ABSTRACT
Recently we developed a novel optical detection system that allowed real-time in-situ simultaneous monitoring of dissolved oxygen and materials movements at a vicinity of micrometers from an aquatic plant by combining deflection of a probe beam and fluorescence quenching. In the detection system, dissolved oxygen (DO)-quenched fluorescence was monitored together with beam deflection signals at the vicinity of the plant, then DO concentration was calculated from the fluorescence intensity. Here, three calculation methods for obtaining DO concentrations from the fluorescence intensity were examined. Results showed that exponential equations method, where fluorescent quenching coefficient and measured fluorescence intensity either with or without DO were approximated with exponential equations of temperature, gave the closest results to the measured ones by a DO sensor. The method was further applied to monitoring of oxygen and materials movement across the aquatic plants surface.

INTRODUCTION
Research on plants is becoming more and more important due to not only by population growth but also environmental problems such as the greenhouse effect. Most studies on plants are carried out by analyzing components involved in the plants activities with various analytical methods. For example, concentration changes of CO$_2$ and O$_2$ due to plants activities have been determined by Infrared spectroscopy$^1$ and fiber optic micro-optrode$^2$, respectively. However, only average concentration changes over a defined sampling interval and within a sample-cell or sample-vessel that hold the plants have been obtained. The resulting average concentration changes reflect spatial- and temporal-average changes caused by the whole plants. It is difficult to distinguish the concentration changes caused from various organs such as leaves from stems. However, activities of the different organs in a plant are usually different.

Keyword: fluorescence quenching, deflection, dissolved oxygen, aquatic plant, in situ monitoring
Recently, we have developed a novel optical detection method which allows real-time in-situ simultaneous monitoring of the dissolved oxygen and material movements at a vicinity of micrometers from the aquatic plant surface.\(^4\) In the method, a blue semiconductor diode-laser was used as the light source of both the probe beam and excitation light for fluorescence of Ru-complex (Tris (2, 2'-bipyridyl) ruthenium (II) chloride). The dissolved oxygen (DO) quenched fluorescence and material movement-induced deflection signals at a vicinity of a plant \textit{Egeria densa} Planch were monitored simultaneously and compared at different distances from the aquatic plant surface. The results show that the optical detection system can monitor DO and the material movements at the vicinity of the aquatic plants not only much more sensitively, but also much closer to real time than those analytical methods that monitor concentration changes in the bulk solution.\(^4\)

In the method\(^4\), DO concentration was calculated from the DO-quenched fluorescence according to the Stern-Volmer equation:

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

(1)

Where \(C_{DO}\) is the DO concentration, \(F_0\) and \(F\) are measured fluorescence intensities of the Ru-complex solution without and with DO, and \(K_{SV}\) is the quenching coefficient, respectively.

Before measuring \(F\) at a vicinity of the plant, \(K_{SV}\) has to be known. Because temperature \(T\) affects \(F_0\), \(F\), and \(C_{DO}\), \(K_{SV}\) is depend on \(T\). The following algorithm for obtaining \(K_{SV}(T)\) was used.\(^4\) Firstly, \(C_{DO}(T)\) and \(F(T)\) in a 10\(^{-6}\) M Ru-complex solution without the aquatic plant were monitored for 2 hours. The values of \(C_{DO}(T)\) and \(F(T)\) were approximated as a linear and quadric equations of \(T\), respectively. Secondly, \(F_0(T)\) was monitored with an anaerobic 10\(^{-6}\) M Ru-complex solution for 2 hours, and the measured \(F_0(T)\) was also expressed by a quadric equation of \(T\). Thirdly, with above \(F_0(T), F(T)\) and \(C_{DO}(T), K_{SV}(T)\) was calculated by

\[
K_{SV}(T) = \left(\frac{F_0(T)}{F(T)} - 1\right) / C_{DO}(T)
\]  

(2)

Then, \(F\) and \(T\) at the vicinity of the aquatic plant were monitored, and \(C_{DO}\) at the vicinity were calculated by Eq. (1) from the monitored \(F(T)\) and calculated \(F_0(T), K_{SV}(T)\). The previous experiments were carried out over a temperature range of 25–27\(^\circ\)C. However, further experimental results showed that the calculation of \(C_{DO}\) had relatively larger experimental error, especially when temperature was exceeded 25 – 27 \(^\circ\)C in summer season. Therefore, the calculation method has to be improved. Here, three different calculation methods for \(C_{DO}\) were tried and compared. Furthermore, the detection system with the improved calculation method is applied to monitor changes of DO and materials movement-induced deflection signal at vicinities of aquatic plant \textit{Cabomba}.

