Diurnal fluctuations in oxygen release from roots of *Acorus calamus* Linn in a modeled constructed wetland

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ABSTRACT

The detailed mechanisms of oxygen release from the roots of plants in constructed wetlands (CW) remains unclear. This study investigated the variation of root oxygen release rate, and the effect of photosynthesis during day and night periods on the rate of oxygen release from the roots of *Acorus calamus Linn* in a model CW. The maximum oxygen release rate was recorded to be in the range of 215.2-750.8 μmolg^{-1}h^{-1} and this occurred at 15:00. The maximum value of photosynthetically active radiation

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(PAR) ranged from 1281.8-1712.0 mmolm^{-2}s^{-1}, and this occurred at 13:00. It was observed that both the oxygen release rate and PAR approached zero at night. The results indicate that the rate of oxygen release depends largely on the light intensity, which exhibits a diurnal periodic variation. Accordingly, there are two time intervals namely: day time and night time, during the former period oxygen is released by plants. This study on dynamics of plant root oxygen release distribution has shown that the variation of root oxygen release during daytime followed the Gaussian function. The Gaussian function can be used to predict the root oxygen release rate in constructed wetlands.

Keywords Constructed wetlands; diurnal variation; oxygen release rate; plant; root

INTRODUCTION

Constructed wetlands (CW), as low-cost wastewater treatment technology, have been gaining increased international interest and application because of their good treatment efficiency and their natural fit into the landscape. Aerobic decomposition of organic matter and nitrification are affected significantly by the oxygen levels in CW during wastewater treatment. It has been reported that subsurface flow wetland systems have not been successful (in some cases) in removing nitrogen mainly due to lack of dissolved oxygen caused by permanent saturation.[1,2]

Plants in CW are known to transport and release oxygen into their root-zones, thus enhancing aerobic processes [3]. Oxygen released by the root systems of wetland plants is one of the important oxygen sources. Brix [4] stated that reeds transport oxygen into the rhizosphere, thereby creating aerobic microsites adjacent to the roots and rhizomes. The ability of reeds to transport oxygen and thereby to support a population of aerobic microorganisms in the rhizosphere is one of the key mechanisms for efficient BOD and nitrogen removal. The oxygen release rate into the rhizosphere by a plant can be determined under various light intensities [5]. Ojeda et al. [6] reported high rates of plant oxygen transfer, suggesting a general view that macrophytes played a considerable role in wastewater treatment. Mitsch [7] reported that the rate of oxygen released by plant roots varied from 0 to $45gO_2m^{-2}d^{-1}$. Sorrell and Armstrong [8] measured the root oxygen release by bathing whole root systems of *Juncus ingens* in titanium (Ш) citrate buffer. The results show that the rate of root oxygen release ranged from 0 to 121.6 $\mu molh^{-1}g^{-1}$ root dry wt. It was noted that the root oxygen release rate is highly variable. Therefore, further study of the root oxygen release behaviour is desirable.

It is well recognized that the rate of oxygen released by the plant root systems is influenced by many factors. Jespersen et al. [9] studied the effect of rhizosphere sediment on oxygen release rate by comparing Typhas grown in two sediments (natural organic sediment and a sediment enriched with acetate), and measuring the root oxygen release using titanium (Ш) citrate buffer. Oxygen demand in the sediment enriched with acetate was higher compared to that of the natural organic sediment. This phenomenon influenced the growth pattern of plants and root shape, and accordingly influenced oxygen release rate, which was about 120-200 $\mu molO_2h^{-1}g^{-1}$ root dry wt. Sasikala et al. [10] investigated the effects of water level fluctuation on plant radial oxygen loss (ROL), root porosity, plant growth performance, and nitrogen removal in vertical subsurface flow CW. Their results showed that the quantity of oxygen released by the root systems of plants could be significantly decreased by water level fluctuation. Stottmeister et al. [11] described that gas transport from the sections of the plant above the ground through the rhizome into the fine roots was affected by specific areas of tissue formed in the plant known as the aerenchyma. Other factors include rhizosphere-specific parameters such as the redox state, pH, oxygen concentration, chemical characteristics, and plant-specific parameters such as the mass, the plant species and stage of development of plants, as well as variation of climate and different testing conditions.

