Real-Time Monitoring of Dissolved Oxygen at a Vicinity of an Aquatic plant

Kansuk Patthamawan, Tamura Keisuke and Xing-Zheng Wu

Life, Environment and Applied Chemistry, Fukuoka Institute of Technology, Fukuoka, Japan

mbm18202@bene.fit.ac.jp, tamy138045c@i.softbank.jp and wu@fit.ac.jp

Abstract. Real-time monitoring of dissolved oxygen (DO) concentration at a vicinity of an aquatic plant in water by the fluorescent quenching method is discussed. Ru(II) complex (Tris(2,2'-bipyridyl) ruthenium(II) chloride) was used a fluorescent probe, whose fluorescence was quenched by DO. The fluorescence of the Ru(II) complex at a vicinity of the aquatic plant was excited by a semiconductor laser of 405 nm, and detected by a PMT. Experiment results showed that the DO concentration at the vicinity of Egeria Densa decreased with time in the respiration processes, while increased with time in the photosynthetic processes. It was further applied to monitor DO at vicinities of the aquatic plant in water environments containing heavy metal ions such as Cu²⁺.

1. Introduction

Dissolved oxygen (DO) is an important parameter to measure water quality as the DO level either too high or low can harm aquatic life and affect water quality [1]. The DO in water varies with temperature, pressure, and saltiness. It is well known that DO decreases as the temperature increases.

Aquatic plants play important roles in the water quality and water environment. They absorb or degrade toxic substances such as heavy metal ions, salicylic acid, carbonic acid, etc. [2]. Many countries use some aquatic plants to treat water pollution problem [3].

Normally, to monitor the physiological activities of plants, oxygen or carbon dioxide concentration in certain container holding the plants is monitored by an oxygen or carbon dioxide sensor. However, the monitored oxygen or carbon dioxide is spatially averaged, which reflects the average concentration of oxygen or carbon dioxide released or absorbed by the whole plants in the container. It is difficult to monitor and distinguish the oxygen or carbon dioxide released or absorbed by different organs (e.g., leaves, stems, or roots) of the plants. Therefore, it is nearly impossible to distinguish the physiological activities of each part of the plants in the conventional method.

Recently, we have developed the optical detection system that could real-time in-situ simultaneous monitoring of the DO at a vicinity of micrometres from an aquatic plant surface [3-5]. Moreover, the system could monitor in both the respiration and photosynthetic process [3]. This real-time method allows to monitor DO change in physiological activity at each part of a plant.

Here, we used Excel macro to proceed the calculation of this work. Here, we apply this method to monitor DO change at vicinities of aquatic plant in different water environment.

2. Experimental

2.1. The optical detection system for plant monitoring
The optical detection system for plants monitoring was almost same as reported before \cite{3-5}. This system was placed in the darkroom. A wavelength of about 660 and 450 nm red-blue LED and electric power supply were placed outside the darkroom to illuminate the aquatic plant for the photosynthetic process. Light from a semiconductor laser with a wavelength of 405 nm was reflected by the dichroic mirror and then was focused to a vicinity of the aquatic plants in the culture dish with a culture solution by the objective lens. The culture dish was filled with 20 ml of 10^{-6} M Ru(II) complex (Tris(2,2'-bipyridyl) ruthenium(II) chloride) solution either without or with Cu^{2+} (10^{-4} M). Fluorescence of the Ru(II) complex passed through the dichroic mirror and was detected by a photomultiplier (PMT). Also, an interference filter with a wavelength of 589 ± 25 nm was placed in front of the PMT. In addition, a commercial temperature sensor and DO sensor were inserted into the culture dish. Temperature, DO, and fluorescence signal were simultaneously monitored.

A piece of slide glass was covered on the aquatic plants to prevent possible movement. *Egeria Densa* was used as a model aquatic plant.

![Figure 1. The optical detection system for plant monitoring](image)

### 2.2. Preparation of sample solutions

Stock solution of the 10^{-4} M Ru(II) complex was prepared by dissolving 0.0075 g of the ruthenium complex in 100 ml water. The stock solution was diluted by 100-fold to prepare a 10^{-6} M Ru(II) complex solution. Preparation of a mixed solution of 10^{-6} M Ru(II) complex and 0.008 M sodium sulfite (Na_{2}SO_{3}) was prepared by adding 1 ml of the Ru(II) complex stock solution and 0.0504 g of Na_{2}SO_{3} to 100 ml volumetric flask, then the water was added to 100 ml. Stock solution of 0.02 M Cu^{2+} solution was prepared by dissolving 0.3929 g of the CuSO_{4} \cdot 5H_{2}O in 100 ml water. Then, the stock solution of Cu^{2+} was diluted to desired concentration when adding to the culture dish.

### 3. Results and Discussions

Quenching of the Ru(II) complex fluorescence by DO is described by the Stern-Volmer equation:

\[
\frac{F_0}{F} = 1 + K_{sv}C_{DO}
\]  

Where, \(F_0\) is the fluorescence intensity of the Ruthenium complex solution without DO, \(F\) is the fluorescence intensity of the Ruthenium complex solution with DO, \(K_{sv}\) is the Stern-Volmer-constant and \(C_{DO}\) is the DO concentration. This equation is transformed as follows:
\[ C_{DO} = \left( \frac{F_0}{F} - 1 \right) K_{sv}^{-1} \]  

(2)

As shown in equation (2), \( C_{DO} \) can be calculated from \( F_0 \) and \( F \) of a sample solution as long as \( K_{sv} \) is known beforehand.

