Quantification of Oxygen Release by Bulrush (*Scirpus validus*) Roots in a Constructed Treatment Wetland

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Abstract: Amount of oxygen released by bulrush (*Scirpus validus*) roots has been quantified based on the radial oxygen loss (ROL) exhibited by the roots, the number and the length of active lateral roots, and the field plant density. It was found that wetland bulrush contains two types of active lateral roots (showing ROL), viz., laterals of brown and white main roots. The two laterals have distinct oxygen release characteristics. Based on the dissolved oxygen (DO) microprofiles of brown and white laterals, the ROLs were found to be ≈ 61 ng O_2 cm^{-2} root surface min^{-1} and ≈ 68 ng O_2 cm^{-2} root surface min^{-1}, respectively, at bulk 5-day biochemical oxygen demand (BOD_5) of 76 mg L^{-1}. The respective average active root lengths of the brown and the white laterals were ≈ 40 and ≈ 1676 μm. Based on field and laboratory measurements, the average amount of oxygen released by bulrush was found to be 2.30 mg O_2 m^{-2} wetland surface d^{-1}; of this $\approx 71\%$ is from the white roots. The results of this study indicate that plants do not release enough oxygen to meet the total oxygen demand of bulk wastewater, and therefore, constructed wetlands should be designed as an anaerobic or an aerobic–anaerobic hybrid system rather than as an aerobic system. However, the results of this study should be viewed in the background of possible errors (including a reactor design flaw), which might have made the measured oxygen release significantly lower than what plant roots actually release. Further studies are needed to quantify wetland plant oxygen release based on micro-scale measurements.

Keywords: constructed wetland; plant oxygen release; microelectrode; active lateral root; radial oxygen loss

INTRODUCTION

Presently, constructed treatment wetlands are designed as either an aerobic, or an anaerobic, or a hybrid (aerobic–anaerobic) system. Earlier research has shown that plants leak out some amount of oxygen through the roots to the immediate external environment [Armstrong et al., 1990; Bezbaruah, 2002; Bezbaruah and Zhang, 2004; Brix and Schierup, 1990; Christensen et al., 1994; Colmer, 2003a; Gersberg et al., 1991; Reed et al., 1995; Sorrel and Armstrong, 1994; United States Environmental Protection Agency (U.S. EPA, 2000)]. However, there are disagreements about the wetland plants' ability to effectively create an oxic (i.e., with DO) environment in the plant rhizosphere. In constructed wetlands for wastewater treatment, the plants may not release enough oxygen to meet the oxygen demand of the bulk wastewater (U.S. EPA, 2000). The lack of information on the amount of oxygen released by plants necessitates the assumption that plants are passive in regard to root oxygen release while designing a constructed wetland (U.S. EPA, 2000).

That plant roots release oxygen to their immediate environment has been established beyond doubt in our earlier work (Bezbaruah and Zhang, 2004), and the amount of oxygen released by the roots varies with the amount of oxygen stress (e.g., biochemical oxygen demand, BOD) present in the immediate rhizosphere environment (Sorrel and Armstrong, 1994). However, it is not clear whether all roots of a wetland plant will be able to actively release oxygen like the roots we reported previously (Bezbaruah and Zhang, 2004). Obviously, if the number of active roots along with their active lengths and the radial oxygen loss (ROL) are known, the amount of oxygen released by wetland plants can be quantified by correlating root oxygen release to the wetland surface area based on relevant field measurements. Our objective in this article is to present the experimental procedure, the theoretical basis for calculation, and the estimated amount of oxygen released by bulrush (*Scirpus validus*) per unit surface area of a field constructed treatment wetland. We also compare the results of this study with those of previous studies.