In this study, the daily change in the root oxygen release rates were carefully examined using a titanium (Ш) citrate buffer. The effect of light intensity on root oxygen release rate was studied. A model (using a Gaussian distribution) to describe the root oxygen release behavior was developed based on the experimental data. The model enables the prediction of the diurnal fluctuation of root oxygen release rate.

MATERIALS AND METHODS

Experimental Materials and Procedures

The plants of young *Acorus calamus Linn* used in this study were collected from a natural wetland located in Xuanwu Lake, Nanjing, China. After collection, the plants were transplanted to individual plastic pots filled with respective nutrient solutions (self-made nutrient solutions with average concentrations of COD and TN of 50mg/L and 15mg/L, respectively) for three weeks before sowing. The plants were removed from the pots and their roots were gently washed free of debris twelve hours before the commencement of experiments. All plants had 20-40 adventitious roots which varied in length from 12-21cm and were up to 0.087±0.029cm (n=118) in diameter. Their height above ground was 41-58cm.

Oxygen release from the roots was examined using a non-phytotoxic titanium (Ш) citrate buffer, which allows root oxygen release measurements in a reducing, oxygen-scavenging solution with a low redox potential [8,9]. The 1000mL jar was initially filled with 900mL of distilled water, and the water was then sparged with N_2 gas for 60 minutes to remove any oxygen dissolved in the water. Sparging with N_2 gas was continued while titanium (Ш) citrate stock solution (made by 0.2249g citric acid and 8mL $TiCl_3$) was added. The basal part of the shoot was wrapped with tinfoil to prevent the oil from infiltrating the aerenchyma. The stirring from the sparging was necessary to ensure complete mixing. The roots of *Acorus calamus Linn* were submerged in the solution. A 5mm thick layer of paraffin oil was placed on top of the solution to prevent re-aeration from the atmosphere. It ensured that the roots were the only possible source of oxygen entry into the chamber. The root chamber was shielded from light using a tight-fitting tinfoil cover. Blank jars without plants were also prepared in a similar way to serve as negative control. Figure 1 shows the experimental device used in the investigation of the root oxygen release rate. The experimental device was exposed to the open air and natural light in a sealed area outside the laboratory building. Light intensity was measured every 1 hour using a luminometer (MODEL ZDS-10F-2D). The unit of light intensity is lux. Details of the experimental set-up are shown in table 1.

Sampling and Analytical Methods

Since the oxygen released from the roots was oxidized by Ti^{3+} in titanium (Ш) citrate buffer, rates of root oxygen release could be calculated from the rate of decrease in the concentration of Ti^{3+} in the jars. As the brown titanium (Ш) citrate solution gradually became clear during oxidation, the samples were taken every 1 hour using a small syringe and the absorbance at 527nm was measured immediately using a spectrophotometer. The absorbances of the samples were compared to those of solutions with a known concentration of Ti^{3+}. At the same time, the light intensity, temperature and humidity were examined. The relation of Ti^{3+} and O_2 is described in the Equation 1. It is seen that 1mol O_2 is consumed when 4mol Ti^{3+} were reduced. The oxygen consumption (ΔO_2, mg) is thus calculated using Equation 2. Thereafter, the root oxygen release rate (V_O, μmolg^{-1}h^{-1}) could be calculated using Equation 3.

$$O_2 + 4Ti^{3+} + 4H^{+} = 4Ti^{4+} + 2H_2O \tag{1}$$

$$\Delta O_2 = \frac{32 \times V \times (C_0 - C_e)}{4 \times 47.73} \tag{2}$$

where V is the volume of titanium (Ш) citrate buffer, 0.9L. C_0, C_e are the initial and end Ti^{3+} concentration, respectively.

$$V_O = \frac{\Delta O_2 \times 1000}{24 \times 32 \times Root\ dry\ weighs} \tag{3}$$

RESULTS

Plant Root Oxygen Release Rate

Figure 2 illustrates the daily changes of titanium(Ш) citrate concentration on 22nd April, 2009. The precision of the detecting device is confirmed by comparing the measured Ti^{3+} concentration with that in blank jars. Titanium (Ш) citrate concentration in the blank jars did not change during the whole experiment. This suggests that the variation of Ti^{3+} concentration was caused by the oxygen released by the plants in the jars.