Basic considerations of the novel calculation methods

\textbf{Cubic equation (CE) method}

As stated above, quadric equations were used to approximate \(F_0(T), F(T)\) and \(K_{SV}(T)\) in previous work. Mathematically, when using polynomial equations to fit experimental data, the higher the polynomial degree is, the more the accuracy can be obtained. Therefore, cubic equations were tried to approximate the experimental relationship of \(F, F_0\) and \(K_{SV}\) to \(T\).

\textbf{Exponential equation (EE) method}

It was reported\(^5\) that \(\ln(K_{SV})\) was linear to \(1/T\). The measured relationships of \(F_0\) and \(F\) to \(T\) were found closer to exponential equations than polynomial ones. Hence exponential equations were used to fit the measured \(F_0(T), F(T), \) and the calculated values of \(K_{SV}(T)\).

\textbf{Three dimensional diagram (3DD) method}

In phase fluorometric technique\(^7\)\(^8\), fluorescence lifetime \(\tau\) or phase shift of fluorescence was a function of \(C_{DO}\). A three dimensional diagram (3DD) of phase shift, \(C_{DO}\) and temperature \(T\) was proposed to obtain \(C_{DO}\) from measured phase shift and temperature.\(^9\) Here, a 3DD of \(F_0/F, C_{DO}\), and \(T\) was tried to obtain \(C_{DO}\). Moreover, the 3DD was tried to be expressed by a binary quadratic equation.

\textbf{Experimental}

Experimental setup was similar to the previous one\(^4\). As shown in Fig. 1, a semiconductor laser (wavelength 405nm) was used as the source of both the excitation light and the probe beam. The
laser light was reflected by a dichroic mirror and focused at a vicinity of a short piece of aquatic plant *Cabomba* (about 3.0 cm long) in a culture dish with 20 ml of $10^{-6}$ M Ru-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. The fluorescence of the Ru-complex passed through the dichroic-mirror was detected by a PMT. Simultaneously, deflection signal of laser beam was detected by a deflection detector. A commercial DO/Temperature sensor (pyro science GmbH Hubertusstr. 35 D-52064 Aachen) was placed in the culture dish.

As the experimental procedures, $C_{DO}$, $F$, and $T$ were firstly monitored for 2 hour with a $10^{-6}$ M Ru-complex solution without the aquatic plant. Secondly, $F_0$ and $T$ were monitored for 2 hours with anaerobic $10^{-6}$ M Ru-complex solution without the plant. Thirdly, $F$ and $T$ at vicinities of the aquatic plants in the $10^{-6}$ M Ru-complex solution were monitored for 2 hours in a dark room. In all experiments, DO concentration at 1cm away from the plants was monitored by the commercial DO sensor too.

Preparations of either air-saturated or anaerobic Ru-complex solutions were prepared as previously described.⁴)

**Results and discussion**

**CE method**

Figure 2(a) showed the monitored $C_{DO}$ in the Ru-complex solution without the aquatic plant by the commercial DO sensor at room temperature. The experiments were carried out three times in different days, and every time the monitoring was carried out for 2 hours. The room temperature changed in 19.7 ~ 22.0 °C in winter season, much different from previous experiments in summer season. The result showed that the $C_{DO}$ linearly changed with $T$. The experimental relationship between $C_{DO}$ and $T$ was expressed as follows:

$$C_{DO}(T) = -0.125T + 11.2 \quad (3)$$

Figures 2(b) and 2(c) showed the monitored $F$ and $F_0$ for an air-saturated and anaerobic $10^{-6}$ M Ru (II) complex solution, respectively. The experiments were also carried out 3 times on different days. Both $F$ and $F_0$ were found to decrease with temperature. Relationships of $F$ and $F_0$ to $T$ were approximately expressed by cubic equations (4) and (5), respectively.

![Fig. 1 Experimental setup of the optical system for deflection/fluorescence measurement.](image)
Then, $K_{SV}(T)$ was calculated by Eq. (2) with above $F_0(T)$, $F(T)$ and $C_{DO}(T)$ at 19.7~22.0°C. Figure 2 (d) showed results of the calculated $K_{SV}(T)$. The values of $K_{SV}(T)$ also decreased with temperature. The calculated $K_{SV}(T)$ was fitted with a cubic equation as follows:

$$K_{SV}(T) = -0.000900T^2 + 0.0668T - 1.567 + 12.1$$  \hspace{1cm} (6)

With above $K_{SV}(T)$ and $F_0(T)$, $C_{DO}$ at vicinity of a plant or another Ru-complex solution could be calculated by Stern-Volmer equation from monitored fluorescence intensity $F$.

