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

C. DONG¹,², W. ZHU¹,², M. GAO², L. F. ZHAO², J. Y. HUANG² and Y. Q. ZHAO³

¹National Engineering Research Center of Water Resources Efficient Utilization and Engineering Safety, Hohai University, Nanjing P.R. China
²College of Environmental Science and Engineering, Hohai University, Nanjing, P.R. China
³Centre for Water Resources Research, School of Architecture, Landscape and Civil Engineering, University College Dublin, Belfield, Dublin, Ireland

Oxygen is known to be released from plant roots, but has seldom been quantified for wetland plants. Our study aims to quantify oxygen release from the roots of one wetland species in China, and use this knowledge as a basis for future modeling. We measured diurnal fluctuations in oxygen release from the roots of *Acorus calamus* Linn in a modeled constructed wetland (CW) using a titanium (III) citrate buffer. Oxygen release was monitored every two hours. Maximum oxygen release was recorded in the range of 215.2–750.8 µmol g⁻¹ h⁻¹ and occurred around 15:00. The maximum value of photosynthetically active radiation (PAR) was in the range of 1281.8–1712.0 mmol m⁻² s⁻¹ and occurred around 13:00. Both the oxygen release rate and PAR were found to approach zero at night. Our results indicate that oxygen release depends largely on light intensity and exhibits a diurnal periodicity with release occurring only during daytime. Rate of root oxygen release varied during the daytime and this temporal variation was well described by the Gaussian function. While further validation is needed, we suggest that the Gaussian function may be used as the basis for modeling root oxygen release in natural and constructed wetlands.

Keywords: Wetland plants, constructed wetlands, diurnal fluctuation, photosynthetically active radiation, oxygen release rate, Gaussian function.

Introduction

Constructed wetlands (CW) are one method of low-cost wastewater treatment. They have been the focus of increasing international interest because of their water treatment efficiency and their natural appearance. Aerobic decomposition of organic matter including nitrification, is dependent on oxygen within a wetland, and is a key process within a CW. Oxygen can enter the wetland in three ways: as dissolved oxygen in the inflow liquid, as direct surface reaeration from the atmosphere, and as root release from macrophytes. Plants in natural and constructed wetlands are known to transport oxygen to their roots and release it into their root zones. Rhizosphere oxidation activates biochemical reactions and biological processes including degradation of organic compounds and nitrification by rhizospheric microorganisms. Although oxygen is known to be released from the root systems of plants, the extent has not been quantified.

It is recognized that the rate of oxygen release by plant root systems varies in accordance with environmental factors. These include rhizospheric characteristics such as redox state, pH, oxygen levels, and chemistry, and plant characteristics such as plant mass, species, and stage of development, as well as climate. Other factors have been reported. For example, Jespersen et al. studied the effect of surrounding sediment on the oxygen release rate of roots, by comparing *Typha* plants grown in two sediments (a natural organic sediment and an acetate-enriched sediment). Oxygen demand in the acetate-enriched sediment was higher than that in the natural sediment. The sediment type influenced growth pattern and root shape, and thus the oxygen release rate. Sasikala et al. investigated the effects of water level fluctuations on radial oxygen loss (ROL) of plants in a CW subjected to vertical subsurface flow. They found that the quantity of oxygen released by root systems could be significantly reduced by fluctuating the water level. Stottmeister et al. described how gas transport from above-ground sections of the plant to the roots was aided by tissue known as aerenchyma.
that forms within the rhizomes and roots of submerged plants.

The dominant process of oxygen release is, or course, photosynthesis; most oxygen transported to roots is of photosynthetic origin.[7] Oxygen is transferred from leaves to roots by molecular diffusion and convection. Changes in photosynthetic rate in response to environmental factors directly influences oxygen-production and transportation; thus ultimately affecting wetland function.[6] Oxygen release rates are known to be several times greater in the light than in the dark.[7] Effects of weather conditions on oxygen release rate need elucidation; this would enable improved design and operation of aquatic, plant-based, wastewater treatment systems.[2] The influence of light intensity on oxygen release needs to be examined in detail, as do diurnal patterns in release rate.

We used young Acorus calamus plants to investigate the effects of light intensity on oxygen release and diurnal patterns in release rate. We modeled a constructed wetland using a titanium (III) citrate buffer. We developed a model based on a Gaussian distribution to describe observed root behavior. This model enables prediction of diurnal fluctuation in root oxygen release.