MATERIALS AND METHODS

General Approach

The radial oxygen loss (ROL) from a wetland plant root to the rhizosphere can be calculated from the gradient

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of the oxygen microprofile using Eq. (1) (Armstrong et al., 2000).

$$\text{Radial oxygen loss (ROL)} = \frac{60 D_w (C_s - C_r)}{r_s \ln(r_r/r_s)} \text{ g O}_2 \text{ cm}^{-2} \text{ (of root surface) min}^{-1} \quad (1)$$

where D_w is the oxygen diffusion coefficient in bulk wastewater ($\cong 2.267 \times 10^{-5}$ cm^2 s^{-1} for water at 23°C; Armstrong et al., 2000). C_s is the oxygen concentration at root surface (g cm^{-3}) and C_r is the oxygen concentration at a small distance (r_r) where ROL is to be calculated (g cm^{-3}). r_s is the radius of root (cm) and r_r is the distance from the center of the root to the point where ROL is to be calculated (cm).

The root surface area can be calculated based on the root diameter and the active (showing ROL) root length (root surface area, $a = \pi \cdot$ root diameter $\cdot$ active root length); the averages of both parameters can be found for a specific plant species. The number of roots associated with each shoot or each plant and the number of plants or shoots per unit wetland surface area can be measured in the field. Thus, plant oxygen release can be calculated as follows (Bezbaruah, 2002):

$$ORWP = 1440 \cdot \pi \cdot (ROL) \cdot L_a \cdot d_r \cdot N_a \cdot N_r \cdot N_p \cdot f \quad (2)$$

where $ORWP$ is the O_2 released by wetland plants [g O_2 m^{-2} (wetland surface area) day^{-1}]; ROL is the radial oxygen loss [g O_2 cm^{-2} (root surface area) min^{-1}]; L_a is the average active root length (cm); d_r is the average root diameter (cm). N_a is the number of active roots per main root; N_r is the number of main roots per shoot; N_p is the number of shoots per unit wetland surface (m^{-2}). f is the fraction of the day time during which plant roots release O_2 actively (= 0.5 based on a 12/12 h day/night cycle).

Field Wetlands

Plants for the present experiment were collected from the Hanson Lakes subsurface flow constructed wetland in Sarpy County, Nebraska. The wetlands were designed to serve 350 households (1750 people) with a design flow of $\approx$400 m^3 per day. The raw wastewater contained BOD_5 $\cong$300 mg L^{-1}, total nitrogen $\cong$ 40 mg L^{-1}, and total suspended solids (TSS) $\cong$ 500 mg L^{-1}. The treatment sequence for the system started with a primary sedimentation/equalization tank, and then the wastewater was distributed equally into four individual biofilters. The effluent from each of these biofilters was then fed to one of the four cells of the Hanson Lakes constructed wetland.

The four wetland cells (50.3 m × 30.8 m × 0.45 m, length × width × depth, each cell) had pea gravel and limestones as the media. Cattail (*Typha spp*.) was planted in the first 8.3 m (after the 1.8 m of the inlet zone) of the wetland, followed by an 8.3 m patch of bulrush (*Scirpus validus*). Reeds (*Phragmites australis*) occupied the rest of the wetland (i.e., 32 m). The plants had grown for two full growing seasons before samplings for the present study were started, and hence, the wetland could be considered as matured (Reed et al., 1995). During the sampling months, the average flow was $\approx$246 m^3 per day, and the HRT of each wetland cell was $\approx$4 days.

Plants Used for Active Root Count

Due to the difficulties encountered in the field measurements, we counted plants' active roots and their lengths in the laboratory. To do so, bushes of bulrush (*Scirpus validus*) were collected from the field wetland with adequate care so as to ensure minimal damage to their roots. The gravel media attached to the roots was removed manually under slowly flowing tap water in the laboratory. Then, the bushes were individually planted in 25 cm plastic flower pots (commercially available in the market) filled with expanded clay balls (Hydroton, Germany). These pots were then placed in 51 cm × 41 cm × 13 cm plastic trays (Rubbermaid) with enough tap water to maintian hydroponic conditions. Measured amounts of nutrients (FloraGro, General Hydroponics, Sebastopol, CA; *solution 1:* NH_4^+-N 0.25%, NO_3^--N 1.75%, P_2O_5 1.00%, K_2O_6 6.00%, Mg 0.5%; and *solution 2:* NH_4^+-N 0.30% NO_3^--N 4.70%, K_2O_6 1.00%, Co 0.0005%, Fe 0.1%, Mn 0.05%, Mo 0.0008%; and *solution 3:* P_2O_5 5.0%, K_2O_6 4.0%, Mg 1.5%, S 1.0%) were added to the water. The water loss due to evaporation and/or evapotranspiration was compensated daily by adding adequate amount of water. Artificial light sources (4, 120 W plant lights; General Electric, Cleveland, OH) were used with a timing device (12/12 h light/dark cycle) to stimulate photosynthesis.

