Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2012.748452

51 (2013) 3026–3031
March

Taylor & Francis
Taylor & Francis Group

Internal oxygen dynamics in rhizomes of *Phragmites australis* and presence of methanotrophs in root biofilms in a constructed wetland for wastewater treatment

Anna Faußer[a,*], Jiří Dušek[b], Hana Čížkova[b,c], Michael Hoppert[d], Paul Walther[e], Marian Kazda[a]

[a]*Institute of Systematic Botany and Ecology, Ulm University, Ulm, Germany*
Tel. +49 (0) 731 5023300; Fax: +49 (0) 731 5023313; email: anna.fausser@uni-ulm.de
[b]*Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, v.v.i. České Budějovice, Czech Republic*
[c]*Faculty of Agriculture, University of South Bohemia, České Budějovice, Czech Republic*
[d]*Institute of Microbiology & Genetics and Courant Research Centre Geobiology, Georg-August-University Göttingen, Göttingen, Germany*
[e]*Central Electron Microscopy Unit, Ulm University, Ulm, Germany*

Received 10 December 2011; Accepted 12 June 2012

ABSTRACT

Oxygen partial pressures (O2pp) inside rhizomes of *Phragmites australis* were measured to monitor diurnal dynamics. Root-associated biofilms were characterised regarding methane-oxidising bacteria (MOB) by immuno labelling. Runs of O2pp showed distinct diurnal patterns repeating day-to-day. Soon after sunrise O2pp increased steeply to around 185 hPa, remained on this level over mid-day and decreased exponentially over night to about 80 hPa. Root surfaces were densely packed with bacteria of which 34–37% accounted for potential MOB. This emphasises the importance of the oxic rhizosphere in the degradation of organic matter and methane oxidation and diurnal fluctuation of O2pp may implicate for variation of biogeochemical processes involved in wastewater treatment.

Keywords: Aerenchyma; Macrophyte; Constructed wetland; Internal oxygen partial pressure; Methane-oxidising bacteria

1. Introduction

Wetlands are a challenging environment for plant growth due to anoxic and often reducing conditions in soil [1]. Plants growing effectively in waterlogged conditions possess the major characteristic to develop an enlarged gas-space continuum running through all plant organs including the shoots, roots and the extensive rhizome system interconnecting shoots [2,3]. This aerenchyma system allows emerged wetland plants to supply oxygen for submerged tissue and to provide partly oxic conditions in the rhizosphere [4–6]. Wetland plants enhance their internal ventilation

---

*Corresponding author.

Third International Conference on Environmental Management, Engineering, Planning and Economics (CEMEPE 2011) & SECOTOX Conference, 19–24 June 2011, Skiathos Island, Greece

based on diffusion additionally by pressurised air flow through the aerenchymatic tissue in shoots and rhizomes [7–9]. Pressurised gas flow is achieved by gradients of temperature (thermal-transpiration) or humidity (humidity-induced convection) over a porous partition (diameter $<0.1\,\mu m$) between the inner and the outer atmosphere enabling Knudson-type diffusion [4,9]. In common reed (*Phragmites australis* (Cav.) Trin. ex. Steud.), additional through flow can occur as a result of wind blowing through the stand by which pressure gradients (venturi effects) are created between young and old or dead shoots and air is sucked through the pith cavities of shoots and rhizomes [10]. The through flow of air raises the internal oxygen partial pressures (O2pp) which can reach levels close to ambient air [9]. Root aeration operates by diffusion only, but profits from elevated oxygen levels in the rhizomes [4,5,9].

