Light irradiance and spectral distribution effects on cyanobacterial hydrogen production

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Abstract. Light is an essential energy source for photosynthetic cyanobacteria. Changes in both light irradiance and spectral distribution will affect their photosynthetic productivity. Compared to the light irradiance, little investigations have been carried out on the effect of light spectra towards cyanobacterial hydrogen production. Hence, this work aims to investigate the effects of both light quantity and quality on biohydrogen productivity of heterocystous cyanobacterium, \textit{A. variabilis}. Under white light condition, the highest hydrogen production rate of 31 µmol H\textsubscript{2} mg chl \textsubscript{a} \textsuperscript{-1} h \textsuperscript{-1} was achieved at 70 µE m\textsuperscript{-2} s\textsuperscript{-1}. When the experiment was repeated at the same light irradiance but different light spectra of blue, red and green, the accumulations of hydrogen were significantly lower than the white light except for blue light. As the light irradiance was increased to 350 µE m\textsuperscript{-2} s\textsuperscript{-1}, the accumulated hydrogen under the blue light doubled that of the white light. Besides that, an unusual prolongation of the hydrogen production up to 120 h was observed. The results obtained suggest that blue light could be the most desirable light spectrum for cyanobacterial hydrogen production.

1. Introduction
Cyanobacteria, also widely known as blue-green algae, are oxygenic photosynthetic prokaryotes with a long evolutionary history. The ability of cyanobacteria to fix nitrogen and to adapt to low light allow them to enter into a very wide range of symbiotic associations [1]. Cyanobacteria are also useful in the carbon sequestration outlook, since they could act as carbon sinks by capturing and transforming CO\textsubscript{2} into organic chemicals and many other valuable materials. In addition, some heterocystous cyanobacteria are well adapted for diazotrophic growth, and are able to synthesize hydrogen under some prerequisite conditions [2]. Both nitrogen fixation and hydrogen production are catalyzed by the O\textsubscript{2}-sensitive nitrogenase enzymes which are confined in the heterocyst.

Like other photosynthetic organisms, cyanobacteria are able to acclimate to changes in ambient light irradiance and spectral distributions. Their responsiveness towards light treatment correlates with the changes of the contents of both light harvesting pigments and photosystems in the cells [3]. It has also been proven that for a given cell density and light irradiance, light spectra conversion gives significant
effect towards photosynthetic pigments composition, cell growth rates and nitrogenase activity [4-7]. However, information on its effect towards cyanobacterial hydrogen production which is also light-dependent is still limited. Considering the essentials of both light quantity and quality toward the complex cellular regulatory systems, this study aims to investigate hydrogen production performance of *A. variabilis* incubated under diversified light irradiances and wavelength distribution.

2. Methodology

2.1 Propagation of *Anabaena variabilis*.

Stock cultures of *Anabaena variabilis* ATCC 29413 were propagated aerobically in 500 mL Erlenmeyer flask and maintained in BG11o medium, as described elsewhere [2]. Cell concentration was monitored according to the optical density (A683) using a UV spectrophotometer.

2.2 Hydrogen production experiment

Cells were collected during mid-to late-exponential growth stage and concentrated using a centrifuge. The supernatant was removed after centrifugation, and the cells pallet was washed twice with fresh BG11o medium. An anaerobic condition with 5% of CO2 was generated prior to cell suspension. The initial inoculum concentration was standardized at 2.32 µg chl a mL⁻¹. The serum bottles were incubated horizontally on an orbital shaker operated at a speed of 120 rpm. Twelve fluorescent lamps (Philips TL-D 18W/865 1SL) were placed slightly above the serum bottles, providing uniform light irradiance of 35-350 µE m⁻² s⁻¹, measured using a quantum sensor (Licor LI-190, USA). Monochromatic lights of blue, red and green were provided using 3W RGB Power LED lights (Vollong Electronics Co., Limited, China). The calculated surface area of one side of the culture bottle (assuming it was transacted by a horizontal plane) was 45 cm². 200 µml of headspace gas was taken out periodically using an airtight syringe and then manually injected to a gas chromatography (Perkin Elmer AutoSystem XL) equipped with a thermal conductivity detector (TCD). Hydrogen production experiment was done in triplicates. The efficiency of photosynthetic conversion of light to hydrogen was calculated as:

\[
\eta_{H_2} \text{(%) = } \frac{\text{Energy content of } H_2}{\text{Absorption of light energy}} \times 100
\]

In full expression, Equation 1 is in the form:

\[
\eta_{H_2} \text{(%) = } \frac{\Delta G^\circ_{H_2} R_{H_2}}{E_a A} \times 100
\]

where \(\Delta G^\circ_{H_2}\) is the free energy available from the heat of combustion of hydrogen which is 0.24 kJ/mmol at 25 °C [8], \(R_{H_2}\) is the amount of accumulated \(H_2\), \(E_a\) is light energy radiation, and \(A\) is the illumination area [9].

3. Results and Discussion

3.1 Effect of Light Irradiance

Figure 1 illustrates the effect of light irradiance towards hydrogen production rate. The maximum rate of 31 µmol H2 mg chl a⁻¹ h⁻¹ was achieved at a moderate light irradiance of 70 µE m⁻² s⁻¹. Further increase of the light irradiance beyond this value did not improve hydrogen production rate. Furthermore, the light conversion efficiency was also significantly reduced by 7 times from 70 µE m⁻² s⁻¹ to 350 µE m⁻² s⁻¹ which were 0.12% to 0.017% respectively. This implies that the light harvesting pigments had already been saturated at 70 µE m⁻² s⁻¹.
3.2 Effect of Light Spectra

The hydrogen production versus time profile in Figure 2(A) depicts the effect of light spectral distribution on hydrogen production. For all light colours except for blue, hydrogen production were significantly lower as compared to control (white) light. Cells incubated under the red light condition demonstrate the lowest hydrogen production. Meanwhile, the production of hydrogen under the green light starts to drop the earliest which is at the 47th hour. The increased production of hydrogen in blue light condition might be the result of enhanced light energy harvested by the chlorophyll $a$ pigments which absorbs light most strongly in the blue spectrum (430-445 nm) as well as strong stimulation of nitrogenase activity within this wavelength range [7].

Figure 2. Accumulation of hydrogen over time under (A) moderate light irradiance of 70 $\mu$E m$^{-2}$ s$^{-1}$ for white (control), blue, green and red lights and (B) high light irradiance of 350 $\mu$E m$^{-2}$ s$^{-1}$ for white and blue lights.
The experiment was then repeated at high light irradiance of 350 µE m$^{-2}$ s$^{-1}$ for both white and blue lights. As demonstrated in Figure 2(B), although the hydrogen production rate for cells incubated under blue light is initially lower than the white light during the first 44 h (21.4 µmol H$_2$ mg chl $a$ $^{-1}$ h$^{-1}$ compared to 24.5 µmol H$_2$ mg chl $a$ $^{-1}$ h$^{-1}$), the rate is however maintained throughout the experiment. Furthermore, significant prolongation of hydrogen production can be observed. The production did not cease and kept increasing even after 120 h or 5 days of incubation. The maximum hydrogen accumulation obtained under blue light condition was 275 µmol H$_2$, a two-fold increase compared to the white light condition.

4. Conclusion

In conclusion, the specificity of A. variabilis response toward variations in light conditions proved that cyanobacteria are light-sensitive and therefore, proper tunings of the light irradiance and wavelength distribution are essential in order to maximize the hydrogen productivity. The improvements in the accumulation of hydrogen and production duration suggest that blue light could be the most desirable light spectrum for hydrogen synthesis by this species.

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