During the first seven days, the water in the trays was aerated continuously with compressed air, and stopped thereafter. After a week without aeration, the tap water (with the aforementioned nutrients) in the trays was replaced with primary settled municipal wastewater (Bezbaruah and Zhang, 2004). The plants were allowed to grow in wastewater environment (without aeration) for at least another 5–6 weeks before they were transferred to another reactor (see below) for active root count. A 7–8 week period was felt necessary to give enough time to the plants to adapt to the new environment. It was assumed that plant roots damaged during the process of transfer from the field to the laboratory would recover during this period.

Reactor, Microelectrodes, and Procedure for Active Root Count

In this study, a root (lateral or main root) was counted as active if it released a measurable concentration of oxygen, and the active root length was assumed to be the length over which there was a measurable oxygen concentration (i.e., > the detection limit, 0.05 mg L^{-1}, of the DO microelectrode) on the root surface. The active root length

of each lateral or main root was measured using oxygen and ORP microelectrodes. These microelectrodes were constructed and tested using methods described elsewhere (Bezbaruah, 2002; Bezbaruah and Zhang, 2004; Linsenmeier and Yancey, 1987; Revsbech, 1989; Revsbech et al., 1983, 1988). While the DO microprofiles of the lateral roots were used to determine the active root lengths, the ORP microprofiles were used just to verify the DO measurements. The DO microelectrodes had a noise level of $\approx 0.1 \times 10^{-12}$ amperes, and the corresponding DO value was about 0.01 mg L^{-1}. Therefore, the detection limit of the DO microelectrode used in this study was ≈ 0.05 mg L^{-1} (Bezbaruah, 2002; Zhang, 1994), this is 5 times higher than the average noise level (APHA et al., 1995).

A reactor shown in Figure 1 was constructed and used for active root count and for finding out the active root length. The reactor was made from a transparent plastic storage box (36 cm × 25 cm × 13 cm, Rubbermaid) with two removable acrylic plastic covers fitted with velcro tapes. The two covers were 36 cm × 10 cm. Once fitted there was a 5 cm × 36 cm opening in the middle of the reactor. This opening was necessary for microelectrode insertion and positioning. Small acrylic plates (each one 11 cm long and 1.3–2.5 cm wide) were used to cover the 5 cm wide central opening as and when necessary. Velcro tapes were again used to securely place the small plates. As shown in Figure 1, the reactor was so designed that both wastewater and nitrogen stream could be introduced continuously into the reactor. In addition, an artificial light source (1, 120 W plant lights; General Electric, Cleveland, OH) was used with a timing device (12/12 h light/dark cycle) to stimulate photosynthesis. The procedure for active root count and for finding out the active root length was as follows:

