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Effect of Light/Dark Cycle on Bacterial Hydrogen Production by *Rhodobacter sphaeroides* RV

From Hour to Second Range

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Abstract

Hydrogen production by photosynthetic bacteria provides an efficient energy conversion method under low light intensity. However, under strong illumination, such as midday sunlight, the efficiency drops. This prevents the method from being applied industrially. To overcome this problem, we examined a method to thin out the excessive illumination. Light was given intermittently to reduce the total energy flux. The on/off ratio was set at 1/1 throughout the study, so that the time average of the light energy flux became half the continuous illumination. By keeping the time-average light flux constant (0.6 $kW \cdot m^{-2}$), the effects of the cycle period were examined in the range of hours to seconds. The hydrogen production rate was greatly affected by the cycle period, but cell growth and substrate consumption rates remained almost constant. The 30-min light/dark cycle (30 min on and 30 min off) provided the highest rate of hydrogen production (22 $L \cdot m^{-2} \cdot 24 h^{-1}$). At the shorter cycles, the rate decreased except that there was a suboptimum at about 40 s. Under excessive light intensity (1.2 $kW \cdot m^{-2}$), the light-to-hydrogen conversion efficiency was greatly enhanced. The hydrogen production rate during the 30-min cycle was twice as high as during a 12-h cycle under the same conditions.

Index Entries: Photosynthetic bacteria; hydrogen production; *Rhodobacter sphaeroides*; light/dark cycle; intermittent irradiation.

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Introduction

Hydrogen, which forms only water after combustion, is thought to be the mainstream of the clean energy network for the next generation *(1,2)*. In hydrogen is produced from renewable energy sources, energy use will truly become harmonized with the environment. Among such energy sources, sunlight can meet worldwide energy demands with only 40 min of irradiation *(3)*. However, the low-density energy prevents its use. Another problem is the transition of intensity in a day. The profile of the transition should be a Gaussian-like distribution with the maximum intensity of 1.0 $kW·m^{-2}$ at meridian transit.

Photosynthetic bacteria produce hydrogen by using sunlight as an energy source *(4)*. The technological preparations for bacterial hydrogen production should be quite simple because the cells perform the complicated reaction and reproduce by themselves. Thus, hydrogen production by photosynthetic bacteria has been investigated intensively *(5)*. The effect of light on hydrogen has been studied from the viewpoint of efficiency *(6)*, light wavelength *(7,8)*, and reactor design *(9)*. However, only a few reports have addressed sunlight *(10)*. Some reports discuss trials that simulate sunlight indoors for biological hydrogen production using artificial light *(11)*.

Based on the results of indoor and outdoor experiments, the sunlight intensity of 1.0 $kW·m^{-2}$ is too strong to produce efficiently hydrogen by photosynthetic bacteria *(12)*. The conversion efficiency from light to hydrogen decreases severely under the peak intensity of sunlight.

In this study, we investigated a method to thin out the energy. The light shone intermittently to reduce the energy flux to bacteria at excessive light intensity. The effect of the period of light/dark cycle was examined from hours to seconds. A 30-min light/dark cycle (30 min light and 30 min dark) gave the best conversion efficiency under light energy supply constant per day. Under the excessive light intensity at 1.2 $kW·m^{-2}$, the 30-min light/dark cycle induced hydrogen production at about twice the rate as did the 12-h cycle. We propose a combination with solar cells to use the chopped light to increase the total efficiency.

Methods

Bacterial Strain

Rhodobacter sphaeroides RV strain, which has steady and high hydrogen production capacity, was used *(12)*.

Cultivation

R. sphaeroides strain RV was precultured using aSy medium *(13)*. The precultured cells were main cultured under anaerobic light (30°C, 0.8 $kW·m^{-2}$) using gL medium containing 50 m*M* sodium lactate as its carbon source and 10 m*M* sodium glutamate as its nitrogen source *(13)*. After being kept in the dark for 12 h, the cells were used for hydrogen production experiments. Dark incubation was carried out to avoid the influence of light in main cultivation.

Light Conditions

An M-26 halogen lamp (Philips Japan, Tokyo) was used as the light source throughout this study. M-26 has a wavelength-selective dichroic mirror and a spectrum similar to the sunlight spectrum that does not have infrared light.

Light intensity was set to 0.58 $kW \cdot m^{-2}$, total light energy in 24 h to 7.0 $kWh \cdot m^{-2}$, and the irradiation period to 12 h. Light energy was measured with a radiometer (model 4090, Springfield Jarco, Instruments, OH).