According to the experiments conducted on 22nd, 25th and 26th April, 2009, respectively, variation of the root oxygen release rates could be obtained based on Ti^{3+} concentrations in tested jars using the Equations (1)-(3). The dry weights of plant root were measured after drying for 24h at 105 ℃. Daily changes of oxygen release rate and PAR are illustrated in Figure 3. The results reveal a significant difference in the root oxygen release rate during day and night. Oxygen release increased gradually with increasing light intensity in the morning. However, a decrease in the oxygen release rate occured following the decreased light intensity in the afternoon, and approached $0\mu molg^{-1}h^{-1}$ at night. These variations indicate a significant time dependency for oxygen release by plants during the day and night. For all three sets of experiments, the start time and end time of oxygen release were closely related to light. The maximum oxygen release rate ($215.2\text{-}750.8\mu molg^{-1}h^{-1}$) was observed during the daytime at 15:00 hrs while the maximum light intensity was observed at 13:00 hrs. The maximum value of PAR ranged from 1281.8 to 1712.0 $mmolm^{-2}s^{-1}$. Clearly, the peaks of root oxygen release occurred after the peak of light intensity.

Daily Variations of Root Oxygen Release and Light Intensity—Application of a Gaussian Function

The variation of the root oxygen release during the day-night is schematically summarized in Figure 4. There are two time intervals during the day-night cycle, i.e. periods of brightness and darkness. The t_{Ls} and $t_{Le,}$ are the start and end time of the bright period, respectively. They also correspond to the sunrise (t_{Ls}) and sunset (t_{Le}) time. L_{max} is the maximum light intensity at the corresponding time t_{Lmax}. The t_{Os} and $t_{Oe,}$ are the start and end time of oxygen release period, respectively. V_{Omax} is the maximum oxygen release rate at the time of t_{Omax}. Since the time of maximum oxygen release rate occurred at a later period than the time of maximum light intensity, this time difference is termed as lag time (⊿ t). It may be caused by photosynthesis and oxygen transport in the aerenchyma.

In order to describe the daily variation of the root oxygen release in a mathematical way, data obtained from the experimants were preliminarily fitted using several functions (t_{Os} was 4:00 and t_{Oe} was 20:00). The results reveal that the best fitting could be acheived by Gaussion function [13], which is represents unimodal distribution model as shown in Figure 5(a). The goodness of fit (R^2) is 0.7574, 0.5357, 0.6796 with 95% confidence bounds on 22nd, 25th and 26th April, respectively. Based on the form of Gaussion function, diurnal variation of root oxygen release rate could be described as:

$$V_O = ae^{-\frac{(t - t_{Omax})^2}{c^2}} \tag{4}$$

where t is time (4:00~20:00); a (in the Gaussian function) is the maximum value of oxygen release rate in a whole day; t_{Omax} is the location of the symmetry axis in Gaussian function; c expresses the gradient of the Gaussian function. A decrease in c indicates a steep Gaussian function while an increase in c indicates a gentle Gaussian function. Figure 5(a) shows the root oxygen release data (on 26th April) with Gaussian function fitting, where *a, c* and t_{Omax} are 613.1μmolg^{-1}h^{-1}, 3.884 and 15:00, respectively.

Light intensity data during daytime (4:00-20:00) also follow a Gaussian function (Fig. 5(b)). It can be described as:

$$PAR = be^{-\frac{(t-t_{Lmax})^2}{d^2}} \tag{5}$$

where *PAR* is the photosynthetically active radiation, μmol·m^{-2} s^{-1}; *t* is time; *b* is the peak value of *PAR* in a whole day; and *d* is the gradient of the unimodal function. Figure 5(b) shows the light intensity data (on 26th April) with a Gaussian function fitting, where *b, d* and t_{Lmax} are 1702μmol·m^{-2} s^{-1}, 3.672 and 13:00, respectively.