Oxygen is depleted in waterlogged soils by chemical (e.g. oxidation of reduced metals) [1] and biological processes that are involved in soil organic matter degradation. Thus, the redox potential (E_h) can reach low values from -200 to $-400\,mV$ [11,12]. In anoxic layers of saturated soils, organic matter is decomposed anaerobically by reduction processes including denitrification, fermentation and methanogensis. Greenhouse gases (CO_2, CH_4 and N_2O) generated in submerged soils are emitted directly or through the gas-spaces of the wetland plants [13]. The wetland macrohytes release oxygen to the rhizosphere through special porous regions at the root tips [5] to oxidise phytotoxic compounds characteristic for reduced anaerobic soils (i.e. reduced metals). Thus, the redox potentials are increased [12,14] and nutrients become available for uptake. Aerobic soil microbes profit from the partly oxic conditions in the rhizosphere and aggregate in biofilms around the roots in comparison to bulk soil [12,15,16]. Metabolically linked microbial communities develop on the root surface which use the oxygen released to decompose organic matter [12] and oxidise reduced substances like methane [15,17]. Up to 80% of soil borne methane can thus be quenched before reaching the atmosphere [18] and methane-oxidising bacteria in the rhizosphere can be considered as crucial controllers of methane emission.

As mechanisms for internal ventilation of *P. australis* and gas flows in the aerenchyma of several wetland plants are well documented [9], we intended to gain insight in the daily dynamics of oxygen concentration in the central rhizome gas-spaces under natural *in situ* conditions of a constructed wetland (CW) utilising *P. australis* for wastewater treatment. The hypothesis was that oxygen is actively pressurised from shoot to rhizome pith cavities by the above-described ventilation processes. During night, submerged plant tissues and rhizobacteria will consume the oxygen from the rhizomes' gas-spaces in a way that mirrors biological and chemical oxygen demand in the rhizosphere. Furthermore, as anaerobic processes in anoxic layers of the wetland soil produce methane, we hypothesise that parts of the bacterial community are involved in methane oxidation. As a result diurnal variation in oxygen supply to the rhizosphere can influence also biogeochemical processes used for wastewater treatment in CWs.

2. Materials and methods

2.1. Site description and plant species analysed

This study was conducted in August 2009 in a subsurface horizontal flow CW located in Slavošovice in South Bohemia, Czech Republic (48N57´44.34´´, 14E39´39.997´´; 480 m a.s.l., Fig. 1). The CW was created for municipal wastewater treatment and started operating in August 2001. It consisted of a pretreatment (screen, sand trap and sedimentation tank) and two parallel vegetated beds (size of each 17 m; 22 m; and total area $748\,m^2$) planted with

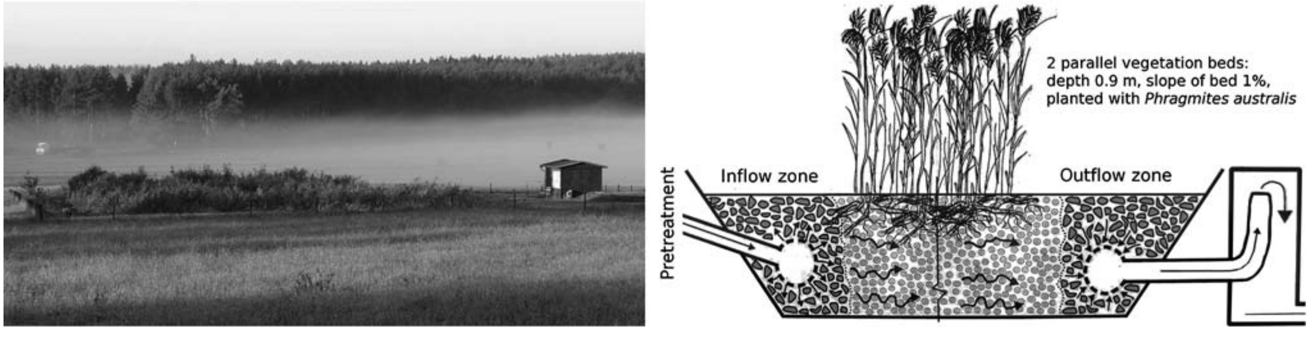

Fig. 1. Diagram and photograph of the subsurface horizontal flow CW in Slavošovice, Czech Republic.

