was then repeated, except for specified intervals of normal photosynthesis that was used to rejuvenate the algae. The interval of time from about 220−240 h and subsequent intervals (see text), for which no hydrogen and oxygen data are recorded, corresponded to a normal period of rejuvenating photosynthesis in which a continuous flow of 400 ppm carbon dioxide in nitrogen was introduced into the reactor.

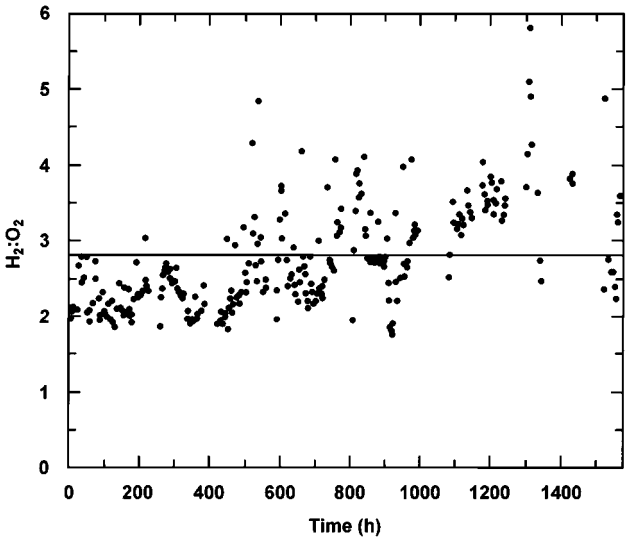

Figure 3. Stoichiometric ratios of hydrogen to oxygen computed from the data of Figure 1. The average ratio was greater than 2 since reducing equivalents for hydrogen production are derived from two immediate sources: real-time water oxidation and the dehydrogenation of endogenous storage products, whose reducing equivalents are derived from an earlier stage of water oxidation.

to a normal period of photosynthesis in which a continuous flow of 400 ppm carbon dioxide in nitrogen was introduced into the reactor. Although prolonged periods of hydrogen and oxygen production did not kill the algae, it was nonetheless a physiological stress, as can be seen by the declining output with time for the yield patterns presented in Figure 2.

The second period of photosynthesis, occurring at 400 h, did not improve the hydrogen and oxygen yields, but the third period at 580 h did. Subsequent intervals of photosynthesis at 790 and 1000 h all improved the hydrogen and oxygen yields following prolonged biophotolysis of water. The longest period of algal rejuvenation, starting at 1000 h and lasting for about 100 h, had a dramatic effect on productivity. The results clearly demonstrate that *C. reinhardtii* is a rugged alga with respect to hydrogen and oxygen production. They also suggest a rational approach to long-term stability and endurance for hydrogen production with algae.[15]

The parallel tracking of the hydrogen and oxygen time profiles presented in Figure 2 indicates a degree of coupling between the two reactions. Since most of the reducing equivalents for hydrogen production are derived from water oxidation,[16] the tracking between the two gases is reasonably close but not perfect. Figure 2 clearly demonstrates that the hydrogen and oxygen profiles echo each other in broad outline, but there are specific differences as well. These differences can be understood by studying the stoichiometric ratios of hydrogen to oxygen over the entire period of data collection.

The stoichiometric ratios are presented in Figure 3. Unlike electrolysis of water, where the stoichiometric ratio of hydrogen to oxygen is precisely two, the data of Figure 3 indicate that algal hydrogen and oxygen photoproduction can deviate from two. While the lowest value is slightly below 2, the maximum is over 5. The average value for the data of Figure 3 is 2.8. The stoichiometric excess, defined as the hydrogen component that causes the stoichiometric ratio to exceed two, represents reducing equivalents that are derived from the dehydrogenation of endogenous storage compounds. The source of reductant for the endogenous compounds, all products of normal autotrophic photosynthesis, is of course water. Oxygen evolution associated with endogenous reductant formation occurred at an earlier stage of photosynthesis, during growth on the rotary shakers prior to the hydrogen production experiments of Figure 2. Whereas electrons provided by these storage compounds in a Photosystem I light-dependent reaction can be expressed as molecular hydrogen, their oxidation products are not oxygen. This is the commonly occurring situation indicated in Figure 3, corresponding to stoichiometric ratios greater than 2. Conversely, the few data points corresponding to ratios less that 2 suggest that under certain conditions not all reducing equivalents are