The peak value of root oxygen release was observed after the maximum light intensity for 2 hrs. The correlation between the light intensity and the oxygen release data (collected 2 hrs later) was analyzed and illustrated in Figure 6. It is clear from Figure 6 that the root oxygen release rate was influenced by light intensity dramatically. Oxygen release rate increased exponentially with increased *PAR* ($R^2 = 0.8689$):

$$V_O = 62.22e^{0.00138PAR} \tag{6}$$

By combining Equations. (4), (5) and (6), the following equation is obtained:

$$ae^{-\frac{(t-t_{Omax})^2}{c^2}} = 62.22e^{0.00138(be^{-\frac{(t-t_{Lmax})^2}{d^2}})} \tag{7}$$

It should be pointed out that, in Equation 7, $t_{Lmax}=t_{Omax}$ since the oxygen release curve (see Figure 4) was shifted for 2 hrs when the light intensity and the oxygen release data was correlated, as described in Equation 6.

In the special case, when the time (t) is equal to the peak time (t_{Omax} or t_{Lmax}), Equation 7 becomes:

$$a=62.22e^{0.00138b} \tag{8}$$

Equation 8 indicates that the parameters of *a* in oxygen release behaviour and *b* in light intensity follow an exponential function.

The parameters *c* and *d* also follow an exponential function. The daily changes of root oxygen release and light were examined. Values of *c* and *d* derived from the experiments were fitted using an exponential function (R^2 is 0.9587). The relationship is as follows:

$$c=0.66e^{0.4856d} \tag{9}$$

Therefore, the Gaussian function can be used to predict the oxygen release rate. The procedure is as follows: (1) obtain light intensity data; (2) fit the data using Gaussian function from which the parameters of *b* and *d* could be obtained using Equation 5; (3) determine parameters *a* and *c* using Equation 8 and Equation 9, respectively; (4) calculate oxygen release using Equation 4.

Validation

The experiments were conducted with the same testing device in October 2009 to validate the model of the proposed Gaussian function. PAR was tested every 1 hour during the daytime (4:00~20:00), and the parameters obtained are tabulated in table 2.

Measured oxygen release rates and the corresponding predicted values using a Gaussian function are jointly illustrated in Figure 7. From the results of the stimulation, it can be seen that the Gaussian function can be satisfactorily used to predict the daily changes of oxygen release by roots of wetland plants. The model data closely correlate with the experimental values.

DISCUSSION

It is well recognized that the oxygen release rate of wetland plants is associated with light intensity. In addition, the oxygen release rate varies with time during natural changes in light intensity. However, no detailed information on the variation of oxygen release rate is found in the literature. In this study, the oxygen release rate of a wetland plant, *Acorus calamus Linn*, was examined in detail. It has been shown that the oxygen release rate for *Acorus calamus Linn* (Fig. 3) appears to be much higher than those reported in the literature although different wetland plants were tested [14,15]. Mei et al., [15] reported oxygen release rate of 7.40-13.24 μmolO_2 $h^{-1}g^{-1}$root dw for *Shengtail* and *Suyunuo*, while oxygen release rate of 1.6μmol$O_2h^{-1}g^{-1}$dw for *Cladium* was reported by Chabbi et al. [14]. It is noted in the literature that even for the same wetland plant, the reported oxygen release rate is significantly different. For example, Sorrel and Armstrong [8] reported oxygen release rate of 126 μmol$O_2h^{-1}g^{-1}$dw for *Juncus ingens*, while a value of 1.5 μmol$O_2h^{-1}g^{-1}$dw for *Juncus bulbosus* was reported by Chabbi [16]. The reason for the variation in reported values may be partially attributed to the light intensity.

Oxygen is produced during photosynthesis [17] and is transferred from the leaves to the roots through the gas-filled tissues of plant by the process of diffusion and convection [18]. Oxygen is then released to the rhizosphere by gas exchange. The photosynthetic rate of plants was highly correlated with light intensity. The photosynthetic characteristics can affect their ability to provide oxygen [19]. The light-dark switch generates a large and rapid fluctuation in the internal oxygen levels of plants [18]. Thus, plants also experience great released oxygen fluctuations. In this paper, the rate of oxygen release was shown to depend largely on the light intensity, which exhibits a diurnal periodic variation. The variation of oxygen release and light intensity followed unimodal distribution and, furthermore, followed the Gaussian function during the daytime. In particular, the maximum root oxygen release rate was shown to occur 2 hours after the occurrence of maximum light intensity (Fig. 3), from which the relationship between the root oxygen release rate and the light intensity was established. Although a recent study has shown that the maximum root oxygen release with up to 35% oxygen saturation at the root surface occurred under light conditions while a decrease of about 30% was observed under dark conditions [5], the present study has given a detailed profile showing the daily changes of root oxygen release rate with natural light.