To obtain a range of light/dark cycles between 12 h and 5 s, the M-26 was alternatively turned on and off by a digital stop timer. Light/dark cycles were set to 12, 6, 3, 1.3, and 1 h; 40, 30, 20, 15, 10, 5, 2, and 1 min; and 45, 30, 15, and 5 s.

In experiments with excessive light energy, light intensity was set to 1.2 $kW \cdot m^{-2}$, and total light energy in 24 h to 14.0 $kWh \cdot m^{-2}$. Light/dark cycles were set to 12 h and 30 min.

Other Conditions

A Roux flask with a 70-cm^2 irradiation area, 200-cm^3 working volume, and 2.5-cm light pass was used as the reactor. The Roux flask was covered with a black sheet (except for the irradiation area) to eliminate the influence of external light (Fig. 1).

Produced hydrogen was collected in a water-filled, inverted, graduated cylinder through a Tygon tube. The volume of produced hydrogen was calculated as the hydrogen production rate per unit of irradiation area ($L \cdot m^{-2} \cdot h^{-1}$).

Analysis

Cell concentration was determined by measuring optical density at 660 nm with a spectrophotometer (Shimadzu UV-160A, Kyoto, Japan). Organic acids concentration was measured by high-performance liquid chromatography (Shimadzu SPD-10AV).

Other Methods

Every experiment was repeated at least four times. All experimental data were calculated by the method of root mean square. Energy conversion efficiency from light to hydrogen was calculated by the following equation:

$$\text{Efficiency (\%)} = (\text{combustion enthalpy of hydrogen}) \times (\text{absorbed light energy})^{-1} \times 100$$