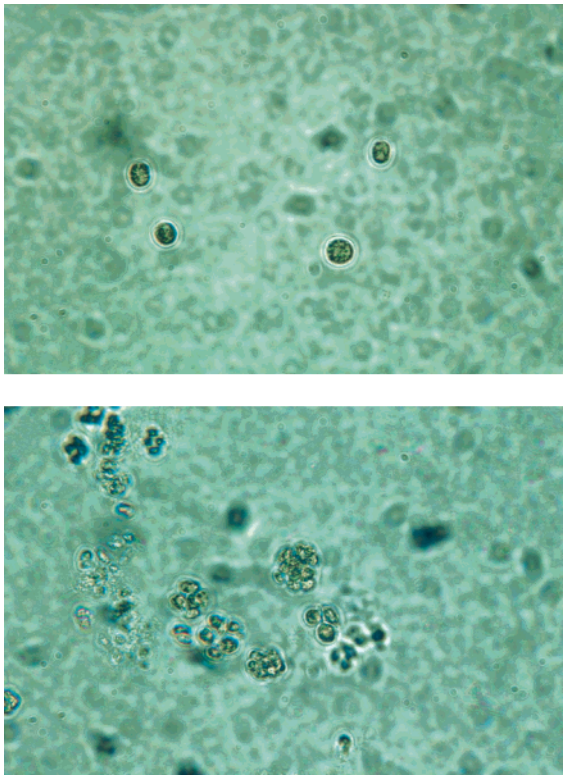

Figure 4. Photomicrographs of *C. reinhardtii*. (a) These algae have not been subjected to anaerobiosis and hydrogen and oxygen production. Note the single, undetached cells. (b) *C. reinhardtii* taken from the photoreactor following the completion of the experiments of Figure 1. Although the cells appear to be intact, they are now aggregated.

expressed as molecular hydrogen. An overlay of the hydrogen and oxygen data with the ratio data indicates that, as expected, the ratios tend to be greater than 2 following extended periods of carbon dioxide fixation by normal photosynthesis. This effect is especially evident at the start of data collection at 1100 h, following a 100-h period of photosynthesis where the stoichiometric ratio of hydrogen to oxygen reaches almost 6.

As mentioned earlier, sustained periods of hydrogen and oxygen represent a physiological stress for the algae. Panels a and b of Figure 4 are photomicrographs of the pre- and post-hydrogen and oxygen evolution production periods. Figure 4a illustrates *C. reinhardtii* prior to anaerobiosis and hydrogen production studies. These correspond to the images that are normally associated with this alga: singled celled and free living. Figure 4b is a photomicrograph of the algae that were removed from the reactor following the completion of the experiments of Figure 2, following more than 1400 h of biophotolysis. The morphology is clearly different. The cells are still intact, but they are now aggregated.

Figure 5 presents the effect of illumination dwell time on hydrogen and oxygen yields. The purpose of these studies was to test the linear relationship between illumination and productivity as well as the stability of the hydrogen and oxygen mixtures in the headspace with respect to hydrogenase inactivation (by virtue of accumulation of oxygen) and the oxy-hydrogen back reaction, $2H_2 + O_2 \rightarrow H_2O$, which is another reaction catalyzed by hydrogenase. As previously mentioned, the data of Figure 2 were taken with 1-h illumination dwell times. The data of Figure 5 indicate that the yields of hydrogen and oxygen are approximately linear up to 3 h. However, it is noteworthy that even for an illumination dwell time of 12 h, the maximum that would be required for a solar photobiochemical process, hydrogen and oxygen coexist in the confined reactor volume without appreciable loss.

Modeling. We present a mathematical model of the processes occurring in the reactor. The time rate of change of the total number of moles of oxygen in the closed experimental vessel is given by