1. One bush of plants grown in one of the 25-cm flower pots was taken out by cutting the plastic pot and letting the clay balls collapse and move away from the roots. The clay balls ensured limited, if any, damage to the roots during this process. Then the plants with bare roots were transferred into the reactor placed in a Faraday cage where all measurements were done.
2. The plants were positioned on a specially fabricated acrylic plastic rack, and the stems (shoots) were secured with a stand fitted with a ring. It was ensured that all the roots were under wastewater. For the purpose of measurement, one individual main root (with adequate number of lateral roots) was isolated using a glass/metal rod and positioned on the rack. Care was taken to ensure the absence of other roots in the immediate vicinity (e.g., 1 cm).
3. Immediately after transferring the plants into the reactor, primary settled wastewater was continuously fed using a prismatic pump (Cole-Parmer). The wastewater depth in the reactor was maintained at 9 cm. The system had a hydraulic retention time (HRT) of ≈ 10 days with a feed rate of 0.795 L d^{-1} at room temperature (23 ± 2°C). Nitrogen gas was continuously streamed (at ≈ 50 kPa at the control gage) over the surface of the wastewater in the reactor during measurements to prevent or at least minimize atmospheric oxygen transfer into the wastewater. The small acrylic plates were then placed over the 5 cm central opening so that nitrogen, instead of air with oxygen, was accumulated in the headspace above the wastewater. Wastewater feeding and nitrogen gas streaming were started at least 2–3 h prior to microelectrode measurements.
4. After the 2–3 h period for stabilization, the measurements were started. A DO or an ORP microelectrode (with a Ag/AgCl reference microelectrode) was positioned vertically above the targeted lateral root (Fig. 1). The small acrylic plates were removed and repositioned as necessary to facilitate the positioning of the microelectrodes. The tip of the DO (or ORP) microelectrode was first positioned manually in such a manner that it was very near to the tip of the lateral root. A microscope (10/63×) with a trinocular head (Model: SMZ-2T,

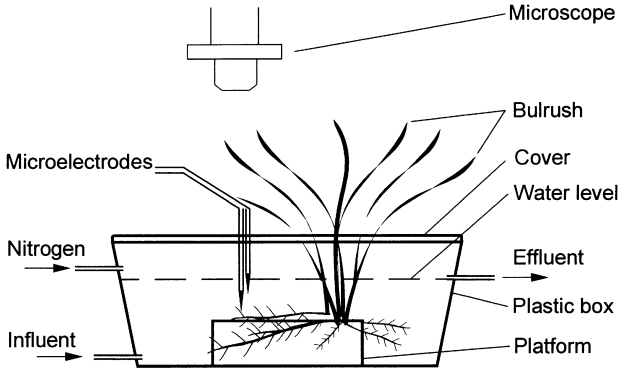

Figure 1. Schematic of the reactor used for active root count. A 3D micromanipulator was used for microelectrode movement. The headspace above the wastewater was streamed with nitrogen gas to prevent diffusional oxygen transfer. A critical evaluation of the reactor design and its possible impact on results are presented in the discussion part of this article.

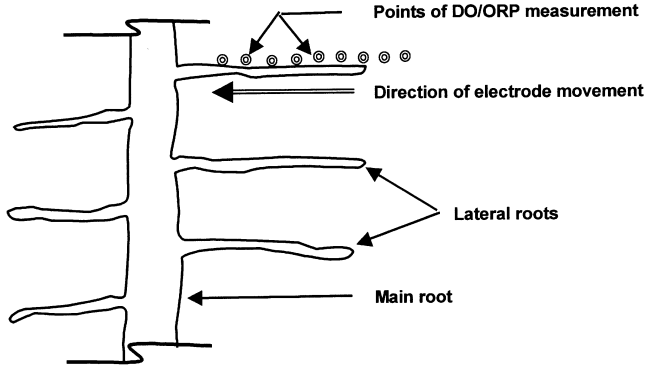

Figure 2. Schematic of a section of a main root with the laterals. The microelectrode (DO or ORP) was first manually positioned at or near the tip of the lateral root and then moved in steps of 5, 10, 50, or 250 μm towards the body of the main root.