More significantly, this study presented a methodology of root oxygen release prediction using a Gaussian function. This allows us to use the light intensity data to calculate the quantity of oxygen likely to be released. However, further studies are still needed to demonstrate the application of the Gaussian function when other wetland plants are tested. It should also be noted that the method of the Gaussian function was established based on the experimental data collected at Nanjing with a unique climate. Thus, validation studies in other places with different natural light should be considered before the methodology established in current study is applied more generally.

CONCLUSIONS

The oxygen release rate of wetland plants exhibited diurnal periodic variation. Light intensity is a major factor influencing oxygen release. In the morning, the oxygen release rate increased with increasing light intensity. Both the values of the oxygen release rate and light intensity decreased gradually in the afternoon, and approached 0μmolg^{-1}h^{-1} at night due to the absence of the light. More significantly, the variation of the root oxygen release rate and light intensity followed a unimodal distribution. The Gaussian function has been demonstrated to well describe the day time variation of the root oxygen release rate. It can also be used for prediction purposes of the root oxygen release rate in constructed wetlands.

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Figure 1. Schematic sketch of the root oxygen release rate detecting device

Figure 2 Daily changes of titanium (Ш) citrate concentration

Figure 3 Diurnal fluctuation of root oxygen release rate: (a) 22nd April; (b) 25th April; (c) 26th April

Figure 4 Schematic indication of the diurnal fluctuation of the root oxygen release

Figure 5 Fitting root oxygen release rate (a) and light intensity (b) using Gaussian function

Figure 6 The effect of light intensity on root oxygen release rate

Figure 7 Comparison of observed and predicted oxygen release rate in October 2009