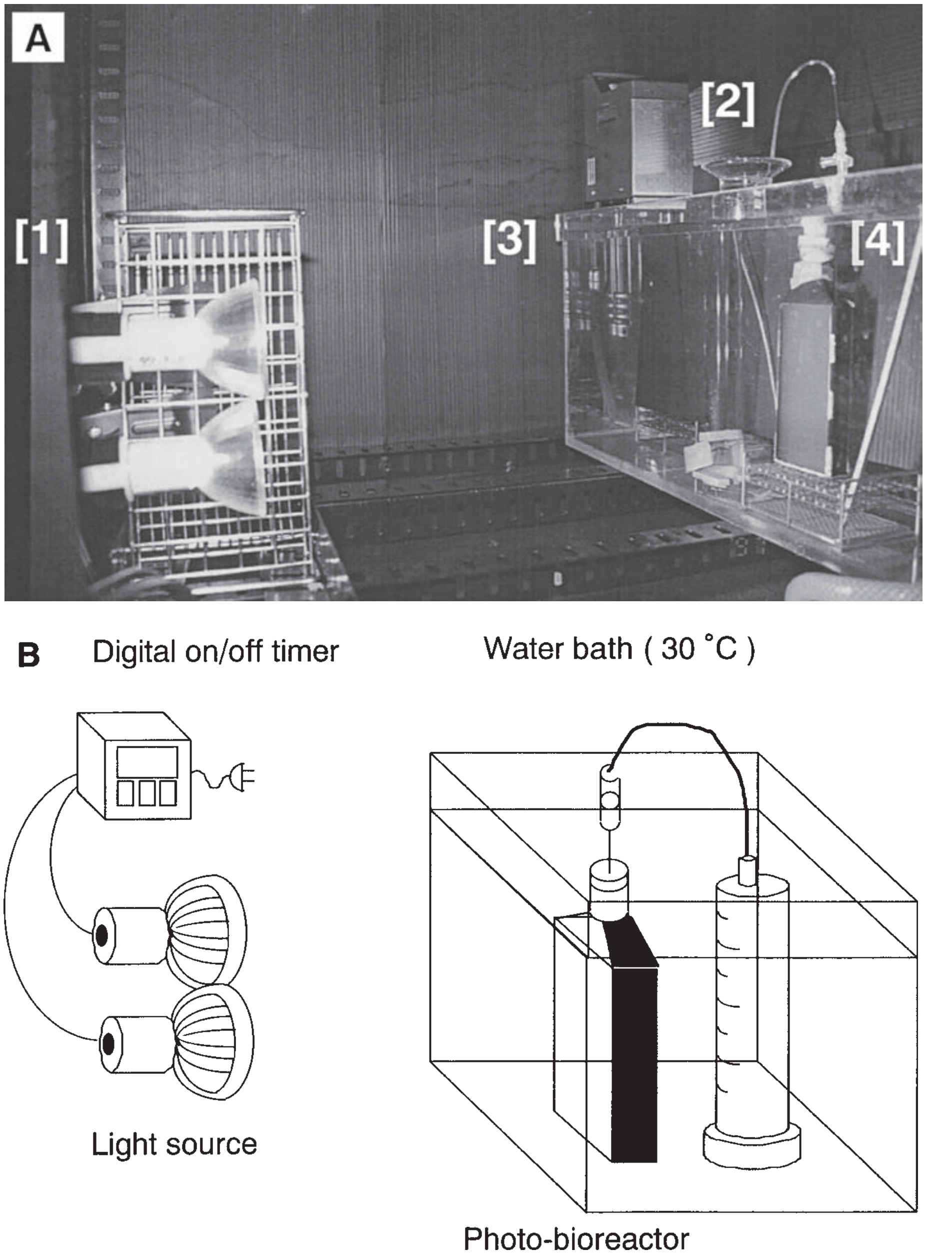

Fig. 1. **(A)** Experimental setup for effect of light/dark cycle. [1], Halogen lamp (M-26) with on/off timer; [2], H_2 collector; [3], water bath (30°C); [4], 200-cm^3 Roux flask. **(B)** Experimental illustration for effect of light/dark cycle.

Results and Discussion

Saturation of hydrogen production rate occurs at excessive light intensities. The effect of a light/dark cycle on hydrogen production was examined for use of excessive light. Thinning out the illumination had a

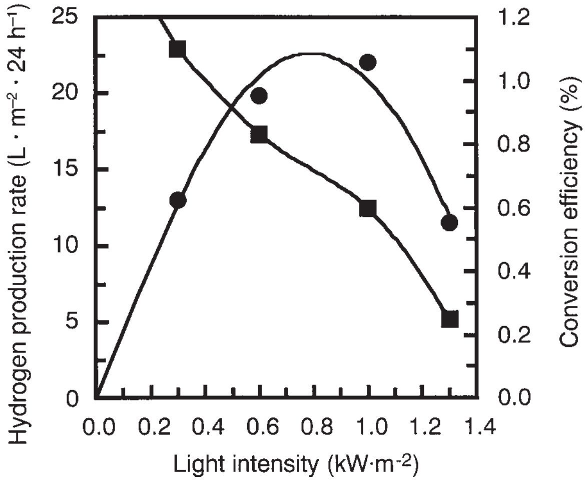

Fig. 2. Effects of various light intensities on hydrogen production. ●, Hydrogen production rate; ■, conversion efficiency.

remarkable effect on the hydrogen production rate under the excessive light condition.

Effects of Various Light Intensities on Hydrogen Production

Indoor experiments mostly used continuous light, whose spectra differ markedly from those of sunlight. Using sunlight to produce hydrogen requires that sunlight, especially daily illumination, be simulated indoors.

The effects of the various light intensities on hydrogen production were studied (Fig. 2). Under a light intensity of about 0.6 $kW·m^{-2}$ or less, the hydrogen production rate was rather high (20 $L·m^{-2}·24\ h^{-1}$). However, under a light intensity of about 1.0 $kW·m^{-2}$ (the same intensity as the sunlight at meridian transit) or higher, the hydrogen production rate significantly decreased. Conversion efficiency also decreased to about 0.2% under excessive light intensity.

We determined that the light intensity of 1.0 $kW·m^{-2}$ is too high for hydrogen to be produced by photosynthetic bacteria. To avoid this problem, we investigated intermittent light for sharing the light energy flux into a reactor. We examined the effects of intermittent light from hour-to-second range by keeping the total energy supply constant (7.0 $kWh·m^{-2}·24\ h^{-1}$). As reference points, continuous light was selected, as was a 12-h light/dark cycle.

Effect of Light/Dark Cycle on Hydrogen Production Rate

The effect of light/dark cycle on the time course of hydrogen production was examined in 72 h with a light intensity of 0.6 $kW·m^{-2}$. Hydrogen production rates per 24 h were plotted against the light/dark cycle (12 h to 5 s) (Fig. 3).

The 30-min cycle provided the highest production rate (22 $L·m^{-2}·24\ h^{-1}$). As a reference, a 12-h light/dark cycle was used together with continuous

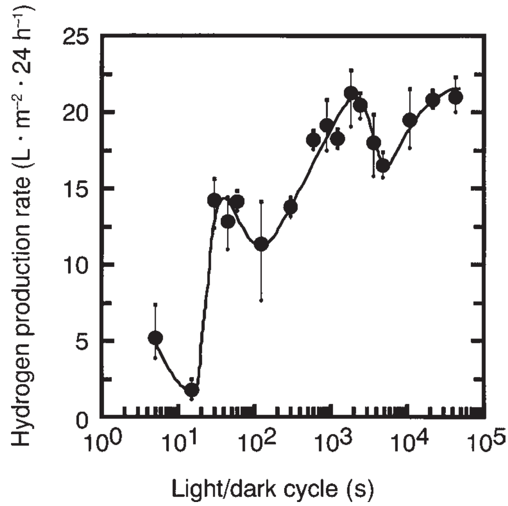

Fig. 3. Effect of light/dark cycle on hydrogen production rate. ●, Hydrogen production rate.

