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Improvements on the Fluorescence Quenching/Deflection Method for Real-time *in situ* Simultaneous Monitoring of Dissolved Oxygen and Material Movement-induced Beam Deflection in the Vicinity of an Aquatic Plant

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Although the newly developed beam deflection/fluorescence detection system for real-time *in situ* simultaneous monitoring of dissolved oxygen (DO) and material movements in the vicinity of aquatic plants was not only much more sensitive but also could be carried out much more closely to real time than conventional analytical methods that monitor the concentration changes at a bulk solution, it could not be applied to the photosynthesis process of aquatic plants. Here, improvements are reported to enable application of the system to the photosynthesis process. A white-light LED, which was used as a light source for photosynthesis in our previous paper, was replaced by a red-blue LED with wavelength of about 660 and 450 nm. Also, an interference filter of 589 ± 25 nm was placed in front of a photomultiplier tube (PMT). Furthermore, the LED and its electric power supply were placed outside of the dark room for preventing great temperature increases in the photosynthetic experiments. Experimental results showed the DO-quenched fluorescence could be sensitively monitored in both the respiration and photosynthesis processes, while only in the respiration process before the improvements. It is successfully demonstrated that the DO change and material movement-induced beam deflection in the vicinity of the plants in both the respiration and photosynthesis processes could be real-time *in situ* monitored with high sensitivity.

Keywords Fluorescence quenching, deflection, dissolved oxygen, aquatic plant, real time, *in situ* monitoring

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Introduction

Aquatic plants play important roles in absorbing or degrading organic or toxic substances such as salicylic acid, carbonic acid, heavy metal ions, *etc.*[1-3] Many countries use aquatic plants to control water pollution and restore the aquatic system from eutrophication.[4] However, purification dynamics and mechanics or material movements during metabolism and life activities of aquatic plants are still not well understood.[5] Most studies on aquatic plants focus on the light- and CO_2- or O_2-saturated photosynthetic rate by using an infrared gas analyzer or O_2 electrode.[6] However, only spatial- and temporal-average changes over a sample cell or sample vessel holding the whole or part of a plant can be obtained in the conventional methods. It is difficult to obtain real-time material movement information across the plant surface. Also, it is impossible to distinguish the difference between different organs such as leaf and stem.[7]

Recently, we have developed a novel optical detection system that allowed real-time *in-situ* simultaneous monitoring of the dissolved oxygen (DO) and material movements within a vicinity of micrometers from an aquatic plant surface.[8] In the system, a blue semiconductor diode-laser light was focused on an area in the vicinity of the aquatic plant/water interface in a culture dish containing Ru(II)-complex (Tris(2,2′-bipyridyl) ruthenium(II) chloride) by an objective lens. Deflection of the laser light and DO-quenched fluorescence of the Ru(II)-complex from the vicinity of the aqueous plants were monitored by a position sensor and a PMT, respectively. Decrease of DO concentration with time in the vicinity of the plant surface during the respiration process was monitored not only much more sensitively, but also much more closely to real time than conventional analytical methods that monitor the concentration changes at a bulk solution. This system can not only obtain the special-dependent concentration changes with time in the vicinity of the aquatic plants, but also distinguish differences in material movements including oxygen transport across surfaces of different organs such as stems and leaves of the aquatic plants.[8]

On the other hand, the optical detection system could not be used for monitoring DO in the vicinity of the aquatic plants during the photosynthetic process, because a white-light LED was used to illuminate the aquatic plants.[8] A part of the white LED light entered the PMT and thus interfered with detection of DO-quenched fluorescence. Also, the monitored DO-quenched fluorescence and deflection signals were not so stable since the white-light LED metal socket and its electric power source generated heat, which caused a temperature increase during the photosynthetic experiments. In this work, the optical system was improved so that it can be applied for the photosynthetic experiments. As a result, it is successfully demonstrated that the DO change and material moment-induced beam deflection in the vicinity of the plants in both the respiration and photosynthesis processes could be real-time monitored with high sensitivity.

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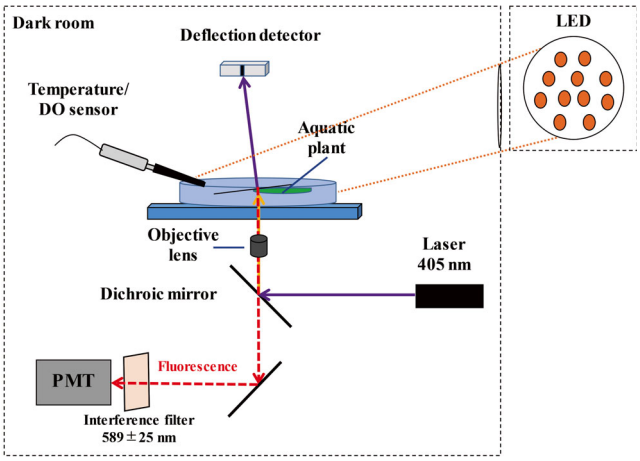

Fig. 1 Improved experimental setup for the deflection/fluorescence detection system.