**Materials and methods**

**Experimental materials and procedures**

Young Acorus calamus plants were collected from a natural wetland located on the shores of Xuanwu Lake, Nanjing, China. These were planted in individual plastic pots filled with purpose-made nutrient solutions (with average concentrations of chemical oxygen demand [COD] at 50 mg/L and total nitrogen [TN] at 15 mg/L) for 3 weeks. The plants were then removed from pots and their roots gently washed free of debris. All plants had 20–40 adventitious roots of 12–21 cm in length, and up to 0.087 g was immediately using a spectrophotometer. Absorbencies were compared with those of solutions with a known Ti$^{3+}$ concentration. Light intensity, temperature, and humidity of the surrounding air were measured concurrently. The reaction between Ti$^{3+}$ and O$_2$ is shown in Equation 1.[10] It can be seen that 1 mole of O$_2$ is consumed when 4 moles of Ti$^{3+}$ are reduced. Oxygen consumption ($\Delta$O$_2$, mg) was thus calculated using Equation 2.

$$\text{O}_2 + 4\text{Ti}^{3+} + 4\text{H}^+ = 4\text{Ti}^{4+} + 2\text{H}_2\text{O}$$

$$\Delta\text{O}_2 = \frac{32 \times V \times (C_0 - C_f)}{4 \times 47.73}$$

**Sampling and analytical methods**

Since oxygen released from roots is oxidized by Ti$^{3+}$ in the titanium (III) citrate buffer, rates of root oxygen release can be calculated from the rate of decrease in Ti$^{3+}$ concentration in the jars. The brown titanium (III) citrate solution was observed to gradually become clear during oxidation. Samples were taken every hour using a small syringe. Absorbance at the wavelength of 527 nm was measured immediately using a spectrophotometer. Absorbencies were compared with those of solutions with a known Ti$^{3+}$ concentration. Light intensity, temperature, and humidity of the surrounding air were measured concurrently. The reaction between Ti$^{3+}$ and O$_2$ is shown in Equation 1.[10] It can be seen that 1 mole of O$_2$ is consumed when 4 moles of Ti$^{3+}$ are reduced. Oxygen consumption ($\Delta$O$_2$, mg) was thus calculated using Equation 2.

| Experimental date | Average PAR ($\mu$mol·m$^{-2}$·s$^{-1}$) | Average Temperature ($^\circ$C) | Average Humidity (%) |
|-------------------|--------------------------------------|-------------------------------|---------------------|
| 22 April 2009     | 444.2                                | 24                            | 38                  |
| 25 April 2009     | 501.1                                | 22                            | 27                  |
| 26 April 2009     | 630.5                                | 22                            | 27                  |

*Sample number is 24. PAR is photosynthetically active radiation. One lux is 0.019 $\mu$mol·m$^{-2}$·s$^{-1}$.[12]
where $V$ is the volume of titanium (III) citrate buffer (0.9 L), $C_0$, and $C_e$ are the initial and end Ti$^{3+}$ concentrations. The root oxygen release rate ($V_o$, $\mu$molg$^{-1}$h$^{-1}$) was calculated using Equation 3.

$$V_o = \frac{\Delta O_2 \times 1000}{24 \times 32 \times \text{Root dry weights}}$$  \hspace{1cm} (3)

Diurnal fluctuations in oxygen release from roots of *Acorus calamus* Linn could be described using a Gaussian function. First, fit the data of the light intensity and oxygen release rate using the Gaussian function, respectively. Second, obtain the relationships between Gaussian function parameters of light intensity and that for oxygen release rate. Third, calculate the oxygen release rate using the light intensity data for other days. Last but not least, the experiments were further conducted in October 2009 to validate the proposed model based on the Gaussian function, and PAR was tested every hour during the daytime (4:00–20:00).