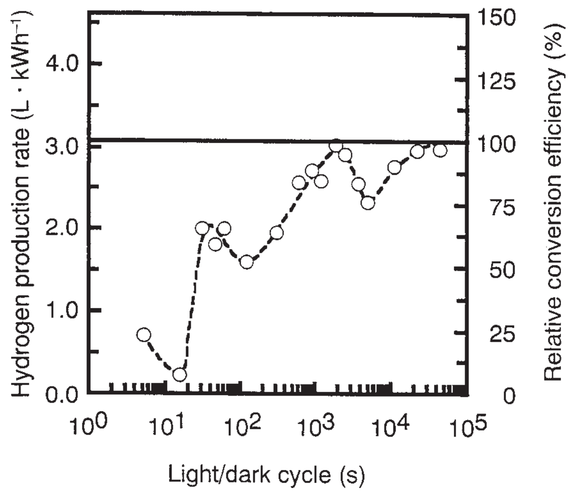

Fig. 4. Effect of light/dark cycle on hydrogen production rate per energy and relative conversion efficiency. ○, Hydrogen production rate and relative conversion efficiency.

illumination. At shorter intervals, the hydrogen production rate decreased to 1.8 L·m^{-2}·24 h^{-1}. At the shortest (5-s) light/dark cycle, the bacterium produced only 5 L·m^{-2} of volume of hydrogen per 24 h. Furthermore, the hydrogen production rate was suboptimum, at about the 40-s light/dark cycle.

The data were replotted per energy and the relative conversion efficiency from light energy to hydrogen was also given (Fig. 4). The relative conversion efficiency was calculated compared with that of the continuous light. The 30-min light/dark cycle gave the highest hydrogen production rate (3.0 L·kWh^{-1}) and the highest relative conversion efficiency. There was

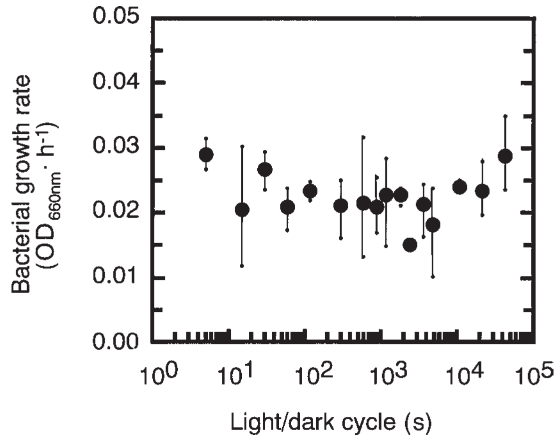

Fig. 5. Bacterial growth rate vs light/dark cycle. ●, Bacterial growth rate.

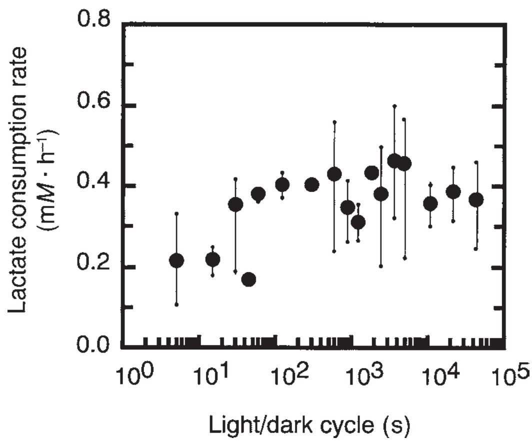

Fig. 6. Lactate consumption rate vs light/dark cycle. ●, Lactate consumption rate.

also a suboptimum point in the hydrogen production rate at about the 40-s light/dark cycle.

Effect of Light/Dark Cycle on Cell Growth and Lactate Consumption

The light/dark cycle had a prominent effect on hydrogen. Then the effects of light/dark cycle on cell growth and lactate consumption were studied. In contrast to hydrogen production, cell growth rate and lactate consumption rate were unaffected by the cycle period. The cell growth rate was not related to the light/dark cycle except for a slight increase in the shorter and longer cycles (Fig. 5). These results differ from the growth kinetics in intermittent light using *Rhodobacter capsulatus (14)*. Lactate consumption was not related to the cycle period (Fig. 6). Some lactate remained in the reactor at the end of the experiments.