common reed (*P. australis* (Cav.) Trin. ex. Steud.). The CW had a purification capacity of 150 person equivalents (PE) with $5\,\mathrm{m}^2\,\mathrm{PE}^{-1}$ and about 100 persons were connected during the study period. Analysis of the water passed the CW showed a high efficiency in removing organic pollution (biological oxygen demand [BOD_5] 82% and chemical oxygen demand 74.0%), total nitrogen (62.6%), total phosphorus (75.4%) and suspended solids (52%) [11]. In average, the wastewater inflow rate was regulated to $0.12 \pm 0.10\,\mathrm{l\ s}^{-1}$, with a maximum of $1.0\,\mathrm{l\,s}^{-1}$ during periods of extremely strong precipitation. Hydraulic retention time ranged from 8 to 16 days [19]. Inflow and outflow zones (1.5 m each) of the treatment beds were filled with coarse gravel (5–20 cm) and the vegetated part were filled with fine gravel (0.5–1 cm).

2.2. General climate and microclimate during the measurement

The annual average air temperature and sum of precipitation at the study site were 7.9°C and 634 mm, respectively. Microclimatic factors (irradiation inside and outside the stand, air and surface water temperature and air humidity) were recorded during the period of measurement via appropriate sensors (all Delta-T, Burwell, UK). Weather conditions during oxygen recording were sunny summer days with more than 12 h sun light (05:45 until 18:15 PFD > $100\,\mu\mathrm{mol\ m}^{-2}\ \mathrm{s}^{-1}$) and almost no clouds. Microclimatic parameters were consistent over the three days of measurement. Air temperatures ranged from 5.4°C at night to 29.8°C at day time. Temperatures of the sediment water were laid between 14.6 and 17.8°C. Relative air humidity (RH) was recorded between 55% at day up to 98% at night.

2.3. Oxygen measurement

Partial pressures of oxygen were measured in the gaseous phase of pith cavities in rhizomes of two *P. australis* plants (A1 and A2). Both plants grew in the outflow half of the CW. After gentle excavation of the rhizome systems, those plants were chosen as they had this year's shoot directly adjacent to a dead-ended vertical rhizome. The rhizomes were one or two years old and emerged over the water tabel of the CW, but were buried inside the gravel and litter layer of the vegetation bed. Optical oxygen sensors (diameter 4 mm, Fibox-type: PreSens, Regensburg, Germany) were implanted into cut ends of the two rhizomes (diameter A1: 13 mm and A2: 16 mm) and sealed airtight. The excavated rhizome systems were covered with aluminium foil to protect them from sunlight. The O2pp from the optodes were recorded every 5 min for several diurnal courses.

The oxygen sensors are designed to show no cross sensitivity for carbon dioxide, hydrogen sulphide, ammonia, pH or any ionic species like sulphide, sulphate or chloride [20]. Values of oxygen measurements were compensated for water temperatures close to the implanted optodes.

2.4. Biofilm characterisation

Adventitious roots (diameter 2–3 mm) were collected from the measured rhizomes from a depth of 7–10 cm below the water table of the CW. Root samples were prepared for transmission electron microscopy (TEM) and immunofluorescence analysis via light microscopy. Fixation was performed chemically with 0.5% glutaraldehyde in isoosmotic phosphate buffer at pH 7.0 and the samples were infiltrated with LR-Gold or Lowicryl K4M [21]. In the immunoassays, polyclonal antibodies directed against methanol dehydrogenase were used to detect methylotrophic and methane-oxidising bacteria (MOB) in the root-associated biofilms. By counting fluorescing cells and total abundances of bacteria, the share of MOB inside the biofilms was evaluated.