**Results**

**Oxygen release rate of plant roots**

Figure 2 displays a typical daily change in titanium (III) citrate concentration. Concentration of titanium (III) citrate in the control jars did not change during the experiment. This suggests that variation in Ti$^{3+}$ concentration was caused by oxygen released by plants in the planted jars. Values for root oxygen release were obtained from Ti$^{3+}$ concentrations measured in the test jars, via the above equations. Diurnal fluctuations in oxygen release and PAR are shown in Figure 3. Our results reveal a significant difference in rate of root oxygen release between day and night. Oxygen release increased gradually with increasing light intensity during the morning. A decrease in oxygen release occurred during the decreasing light intensity of the afternoon. At night, oxygen release rate approached 0 $\mu$molg$^{-1}$h$^{-1}$. In all three experiments, the start and end times of oxygen release were closely related to light. The maximum oxygen release rate (215.2–750.8 $\mu$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 peak of root oxygen release occurred after the peak of light intensity.

**Diurnal fluctuations in root oxygen release and light intensity—application of a Gaussian function**

Daily fluctuations in root oxygen release are schematically summarized in Figure 4. Two time intervals were used during the diurnal cycle; $t_{ls}$ and $t_{le}$ signify the start and end times of the bright period. These also correspond to sunrise ($t_{ls}$) and sunset ($t_{le}$). $L_{max}$ is the maximum light intensity at the corresponding time $t_{Lmax}$. $t_{Os}$ and $t_{Oe}$ signify the

![Fig. 4. Schematic diagram of diurnal fluctuation in root oxygen release.](image-url)
Diurnal fluctuations in O$_2$ release in wetlands

Fig. 5. Fitting root oxygen release (a) and light intensity (b) using the Gaussian function.

start and end times of the oxygen release period. $V_{O_{\text{max}}}$ is the maximum oxygen release rate at the time of $t_{O_{\text{max}}}$. Since the time of maximum oxygen release occurred after the maximum light intensity, the time difference between these, termed lag time ($\Delta t$), is also defined. This time lag may be caused by photosynthesis and consequent oxygen transport through the aerenchyma.

To quantify diurnal fluctuations in root oxygen release, experimental data was preliminarily fitted using several functions ($t_{Os}$ was 4:00 and $t_{Oe}$ was 20:00). The best fit was achieved using the Gaussian function$^{[14]}$; this represents a unimodal distribution (Figure 5a). The goodnesses of fit ($R^2$) were 0.7574, 0.5357, and 0.6796 with a 95% confidence interval, on the 3 days studied. Based on the Gaussian function, diurnal fluctuation in root oxygen release can described as

$$V_O = ae^{-\frac{(t-t_{O_{\text{max}}})^2}{c^2}}$$

where $t$ is time (4:00–20:00); $a$ is the maximum rate of oxygen release in a day; and $c$ expresses the gradient of the Gaussian function for rate of oxygen release. A small value of $c$ indicates a steep Gaussian function, while a large value of $c$ gives a gradually varying Gaussian function. Figure 5a shows root oxygen release data from one day with the Gaussian function fitting, where $a$, $c$ and $t_{O_{\text{max}}}$ are estimated to be 613.1 $\mu$mol$^{-1}$h$^{-1}$, 3.884 and 15:00, respectively.

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

$$\text{PAR} = be^{\frac{-(t-t_{L_{\text{max}}})^2}{d^2}}$$

where $\text{PAR}$ is photosynthetically active radiation in $\mu$mol-m$^{-2}$s$^{-1}$; $b$ is the peak value of $\text{PAR}$ in a whole day; and $d$ is the gradient of the Gaussian function for light intensity. Figure 5b shows the light intensity data on one day with the Gaussian function fitting, where $b$, $d$ and $t_{L_{\text{max}}}$ are estimated to be 1702 $\mu$mol-m$^{-2}$s$^{-1}$, 3.672 and 13:00, respectively.

The peak rate of root oxygen release was observed 2 h after the maximum light intensity. The correlation between light intensity and oxygen release was analyzed (Fig. 6). It is evident that root oxygen release was influenced dramatically by light intensity. The oxygen release rate increased exponentially with increased $\text{PAR}$ ($R^2 = 0.8689$):

$$V_O = 62.22e^{0.00138\text{PAR}}$$

By combining Equations 4, 5 and 6, the following equation was obtained:

$$ae^{-\frac{(t-t_{O_{\text{max}}})^2}{c^2}} = 62.22e^{0.00138\left(be^{\frac{-(t-t_{L_{\text{max}}})^2}{d^2}}\right)}$$

In Equation 7, $t_{L_{\text{max}}} = t_{O_{\text{max}}}$ because the oxygen release curve (see Figure 4) shifted to 2 h later, following the
correlation of light intensity and oxygen release data, as described in Equation 6.