**EE method**

Figures 3(a) and 3(b) showed the exponential fitting of $F(T)$ and $F_0(T)$, respectively. The exponential fitting equations were as follows:

$$F(T) = 1.99 + 6.23 \times 10^{-12} \times e^{\frac{463}{T}} \hspace{1cm} (7)$$

$$F_0(T) = 3.10 + 2.21 \times 10^{-7} \times e^{\frac{297}{T}} \hspace{1cm} (8)$$

Then, values of $K_{SV}(T)$ were calculated from above $F_0(T)$, $F(T)$ and $C_{DO}$ by Eq. (2) and the results were shown in Fig. 3(C). The calculated $K_{SV}(T)$ was also fitted with an exponential equation as follows:

$$K_{SV}(T) = 0.0586 + 7.45 \times 10^{-7} \times e^{\frac{214}{T}} \hspace{1cm} (9)$$

With above $K_{SV}(T)$ and $F_0(T)$, $C_{DO}$ at vicinity of a plant or another Ru-complex solution also could be calculated by Stern-Volmer equation from monitored fluorescence intensity $F$.

**3DD method**

Figure 4 showed the measured 3DD of $F_0/F$, $C_{DO}$ and $T$ from the $10^{-6}$M Ru-complex solution without the plant. Furthermore, a binary quadratic equation was used to fit the 3DD in Fig. 4. A MATLAB program for the fitting calculations gave the following binary quadratic equation:

$$\frac{F_0}{F} = f(C_{DO}, T) = 0.857 \times C_{DO}^2 + 0.0555 \times T^2 + 0.277 \times C_{DO} \times T - 20.8 \times C_{DO} - 6.83 \times T + 144 \hspace{1cm} (10)$$

Then, $C_{DO}$ at vicinity of the plant can be either found from the 3DD or calculated from the Eq. (10) for a measured $F_0/F$ and $T$. 

**Fig. 2** Relationships of $C_{DO}(a)$, $F(b)$, $F_0(c)$, and calculated $K_{SV}(d)$ to T, respectively. The data were obtained from $10^{-6}$M Ru-complex solution without the aquatic plant.
Fig. 3 Exponential fitting results of $F(T)$ (a) and $F_0(T)$ (b), and the calculated $K_{SV}(T)$ (c).

Fig. 4 Three dimensional diagram of the $F_0/F$, $C_{DO}$ and $T$.

Fig. 5 Comparisons of the three calculation methods and the previous method with the DO-sensor measured results.
Comparison of the three methods with the previous one.

A new prepared $10^6$ M Ru-complex solution without the aquatic plant was used for comparison of the three calculation methods. Fluorescence intensity $F$ was monitored, and meanwhile temperature $T$ and DO concentration were also monitored by the commercial DO sensor. Then, $C_{DO}$ was calculated by the three methods, and the results were shown in Fig. 5. Figure 5 also shows calculation results of $C_{DO}$ by the previous method and the measured $C_{DO}$ by the DO sensor.

Figure 5 showed that the EE method gave the closest DO concentration to the measured ones by the DO sensor in the three calculation methods. In Fig 5, results of the FE method were also closer to measured ones by the DO sensor than the previous method. This meant that the EE method was the most accurate to obtain the DO concentration from the fluorescence quenching. Therefore, the EE method was used to determine the DO concentration at vicinity of the aquatic plants.

Monitoring of DO and deflection signals at the vicinity of the aquatic plant

Figure 6 showed a typical example of monitoring results of DO concentration and deflection signals at vicinities of different distance away from the aquatic plant surface in a dark room. As shown in Fig. 6(a), the DO concentration decreased with time at 0 μm. After 2 hours, the DO concentration at 0 μm decreased to about 8.1 ppm from 9.3 ppm. Because respiration processes in the dark room consumed oxygen, DO at the plant surface was consumed firstly, then DO in solution diffused to the plant surface to supply the oxygen consumption. On the other hand, DO changed little at 100 μm away from the plant. The DO concentration at this distance was nearly the same as that at 1 cm away from the plant surface monitored by the DO sensor. Figure 6(b) showed that the deflection signals also changed greatly at 0 μm, while on the similar level as background solution at 100 μm. These results indicated that the monitoring of both the DO and materials movements-induced deflection were with the most highest sensitivity at the plant surface.

Fig. 6 Real time in-situ morning of DO concentration (a) and deflection signals (b) at the vicinity of different distance away from the leaf surface of the aquatic plant in a dark room.
As a conclusion, three calculation methods for obtaining DO concentration at vicinity of a plant from the oxygen-quenched fluorescence were examined. The results show that the EE method gave the best results. The optical detection system with the improved calculation method is more accurate in monitoring of DO at vicinity of the aquatic plants. This method is expected to be powerful in monitoring aquatic plants activities because of its higher sensitivity and more real-time than those conventional analytical methods by measuring the spatial- and temporal-average concentration changes.

REFERENCES