3. Results and discussion

3.1. Diurnal oxygen recording

Oxygen measurements in rhizomes of both plants showed distinct diurnal patterns repeating every day of measurement (Fig. 2). Before sunrise, O2pp were around 80 hPa. With little delay after sunrise and at the moment when RH began to fall, oxygen concentrations increased steeply. Over mid-day when RH was low, O2pp stayed at a plateau level of around 185 hPa until 18:00. As soon as radiation decreased and RH rose again, O2pp in rhizomes began to fall rapidly. The decline of O2 pp was exponential over night and levelled out low before sunrise. Diurnal O2pp courses in both rhizomes showed high negative correlations with RH (A1: $r \leq -0.94$ and A2: $r \leq -0.95$; for all correlations $p < 0.001$).

Pressurisation in emergent wetland plants with cylindrical shoots and linear leaves has been investigated to be mainly driven by humidity-induced convection (*Eleocharis spacelata* R.Br., *Typha domingensis* Pers., *T. latifolia* L., *T. angustifolia* L., *Juncus ingens* N. A.Wakef., and *P. australis* (Cav.) Trin. ex Steud. [7–10, 22–24]). Until now little has been recorded about

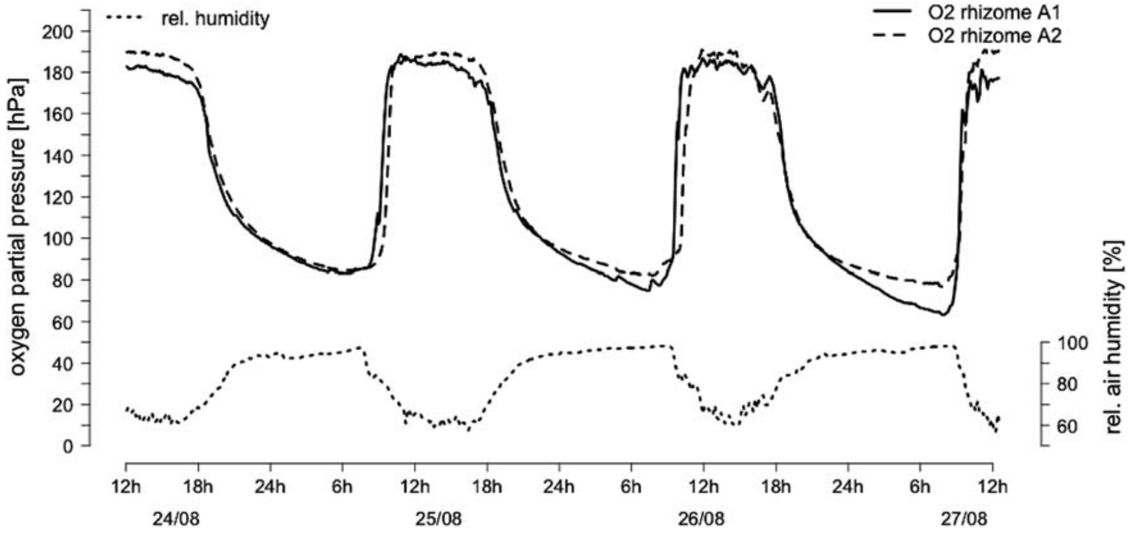

Fig. 2. Diurnal courses of O2pp in rhizomes A1 and A2 and of relative humidity of the ambient air.

dynamics of internal O2pp in the field. The diurnal O2pp courses recorded in this study clearly demonstrate the dependencies between internal oxygen concentration and RH [25] as reflected in close negative correlations. This relationship is thus confirmed also for a CW with an organic load exceeding the natural wetland conditions.