If the time (t) happened to be equal to the peak time (tOmax or tLmax), Equation 7 would become

\[ a = 62.22e^{0.00138b} \] (8)

Equation 8 indicates that parameter a for describing oxygen release behavior is related to b in the light intensity equation by an exponential function.

The relationship between parameters c and d also follows an exponential function. Values of c and d derived from our experiments were fitted using an exponential function (R² = 0.9587). The relationship is as follows:

\[ c = 0.66e^{-0.485d} \] (9)

Based on the above results, the Gaussian function can be used to predict oxygen release rate by the following procedure: (i) obtain light intensity data; (ii) fit the data using the Gaussian function from which parameters b and d can be obtained using Equation 5; (iii) determine the values of a and c using Equation 8 and Equation 9, respectively; and (iv) calculate the oxygen release rate using Equation 4.

**Validation**

Modeling parameters obtained are shown in Table 2. Oxygen release values and corresponding predictions using the Gaussian function are presented in Figure 7. Our model data closely match our experimental values. From the results of this simulation, it can be seen that the Gaussian function can be satisfactorily used to predict diurnal fluctuations in oxygen release by roots of wetland plants.

### Table 2. Modeling parameters.

| Date       | PAR | Oxygen release |
|------------|-----|----------------|
|            | b (µmol·m⁻²·s⁻¹) | d | a (µmol·g⁻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 |

**Discussion**

In this study, the oxygen release rate from the roots of a wetland plant, *Acorus calamus* was examined. We have shown that oxygen release rates for *Acorus calamus* (Fig. 3) appear to be much higher than those reported for other wetland plant species.[11,12] An oxygen release rate of 7.40–13.24 µmolO₂ h⁻¹·g⁻¹·root dry weight (dw) was reported for the rice varieties ‘Shengtai’ and ‘Suyunuo’, [12] while an oxygen release rate of 1.6 µmolO₂·h⁻¹·g⁻¹·dw was reported for the sedge *Cladium*. [11] Reported oxygen release rates may be significantly different even for the same wetland plant. Sorrel and Armstrong, [9] for example, reported an oxygen release rate of 126 µmolO₂·h⁻¹·g⁻¹·dw for *Juncus intergens*, while a value of 1.5 µmolO₂·h⁻¹·g⁻¹·dw was reported by Chabbi for the same species. [13] This variation may be partially attributable to testing conditions, including varying light intensity.

Oxygen is produced during photosynthesis [14] and transferred from leaves to roots through the gas-filled tissues of a plant by a process of diffusion and convection. [15] Oxygen is then released to the rhizosphere by gas exchange. We found that photosynthetic rate is highly correlated with light intensity. Photosynthetic parameters affect a plant’s ability to produce oxygen. [18] The light-dark switch generates a large and rapid fluctuation in the internal oxygen levels of plants. [15] Thus, plants also exhibit great fluctuations in released oxygen. We show that rate of oxygen release depends largely on light intensity and exhibits a diurnal periodicity. Variations in oxygen release and light intensity follow unimodal patterns during the daytime and can be accurately described by the Gaussian function. The maximum root oxygen release was measured 2 h after the maximum measured light intensity (Fig. 3). From this we established the relationship between root oxygen release and light intensity. A recent study has shown that maximum root oxygen release (with up to 35% oxygen saturation at the root surface) for *Myriophyllum spicatum* occurred under light conditions, while a decrease of about 30% was observed in the dark.[7] Our study gives a detailed profile of diurnal fluctuations in root oxygen release correlating with natural light.

Our study also presents a methodology for quantifying root oxygen release using the Gaussian function. This allows use of light intensity data in prediction of the quantity of oxygen likely to be released. Further studies are needed to demonstrate the applicability of the Gaussian function for other wetland plants. It should be noted that our method and predictions are based on experimental data collected at
Diurnal fluctuations in oxygen release in wetlands

Nanjing; this area has a unique climate (temperate). Studies of other plant species, in other climates and differing natural light conditions should be considered before the methodology is applied more generally.