Values of \( K_{sv} \) were obtained as follows. Firstly, \( F \) and \( C_{DO} \) of the \( 10^{-6} \) M Ru(II) complex solution without the aquatic plant were measured, and their relationships with temperature \( T \) were approximated as cubic equations of (3) and (4), respectively.

\[ F = 0.04837T^3 - 3.74319T^2 + 96.55830T - 828.82074 \]  

(3)

\[ C_{DO} = 0.23984T^3 - 18.43152T^2 + 471.95482T - 4018.19559 \]  

(4)

Secondly, \( F_0 \) of the \( 10^{-6} \) Ru(II) complex solution containing 0.008 M \( Na_2SO_3 \) were monitored, and its relationships with \( T \) was also approximated as a cubic equation of (5).

\[ F_0 = 0.00133919T^3 - 0.06924T^2 + 0.89438T + 2.18662 \]  

(5)

Thirdly, \( K_{sv} \) was calculated according to equation (1) from above \( F, C_{DO}, \) and \( F_0 \). Figure 2 showed calculated results of \( K_{sv} \) at 24.9 °C - 25.8 °C. The calculated \( K_{sv} \) was approximated as a cubic function too, as shown in Fig. 2.

![Figure 2. Relationship of \( K_{sv} \) to temperature](image)

In order to verify validity of the above \( K_{sv} \), fluorescence intensity \( F \) of another \( 10^{-6} \) M Ru(II) complex solution without the aquatic plant were monitored for 4 hours, and \( C_{DO} \) was calculated according to equation (2) from the monitored \( F \) and \( K_{sv} \) in Figure 2. The calculation results were further compared with the monitored DO concentration by the DO sensor, and results were shown in figure 3. The calculated \( C_{DO} \) agreed well with the one obtained by the DO sensor, although the calculated ones were with large noise. Therefore, the method can be used for monitoring DO.

Next, the method was applied to monitor DO at vicinity of the aquatic plant. Fluorescence intensity \( F_v \) at vicinities of the aquatic plants were monitored, and \( C_{DO} \) at the vicinities was calculated according to equation (2) from \( F_v \) and \( K_{sv} \) in figure 2. Figure 4A showed an example of the calculated \( C_{DO} \) at vicinities of the aquatic plant in a respiration process (The red-blue LED was off). Both the calculated \( C_{DO} \) at the vicinities (0 μm) and DO concentration obtained from the sensor at 1 cm away from the aquatic plants surface decreased with time. However, the \( C_{DO} \) at 0 μm decreased faster with time than that at 1 cm. Figure 4B showed another example of DO monitored in a photosynthetic process (The red-blue LED was on). The DO concentration at the vicinity of the aquatic plants of 0 μm increased with
time faster than that at 1 cm in the photosynthetic process. The photosynthesis process of aquatic plants produced oxygen, and then spread to the culture solution [3,5]. While the respiration process of aquatic plants absorbed DO firstly at vicinities of the plants. Therefore, DO at vicinities of the plants changed with time faster than that at 1 cm away from the plants.

Figure 3. A comparison of DO concentration change with time obtained by the present method and commercial DO sensor.

Figure 4. DO concentration changes with time in a respiration (A) and photosynthetic process (B).
DO change at vicinities of the aquatic plants in water environment containing heavy metal irons such as Cu$^{2+}$ is being monitored too. Figure 5 showed an example of preliminary experimental results of DO monitoring at vicinities of the aquatic plant in water containing of $10^{-4}$ M Cu$^{2+}$ in a respiration process. It is clear that DO changes at vicinities with time is different from that in Fig. 4A. This suggested that existence of Cu$^{2+}$ might have affected absorption of DO in respiration process of the aquatic plants. Its details and mechanisms are being studied and will be reported later.

![Figure 5](image)

**Figure 5.** DO concentration changes with time in a respiration process containing Cu$^{2+}$ of $10^{-4}$ M.

4. Conclusion

DO concentration at vicinities of an aquatic plant surface were monitored real time and in-situ in both respiration and photosynthetic processes. The method is useful in monitoring of DO change at vicinities of aquatic plant in the different water environment.

References

[1] Fondriest Environmental. Inc. 2013 Dissolved Oxygen Online: http://www.fondriest.com/environmental-measurements/parameters/

[2] Manzatua C, Nagya B, Ceccarini A and Iannelli R 2015 *Mar. Pollut. Bull.* **101** 605

[3] Wu X-Z Wu X and Inoue T 2017 Real-time in-situ Simultaneous Monitoring of Dissolved Oxygen and Materials Movements at a Vicinity of Micrometers from an Aquatic Plants by Combining Deflection of a Probe Beam and Fluorescence Quenching *Anal. Sci.* **33** 351-355

[4] Huang L and Wu X-Y 2017 Real-time in-situ Simultaneous Monitoring of Dissolved Oxygen and Materials Movement at Vicinities of an Aquatic Plants by Fluorescence Quenching/Deflection with an improved calculation method *SDRP Journal of Plant Sci.*, **2**, 1-7

[5] Wu X.-Z and Huang L 2018 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 Plants *Anal. Sci.* **34** 1335-1337