Over night when pressurisation mechanisms were missing and photosynthesis was ceased, only restricted oxygen is available in the gas–space continuum of *P. australis* plants, especially in the rhizomes and roots. Submerged plant tissue and substrate micro-organisms fed on the oxygen reserves generated over the day time. When O2pps dropped below values of 120 hPa, steep course O2pp slopes flattened out. One explanation is that below a certain partial pressure, oxygen cannot cross the barriers for radial oxygen loss [5] to the substrate and thus remains inside the rhizome and root gas-spaces. Another possibility is that substrate related oxygen demand slowed down because plant tissue as well as root-associated micro-organisms reduced their respiration below this level of oxygen availability. A recent study showed that root respiration in pea seedlings declined when cortical O2pp in the root tips decreased below 5–45 hPa [26]. Here, we have measured oxygen concentrations in rhizomes emerging from the water table which did not drop below 70 hPa. The oxygen concentrations in horizontal rhizomes of the same study site decreased below 40% (~45 hPa) during the night [27]. Thus, it can be assumed that O2pp in the roots dropped to values in the range of the proposed critical oxygen pressures of roots in anoxic sediments [26] which results in reduced activity and respiration. A

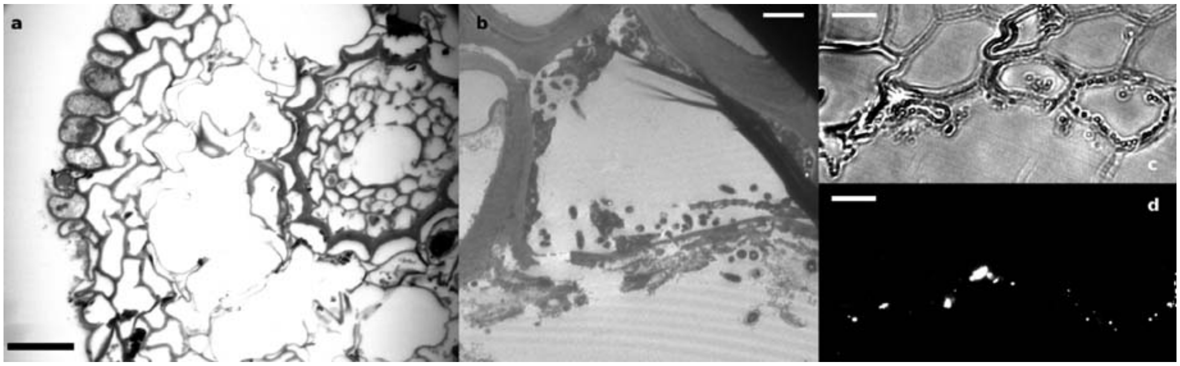

Fig. 3. Root cross sections from *P. australis* showing (a) the organisation of a lateral root, internal gas spaces and colonised exodermis cells, (b) enlargement of root-associated biofilms in TEM, (c) bright-light image, and (d) fluorescing bacteria labelled by antibodies against methanoldehydrogenase. Bars: (a) 20 μm, (b) 2 μm and (c, d) 10 μm.

third explanation is that around 70 hPa resembles the level of oxygen that is reached in this part of the rhizome by diffusion alone through the aerenchymatic tissues of shoots and rhizomes [4,9]. Also, wind driven venturi effects can ventilate the rhizome system at night [9,10].

3.2. Root-associated methanotrophic biofilms

Cross sections of *P. australis* roots revealed high proportions of gas-spaces in the tissue (Fig. 3(a)). Numerous bacteria attached to the root surface were detected in biofilms (Fig. 3(b)). Additionally, up to 45% of root exodermis cells seemed to be colonised with bacteria (Fig. 3(a)). Evaluation of root-associated biofilms revealed 34–37% of the bacteria to be potential methane-oxidising bacteria (Fig. 3(c) and (d)).

By principle, the water purification capacity of CWs depends on the oxygen supply to the rhizosphere, where oxidative processes degrade organic pollutants. Certain microbial communities develop in the rhizosphere of different plant species [28]. On such sites with high organic load and high oxygen demand, many aerobic bacteria rely on spatially restricted oxygen release by roots of aerenchymatic wetland plants. Outside of these oxic zones, methanogenesis is the prevailing process of organic matter degradation. In this context, root-associated methanotrophs play a crucial role in the carbon cycle and in mitigation of methane emissions from the wetland to the atmosphere.