Experimental

Figure 1 shows the improved optical detection system for plant monitoring. A semiconductor laser of 405 nm (Sigma Koki, Japan) was used as the source of both the excitation light and the probe beam. The laser light was reflected by a dichroic mirror and then focused on an area in the vicinity of a short piece of aquatic plant *Cabomba* (about 3.0 cm long) in a culture dish with 20 mL of water or culture solution with 10^{-6} M Ru(II)-complex solution. A short piece of slide glass was placed on the aquatic plants to prevent any possible movement or motion. The culture dish was placed on a holder mounted on an X-Y-Z micro-stage (Edmund Optics). Fluorescence of the Ru(II)-complex passed through the dichroic-mirror was detected by a PMT, in front of which an interference filter of 589 ± 25 nm (Edmund Optics) was placed. Simultaneously, deflection of the laser beam was detected by a position sensor. A commercial DO/temperature sensor (Pyro Science GmbH Hubertusstr., 35 D-52064 Aachen) was placed in the culture dish for monitoring the temperature and DO in the culture solution. The sensor tip was covered with a black pipette to prevent heating by the light illumination in the photosynthetic experiments. The optical detection system was placed in a dark room with a window through which a red-blue LED (8 W, Luxour, Japan) with wavelength of about 660 and 460 nm illuminated the aquatic plants in the photosynthetic process.

Preparation of the 10^{-6} M Ru(II)-complex solution and other experimental conditions were the same as in the previous experiments.[8] The aquatic plant was firstly immersed in the 10^{-6} M Ru(II)-complex solution for 12 h so that the aquatic plant surface was in equilibrium with the Ru(II)-complex solution. Then the probe beam was focused on an area in the vicinity of the aquatic plants at a distance of 0, 10, or 30 µm by the X-Y-Z micro-stage. The distance of 0 µm was defined as the one at which the probe beam was so close to the aquatic plant surface that scattering light of the probe beam by the aquatic plant surface was seen.[8] Thirdly, the red-blue LED was turned off, and monitorings of the deflection, fluorescence, DO and temperature (by the commercial DO/temperature sensor) started. After 2 h, the red-blue LED was turned on, and the monitorings were continued for another 2 h. The monitorings with LED off or on were repeated several times.

Results and Discussion

As stated above, the previous detection system could not be used for photosynthetic study because of the use of a white-light LED. Here, a red-blue LED with wavelength of about 660 and 450 nm, which was originally designed for plant cultures, replaced the previous white-light LED. Also, an interference filter of 589 ± 25 nm was placed in front of the PMT so that only fluorescence of the Ru(II)-complex (maximum wavelength was about 585 nm) entered the PMT.

Furthermore, the red-blue LED and its electric power supply was placed outside of the dark room. A window was opened in the dark room, and the red-blue LED light illuminated the culture cell through the window. The temperature increase in the culture cell was about 0.4 – 0.6°C during 2 h of illumination, which was nearly the same level of temperature change in the laboratory. However, about 2°C of temperature increase was observed during the illumination in the previous detection system. Therefore, influence of the heat generated by the LED electric power supply and the metal socket was greatly decreased.

The algorithm for calculating the DO from the monitored DO-quenched fluorescence by considering the effects of temperature was similar to the previous ones.[8,9] Briefly, DO, temperature T, and fluorescence intensity F were firstly monitored with the 10^{-6} M Ru(II)-complex solution without the aquatic plant at room temperature. Linear and exponential equations were used for fitting temperature dependence of $DO(T)$ and $F(T)$, respectively. Secondly, fluorescence intensity F_0 and T of an anaerobic 10^{-6} M Ru(II)-complex solution were monitored, and $F_0(T)$ was fitted with an exponential equation of T.[9] Thirdly, the Stern–Volmer constant $K_{SV}(T)$ was calculated from the $F_0(T)$, $F(T)$ and $DO(T)$, and was further approximated as an exponential equation of T.[9] Fourthly, fluorescence intensity in the vicinity of the aquatic plant sample and T were monitored, and DO in the vicinity were calculated according to the Stern–Volmer equation[10] by using the $K_{SV}(T)$ and $F_0(T)$.[9]

Figure 2 shows the monitoring results of DO and deflection at different distances from the aquatic plants with the red-blue LED either on or off. When the red-blue LED was on, the photosynthesis process of the aquatic plant was carried out and DO increased with time in the vicinity of the aquatic plant (Fig. 2A). The concentration of DO increased from about 9.0 ppm to about 11.0, 9.3, and 9.2 ppm in 2 h at 0 µm, 10, and 30 µm away from the plant surface, respectively. On the other hand, DO at about 1 cm away from the plant surface monitored by the commercial sensor changed little. In the photosynthesis process, oxygen was produced at the aquatic plant surface, and then diffused to the solution.[8] Therefore, the closer to the plant surface, the higher the DO concentration.