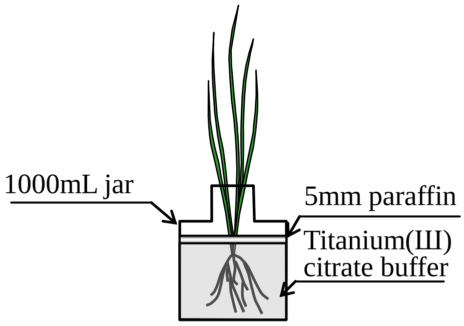

Fig.1

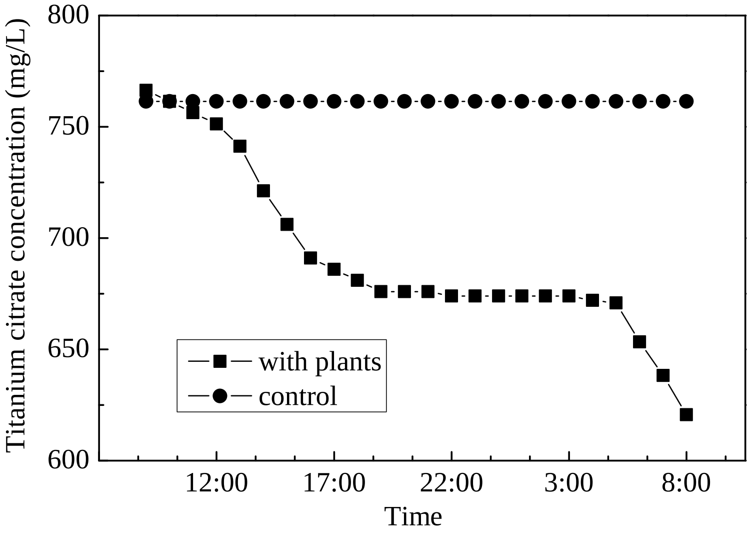

Fig. 2

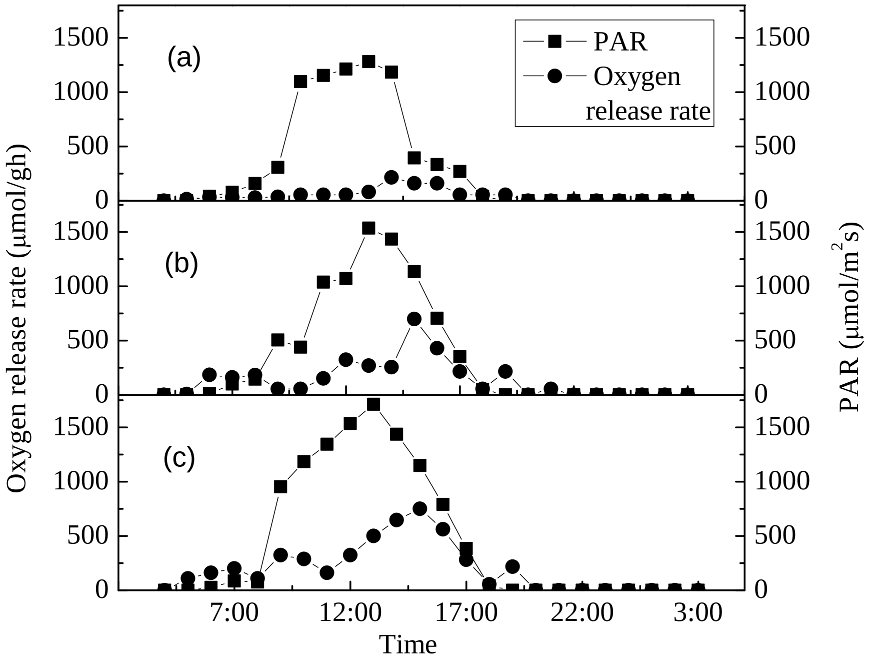

Fig. 3

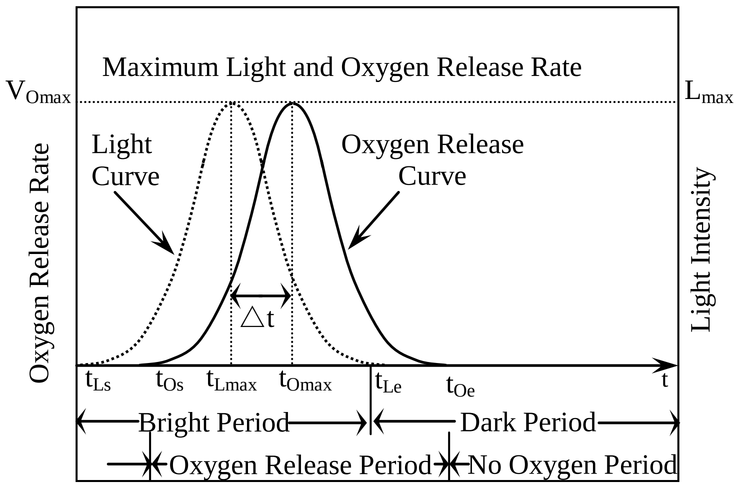

Fig. 4

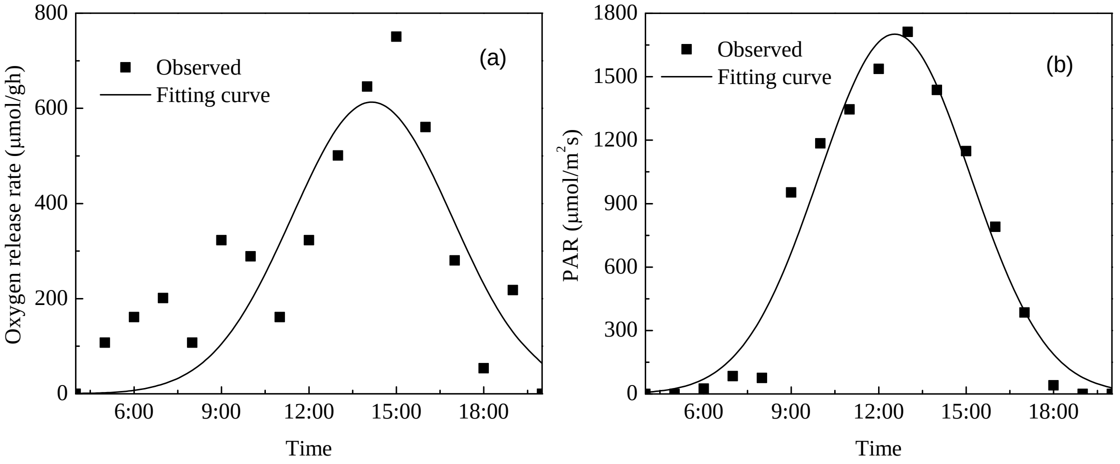

Fig. 5

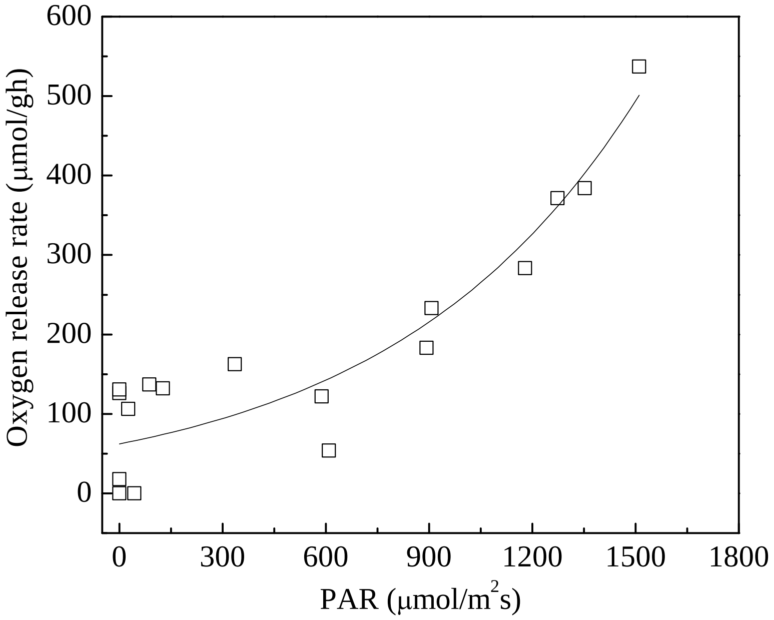

Fig. 6

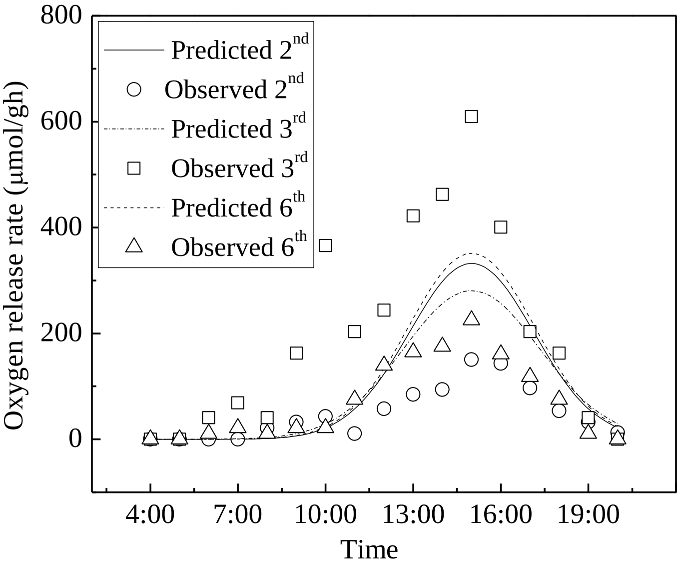

Fig. 7

Table 1. Light intensity, temperature and humidity during experiments

Experimental date	PAR (μmol·m^{-2} s^{-1})		Temperature (℃)		Humidity (%)	
	Average	range	Average	range	Average	range
22 April 2009	444.2	0-1281.8	24	19-28	38	15-61
25 April 2009	501.1	0-1536.4	22	18-25	27	10-52
26 April 2009	630.5	0-1712.0	22	18-25	27	9-55

* Sample number is 24. PAR is photosynthetically active radiation. One lux is 0.019 μmol·m^{-2} s^{-1} [12].

Table 2. The parameters of modeling

Date	PAR		Oxygen release	
	b (μmol·m^{-2} s^{-1})	*d*	*a* (μmolg^{-1}h^{-1})	*c*
2nd October	1214	3.137	332.31	3.020
3rd October	1092	3.33	280.82	3.317
6th October	1254	3.155	351.16	3.047