Conclusions

The oxygen release rate of wetland plants exhibits diurnal fluctuations. Light intensity is a major factor influencing oxygen release. During the morning, oxygen release rate increased with increasing light intensity. Values for both oxygen release and light intensity decreased gradually during the afternoon; and approached 0 μmol g⁻¹ h⁻¹ at night. Fluctuation in root oxygen release and light intensity follow a unimodal distribution. The Gaussian function is demonstrated to accurately describe the observed daytime variation in root oxygen release and may be used to predict root oxygen release in constructed wetlands.

Acknowledgements

The authors gratefully acknowledge Professor Ling Li (The University of Queensland, Australia) for many suggestions and fruitful discussions. This study was supported by the National Natural Science Foundation of China (50979028), and the Ministry of Water Resources of China (200801065).

References

[1] McBride, G.B.; Tanner, C.C. Modeling biofilm nitrogen transformations in constructed wetland mesocosms with fluctuating water levels. Ecol. Eng. 1999, 14(1-2), 93–106.
[2] Soda, S.; Ike, M.; Ogasawara, Y.; Yoshinaka, M.; Mishima, D.; Fujita, M. Effects of light intensity and water temperature on oxygen release from roots into water lettuce rhizosphere. Water Res. 2007, 41(4), 487–491.
[3] Brix, H. Gas exchange through the soil-atmosphere interphase and through dead culms of Phragmites australis in a constructed reed bed receiving domestic sewage. Water Res. 1990, 24(2), 259–266.
[4] Stottmeister, U.; Wießner, A.; Kuschk, P.; Kappelmeyer, U.; Kästner, M.; Bederski, O.; Müller, R.A.; Moormann, H. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnol. Adv. 2003, 22(1–2), 93–117.
[5] Jespersen, D.N.; Sorrell, B.K.; Brix, H. Growth and root oxygen release by Typha latifolia and its effects on sediment methanogenesis. Aquat. Bot. 1998, 61(3), 165–180.
[6] Sasikala, S.; Tanaka, N.; Wah, H.S.Y.W.; Jinadasa, K.B.S.N. Effects of water level fluctuation on radial oxygen loss, root porosity, and nitrogen removal in subsurface vertical flow wetland mesocosms. Ecol. Eng. 2009, 35(3), 410–417.
[7] Laskov, C.; Horn, O.; Hupfer, M. Environmental factors regulating the radial oxygen loss from roots of Myriophyllum spicatum and Potamogeton crispus. Aquat. Bot. 2006, 84, 333–340.
[8] Huang, J.; Wang, S.H.; Yan, L.; Zhong, Q.S. Plant photosynthesis and its influence on removal efficiencies in constructed wetlands. Ecol. Eng. 2010, in press.
[9] Sorrell, B.K.; Armstrong, W. On the difficulties of measuring oxygen release by root systems of wetland plants. J. Ecol. 1994, 82, 177–183.
[10] Kang, Y.B.; Jung, I.H.; Lee, H.G. Critical thermodynamic evaluation and optimization of the MnO–TiO₂–Ti₂O₃ system. Calphad. 2006, 30, 235–247.
[11] Chabbi, A.; McKee, K.L.; Mendelsohn, I.A. Fate of oxygen losses from Typha domingensis (Typhaceae) and Cladium jamaicense (Cyperaceae) and consequences for root metabolism. Am. J. Bot. 2000, 87, 1081–1090.
[12] Mei, X.Q.; Ye, Z.H.; Wong, M.H. The relationship of root porosity and radial oxygen loss on arsenic tolerance and uptake in rice grains and straw. Environ. Pollut. 2009, 157, 2550–2557.
[13] Chabbi, A. Juncus bulbosus as a pioneer species in acidic lignite mining lakes: interactions, mechanism and survival strategies. New Phytol. 1999, 144, 133–142.
[14] Chen, P.C.; Fan, S.H.; Chiang, C.L.; Lee, C.M. Effect of growth conditions on the hydrogen production with cyanobacterium Anabaena sp. Strain CH3. Int. J. Hydrogen Energy 2008, 33, 1460–1464.
[15] Gara, L.D.; Locato, V.; Dipierro, S.; Pinto, M.C. Redox homeostasis in plants. The challenge of living with endogenous oxygen production. Respir. Physiol. Neurobiol. 2010, In press.