Figure 2B shows the deflection signals monitored at different distances in the photosynthetic process of the aquatic plant. The closer to the plant surface, the larger the change of concentration gradient and thus the larger the deflection signals. Figures 2C and 2D show typical monitoring results of DO and deflection during the respiration process of the aquatic plant when the LED was off. It is clear that the DO concentration decreased with time, and the closer to the plant surface, the greater the decrease with time (Fig. 2C). This changing trend was opposite to that of Fig. 2A. Also, Fig. 2D shows that the deflection changed with time in an opposite manner to those in Fig. 2B. Because the respiration reaction was reversed to the photosynthetic one, the direction of the concentration gradients of both DO and CO_2 in the vicinity of the aquatic plant surface

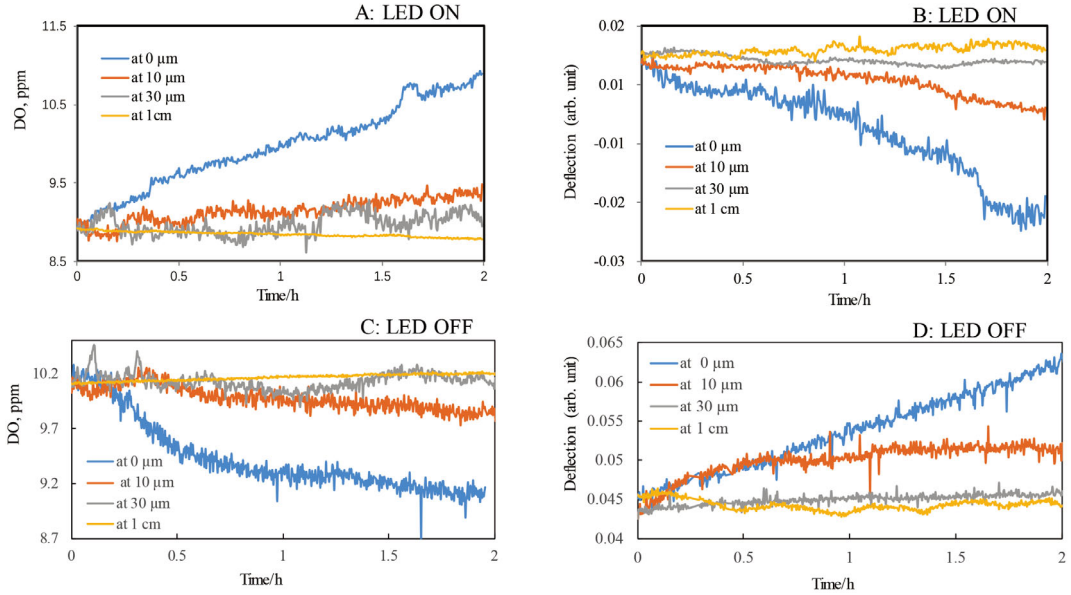

Fig. 2 Typical results of real-time *in situ* monitoring of DO (A and C) and deflection (B and D) at a distance of 0, 10, or 30 μm away from the leaf surface of the aquatic plant *Cabomba* when the LED was on (A and B) and off (C and D).

in the photosynthetic process was opposite to those in the respiration process. This induced opposite change trends of both DO and deflection signals.[8]

As a conclusion, the improved optical detection system is able to achieve real-time *in situ* monitoring of material movements including DO transport in the vicinity of an aquatic plant surface during not only the respiration but also photosynthetic processes. Also, the temperature increase caused by the LED power supply was greatly decreased, leading to stable monitoring of both deflection and DO-quenched fluorescence. The detection system is expected to be useful for exploring material movements across not only aquatic plants but also other living organisms including a single cell. In principle, either horizontal or vertical distributions of DO in the vicinities of the plant surface can be obtained by scanning the probe beam horizontally or vertically to the plant surface. The flux of oxygen from the plant surface, which reflects the photosynthesis activity, should be calculated from the vertical distribution of DO. Also, the detection sensitivity of both the DO and beam deflection may be further improved by use of the lock-in detection technique. These improvements require further study and will be reported later.

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