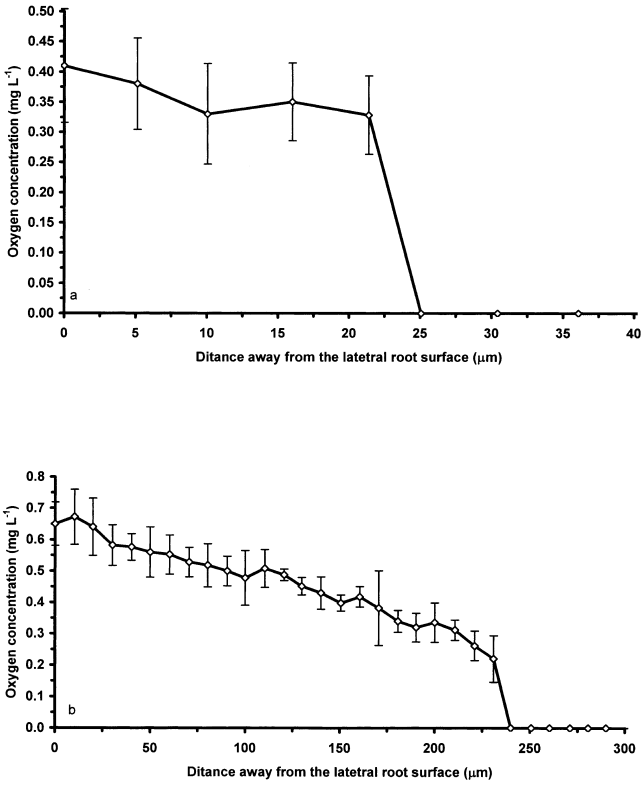

Figure 3. DO microprofiles of (a) a brown lateral and (b) a white lateral as the microelectrode was moved away from the root surface in the radial direction. Error bars representing ±1 standard deviation (*SD*) were calculated from five consecutive stable readings. All measurements were done inside the Faraday cage and in the reactor shown in Figure 1.

Nikon, Japan) was then used in conjunction with a fiber optic dual light microlight (Model: FO-150, Boyce Scientific, Gray Summit, MO) for precisely locating the root and positioning the tip of the microelectrode; the point where the microelectrode was positioned (on or near the lateral root tip) was set as the origin (0, 0).

5. The microelectrode was moved in steps of 5, or 10, or 50, or 250 μm with a 3D micromanipulator (Model MPC-100, Sutter Instruments, Novato, CA) along the axis (i.e., perpendicular to the radial direction) of the lateral root (Fig. 2). The root was observed through the microscope each time the microelectrode position was manipulated; this was to ensure that the microelectrode tip was still touching the root or was very near to it. In case it was found that the microelectrode tip was away from the root, then the micromanipulator was used to reposition it (by moving the microelectrode in the other two directions as necessary). Dissolved oxygen and ORP microprofiles along the axis of the lateral root were obtained through this exercise.

The initial active point on the root, if it was not at the tip of the root, was taken as the point from where a steady DO (> 0.05 mg L^{-1}) signal was received, and the active length ended at the point where there was no DO signal (i.e., < 0.05 mg L^{-1}). It was ensured that two or three consecutive points with no signals followed an "end point." When steps of 250 μm were used, in the case of white roots (see below), it was necessary to trace back along the root in smaller steps (≤50 μm) to locate the exact end point of the active root length.

In this study, the laterals of seven brown and five white main roots were examined for active root count in the aforementioned reactor with a bulk BOD_5 of 76 mg L^{-1}. In addition, DO and ORP microprofiles of the laterals of both brown and white main roots were measured to check (a) the similarity of the experimental conditions between

Table I. Length of active zone in various lateral roots.[a]

	Length of active zone of lateral roots (μm)											
	← Brown main root ID # →							← White main root ID # →				
Lateral root identification number	1	2	3	4	5	6	7	8	9	10	11	12
1	10.04	40.24	49.72	60.56	60.16	41.08	20.12	1399.68	1750.60	1949.84	1750.60	1550.64
2	29.76	30.32	59.48	49.52	60.16	44.96	25.64	1950.52	1849.52	1800.36	1600.52	1750.36
3		30.40	59.68	60.16	50.76	36.00	25.16	1699.72	1500.08	1850.92	1351.04	1550.68
4		20.52	59.76	49.92	60.16	45.08	35.08	1949.68	1750.28	1950.32	1850.44	1402.24
5		29.92	50.04	49.92	39.48	35.08	31.56	1500.36	1850.24	1850.60	1450.68	1250.56
6			70.52	70.48	20.52	40.16			2000.88	1850.40		1602.76
7			50.96		70.16					1650.00		
8			50.28							2050.64		
9										1050.04		
Average number of active lateral roots				5.6 ± 1.9						6.2 ± 1.6		
Average active lateral root length	19.90	30.28	56.31	56.76	51.63	40.39	27.51	1699.99	1783.60	1778.12	1600.66	1517.87
Overall average active length ± Standard Deviation				40.40 ± 15.11						1676.05 ± 115.39		