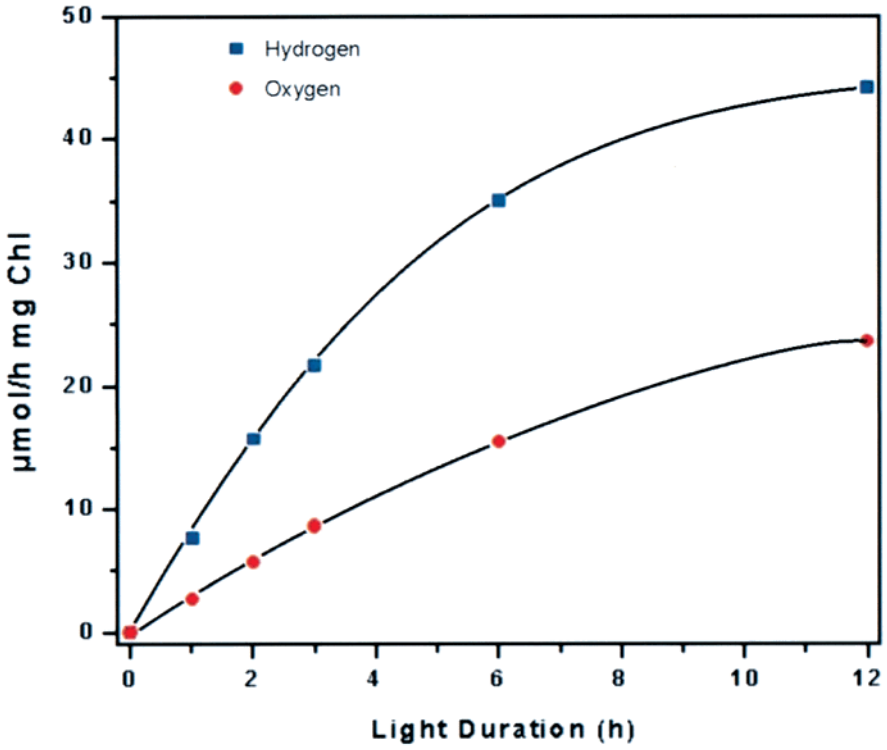

Figure 5. Effect of illumination dwell time on hydrogen and oxygen yields.

$$\frac{d}{dt}\left(c_{O_2}V_l + \frac{p_{O_2}}{RT}V_g\right) = p_{O_2} \quad (1)$$

where c_{O_2} is the molar concentration of oxygen in the liquid phase, p_{O_2} is the partial pressure of oxygen in the gas phase, R is the gas constant, T is the absolute temperature, V_l is the liquid volume, V_g is the gas-phase volume, and r_{O_2} is the rate at which oxygen is produced by algae in the system. The total number of moles of oxygen produced over time is illustrated in Figure 5. If we assume that equilibrium is achieved between the gas and liquid phase, we can relate the partial pressure of oxygen in the gas phase to the liquid-phase concentration through Henry's law

$$p_{O_2} = H\frac{c_{O_2}}{c} \quad (2)$$

where H is the Henry's constant and c is the molar concentration of the solvent (H_2O). The Henry's constant for oxygen in water at 20 °C is 4.01×10^4 atm/mole fraction.[17] For a dilute aqueous phase with c approximately constant, eq 2 can be used to rewrite eq 1 in terms of the liquid-phase concentration

$$\frac{d}{dt}(\kappa c_{O_2}) = r_{O_2} \quad (3)$$

where κ is a constant equal to $V_l + (HV_g/RTc)$. If the production rate of oxygen r_{O_2} is constant over time, we would expect to see a linear relationship in Figure 5. This is approximately true for the first 3 h. However, after 3 h, the production rate appears to slow over time. One explanation for this is an inhibition of the hydrogenase enzymatic reaction due to build-up of oxygen in the liquid phase, thus making r_{O_2} a function of c_{O_2}.

To attribute the behavior observed in Figure 5 to the kinetics of the reaction assumes that there are no mass transfer limitations. This is a reasonable assumption if the time scale for mass transfer is short relative to the time scale over which we observe the time-dependent behavior in Figure 5.

Because the liquid phase is well stirred and the oxygen concentration is well below the saturation level (1.26 mM), we assume oxygen is dissolved in the liquid phase and distributed uniformly throughout with the exception of a small stagnant film adjacent to the gas/liquid interface. Resistance on the gas side of the interface should be much smaller than that on the liquid side because the diffusion coefficient of oxygen in a gas is several orders of magnitude greater than that in a liquid.[18] Thus, an estimate of the mass transfer coefficient across the liquid film should provide a good estimate of the overall resistance. The time constant for mass transfer τ is dependent on the diffusion coefficient D and the mass transfer coefficient k according to

$$\tau = \frac{D}{k^2} \quad (4)$$

The diffusion coefficient for oxygen in water is approximately 2×10^{-5} cm^2/s, and the mass transfer coefficient is estimated at 3.3×10^{-3} cm/s (19), which yields a time constant less than 2 s. This time period is much shorter than the time scale over which we observe changes in the production rate of oxygen. Thus, mass transfer limitations are not expected to play a role.

Conclusion

Data were presented on the simultaneous photoproduction of hydrogen and oxygen in a confined bioreactor in which the gases accumulated in the headspace above the liquid. An elementary model of the gas/liquid interface was developed, and the characteristic time constant for movement of oxygen into the gas phase was calculated. The results demonstrated that to within upper limits of oxygen formation, even for wild-type unicellular green algae such as *C. reinhardtii*, it is possible to accumulate oxygen in the presence of active hydrogenase without severe inhibition of H_2 productivity. Of course, it is recognized that accumulation of hydrogen and oxygen in a confined bioreactor presents an explosion hazard. The safe upper limit of gas accumulation in combination with preservation of hydrogen evolution for the working algae will be a key parameter in the operation of such systems. An experimental determination of the safe upper limit in a water vapor−saturated confined bioreactor is worthy of determination.

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