High shares of potential methanotrophic bacteria detected on roots of *P. australis* demonstrate that the latter micro-organisms play an essential role in the carbon cycle in ecosystems with waterlogged soils [15,18,29]. Especially on sites with high organic load and high oxygen demand like CWs, aerobic bacteria like methanotrophs rely on spatially restricted oxygen release by roots of aerenchymatic wetland plants.

Wetland plants like *P. australis* also provide easily degradable organic substances to the rhizosphere that result in an additional emission of carbon dioxide from the CW [13]. Plant roots release oxygen in a passive process that is restricted to the root tips [4,9] and was thus occasionally regarded as irrelevant for the purification capacity of CW. But root oxygen release is enhanced by convective throughflow that rises the oxygen levels in the rhizomes close to ambient air [5,9]. It could be shown that the oxygen released to the soil is effectively used in the planted CW to oxidise methane and phytotoxic compounds and supports the wastewater purification processes, especially denitrification [12,13]. Oxygen is also provided for bacterial processes in cold periods without active plant growth [13] by diffusion and venturi effects through the submerged rhizomes of *P. australis*.

4. Conclusions

Common reed (*P. australis*) showed high capacity to ventilate oxygen actively to submerged tissues. Diurnal oxygen dynamics in the rhizomes revealed very consistent courses of oxygen supply. Internal oxygen reserves created during the day were consumed rapidly over the night, but not depleted as respiration can be assumed to slow down and diffusion processes sustain a steady-state but low oxygen level. This indicates the huge demand for oxygen in the rhizomes, roots and the rhizosphere. A dense biofilm on the rhizoplane shows that the oxygen released to the rhizosphere is adequate to supply bacterial communities active in purifying the wastewater of the CW. Also methane-oxidation is a common process around the roots, as about one-third of the attached bacteria are potential methane-oxidisers. Through the continuous gas-spaces of *P. australis* oxygen can be provided to below ground even in periods outside the growing season, as can be seen in the high purification efficiency of the studied CW.

References

[1] F.N. Ponnamperuma, The chemistry of submerged soils, Adv. Agron. 24 (1972) 29–96.

[2] H. Končalová, Anatomical adaptations to waterlogging in roots of wetland graminoids: Limitations and drawbacks, Aquat. Bot. 38 (1990) 127–134.

[3] M.B. Jackson, W. Armstrong, Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence, Plant Biol. 1 (1999) 274–287.

[4] W. Armstrong, Aeration in higher plants, Adv. Bot. Res. 7 (1979) 225–332.

[5] W. Armstrong, D. Cousins, J. Armstrong, D.W. Turner, P.M. Beckett, Oxygen distribution in wetland plant roots and permeability barriers to gas-exchange with the rhizosphere: A microelectrode and modelling study with *Phragmites australis*, Ann. Bot. 86 (2000) 687–703.

[6] R. Mainiero, M. Kazda, Effects of *Carex rostrata* on soil oxygen in relation to soil moisture, Plant Soil 270 (2005) 311–320.

[7] W. Armstrong, J. Armstrong, A convective through-flow of gases in *Phragmites australis* (Cav.) Trin. ex Steud, Aquat. Bot. 39 (1991) 75–88.

[8] H. Brix, B.K. Sorrell, P.T. Orr, Internal pressurization and convective gas flow in some emergent freshwater macrophytes, Limnol. Oceanogr. 37 (1992) 1420–1433.

[9] T.D. Colmer, Long-distance transport of gases in plants: A perspective on internal aeration and radial oxygen loss from roots, Plant, Cell Environ. 26 (2003) 17–36.

[10] J. Armstrong, W. Armstrong, P.M. Beckett, *Phragmites australis*: Venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation, New Phytol. 120 (1992) 197–207.

[11] J. Dušek, T. Picek, H. Čížková, Redox potential dynamics in a horizontal subsurface flow constructed wetland for wastewater treatment: Diel, seasonal and spatial fluctuations, Ecol. Eng. 34 (2008) 223–232.