Table 1
Hydrogen Production Rate and Light Energy

Light/dark cycle	Light intensity ($kW·m^{-2}$)	Total energy supply ($kWh·m^{-2}·24\ h^{-1}$)	H_2 production rate ($L·m^{-2}·24\ h^{-1}$)	(%)[a]
None (continuous light)	0.6	14	22	100
12/12 h	0.6	7	21	95
	1.2	14	16	73
30/30 min	0.6	7	22	100
	1.2	14	33	150

[a]H_2 production rates are the average of the experiments for the 72 h.

Lam et al. *(15)* reviewed the time scale for biological activity. Phenomena of the time scale of second and higher order should be related to a biochemical and physiological reaction. Because the cell growth was not affected, the energy capture in the light reaction of photosynthesis should not be lowered by the intermittent light. The rate-limiting factor for hydrogen production could be in the metabolic pathway with the reaction time constant of 30 min and 40 s.

Enhancement of Hydrogen Production Under Excessive Light Energy

The effect of the 30-min light/dark cycle on the time course of hydrogen production was examined for 72 h with the light intensities of 0.6 and 1.2 $kW·m^{-2}$ (Table 1). As a reference, hydrogen production was measured using continuous light and a 12-h light/dark cycle. The 12-h cycle corresponds to the daily light cycle. The light intensity of 1.2 $kW·m^{-2}$ is high enough to make the saturation of hydrogen production in the case of continuous light.

The apparent effect of avoiding the saturation was seen in this condition by using intermittent light (Table 1). At 0.6 $kW·m^{-2}$, below the saturation, the efficiencies were the same for any cycles. On the other hand, at 1.2 $kW·m^{-2}$, the 30-min light/dark cycle improved the efficiency to 150%, whereas the 12-h cycle reduced it to 73% compared to the reference (continuous light). Comparison with the 12-h cycle shows that the rate at the 30-min cycle period was twice as high. The intermittent light had a remarkable effect on the use of excessive light energy on the hydrogen production rate.

We suggest that sharing light energy should be an effective method to avoid photoinhibition under excessive sunlight. The chopped light could be applied to other purposes for other energy conversions. We propose a combination system of biological hydrogen photoproduction with a chopper made of mirror for supplying the reflected light to solar cells (Fig. 7).

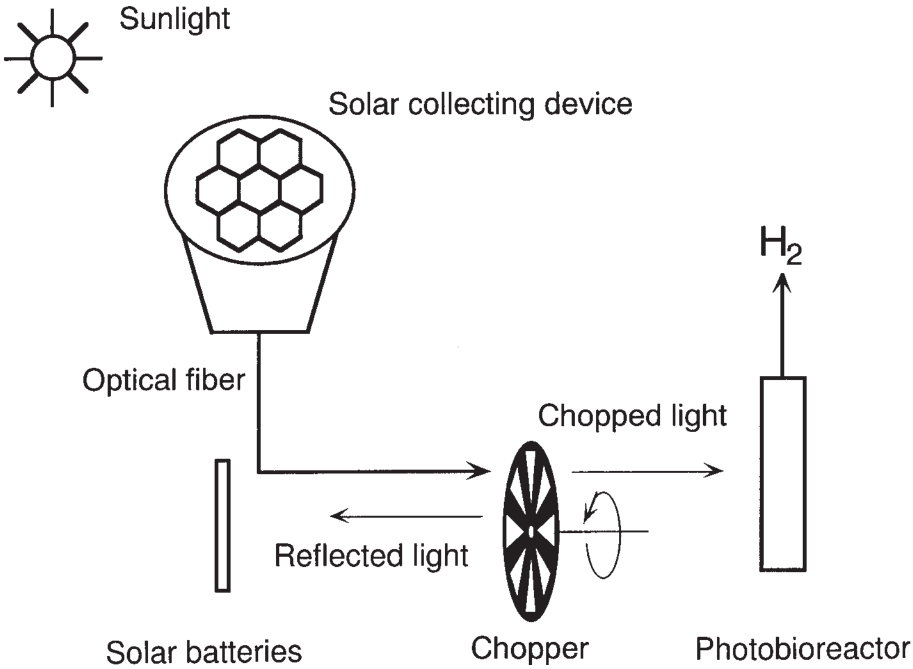

Fig. 7. Conceptual illustration for effective utilization of excessive sunlight.

Acknowledgments

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