[12] A. Wießner, U. Kappelmeyer, P. Kuschk, M. Kästner, Influence of the redox condition dynamics on the removal efficiency of a laboratory-scale constructed wetland, Water Res. 39 (2005) 248–256.

[13] T. Picek, H. Čížková, J. Dušek, Greenhouse gas emissions from a constructed wetland—Plants as important sources of carbon, Ecol. Eng. 31 (2007) 98–106.

[14] H. Flessa, Plant-induced changes in the redox potential of the rhizospheres of the submerged vascular macrophytes *Myriophyllum verticillatum* L. and *Ranunculus circinatus* L, Aqua. Bot. 47 (1994) 119–129.

[15] P.L.E. Bodelier, Interactions between oxygen-releasing roots and microbial processes in flooded soils and sediments, Ecol. Stud. 168 (2003) 331–362.

[16] T. Rudrappa, M.L. Biedrzycki, H.P. Bais, Causes and consequences of plant-associated biofilms, FEMS Microbiol. Ecol. 64 (2008) 153–166.

[17] M. Shrestha, W.-R. Abraham, P.M. Shrestha, M. Noll, R. Conrad, Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of pmoA gene and stable isotope probing of phospholipid fatty acids, Environ. Microbiol. 10 (2008) 400–412.

[18] R. Conrad, Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO), Microbiol. Rev. 60 (1996) 609–640.

[19] V. Holcová, The affect of macrophytes on retention times during summer and winter periods in a constructed wetland for wastewater treatment. M.Sc. Thesis. Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic, 2007 (in Czech).

[20] I. Klimant, V. Meyer, M. Kühl, Fiber-optic oxygen microsensors, a new tool in aquatic biology, Limnol. Oceanogr. 40 (1995) 1159–1165.

[21] J. Roth, M. Bendayan, E. Carlemalm, W. Villiger, M. Garavito, Enhancement of structural preservation and immunocytochemical staining in low temperature embedded pancreatic tissue, J. Histochem. Cytochem. 29 (1981) 663–671.

[22] H. Brix, B.K. Sorrell, H.-H. Schierup, Gas fluxes achieved by *in situ* convective flow in *Phragmites australis*, Aqua. Bot. 54 (1996) 151–163.

[23] M. Bendix, T. Tornbjerg, H. Brix, Internal gas transport in *Typha latifolia* L. and *Typha angustifolia* L. 1. Humidity-induced pressurization and convective throughflow, Aqua. Bot. 49 (1994) 75–89.

[24] T. Tornbjerg, M. Bendix, H. Brix, Internal gas transport in *Typha latifolia* L. and *Typha angustifolia* L. 2. Convective throughflow pathways and ecological significance, Aqua. Bot. 49 (1994) 91–105.

[25] F. Afreen, M.A. Zobayed, J. Armstrong, W. Armstrong, Pressure gradients along whole culms and leaf sheaths, and other aspects of humidity-induced gas transport in *Phragmites australis*, J. Exp. Bot. 58 (2007) 1651–1662.

[26] W. Armstrong, T. Webb, M. Darwent, P.M. Beckett, Measuring and interpreting respiratory critical oxygen pressures in roots, Ann. Bot. 103 (2009) 281–293.

[27] J. Dickopp, M. Kazda, H. Čížková, Differences in rhizome aeration of *Phragmites australis* in a constructed wetland, Ecol. Engin. 37 (2011) 1647–1653.

[28] Y.-K. Yuan, C.-M. Huang, R.-H. Jeng, Investigation of the water purification efficiency of land treatment system by using microbial community as an index, Desal. Wat. Treat. 32 (2011) 153–160.

[29] D.N. Jespersen, B.K. Sorrell, H. Brix, Growth and root oxygen release by *Typha latifolia* and its effects on sediment methanogenesis, Aqua. Bot. 61 (1998) 165–180.