All the lateral roots of each brown and white main root were examined. Only the active ones are recorded here.

the present reactor and the lab-scale vertical flow wetland (Bezbaruah and Zhang, 2004), and (b) the methodologies used for microprofile measurements. The laterals of the brown and the white roots were 5–10 mm long. It is not known whether wastewater inhibited their growth. There is a possibility that their growth was inhibited by wastewater toxicity, but that should not affect our results and oxygen estimation as we can't expect any better root growth in a treatment wetland. In such measurements, the microelectrode tip and the root were routinely observed after each measurement and adjustments in the lateral directions were made as necessary to maintain the path of measurement perpendicular to the root axis.

Methods for Plant Density and Total Main Root Counts

To determine the plant density per unit wetland surface area, five different areas (0.9 m × 0.9 m each) were identified randomly at the field wetland, and the number of green shoots (i.e., stems) in each of these selected areas was manually counted in the field.

To count the number of main roots associated with each shoot, a bush of bulrush in the field wetland was dug out carefully with all the media and transported to the laboratory. Once in the laboratory, media was manually removed as much as possible by putting the plants under slowly flowing tap water and with utmost care to ensure the least damage to the roots. Once most of the media particles were removed, the rhizome attached to a definite number of shoots (say, 4–7 shoots) was cut using a sharp knife. Then, the shoots were separated from the rhizome using the knife or a pair of scissors. To count total number of roots, the rhizome of the bulrush was cut into pieces. The main roots attached to the rhizome were individually cut with a pair of pointed scissors and sorted in separate petri dishes depending on their color. A pair of forceps was used to count the roots individually. The separated shoots were cut to smaller pieces and preserved separately. The shoots, rhizome, and roots were dried in a drying oven at 80°C for 48 h to determine the respective biomass (Le Bot et al., 2001). A microscope fitted with a micrometer scale

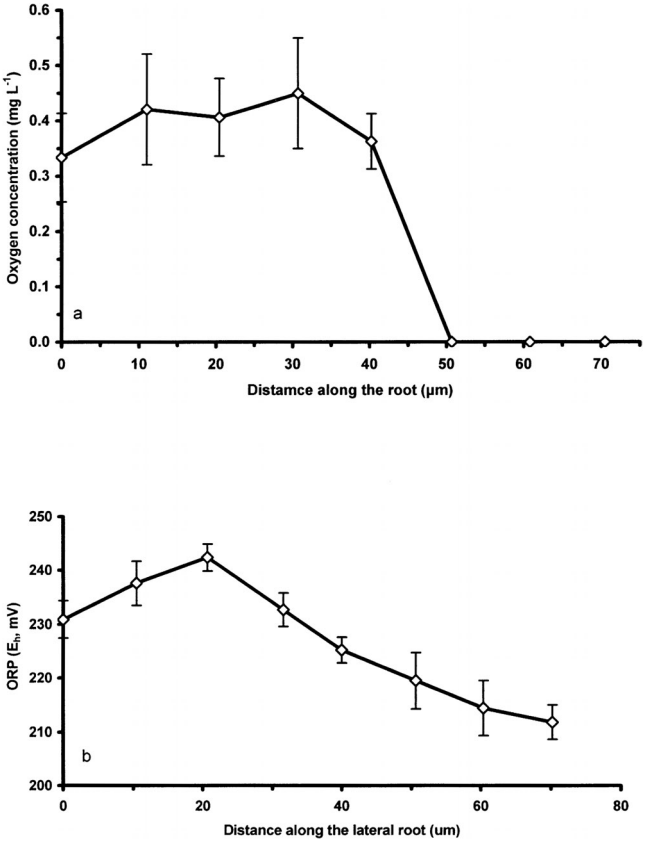

Figure 4. (a) DO, and (b) ORP typical microprofiles along the axis (from the tip side of the root, see Fig. 2) of an active lateral root of a brown main root. Error bars representing ± 1 standard deviation (*SD*) were calculated from five consecutive stable readings. The DO value was assumed to be zero if the reading at a particular point was ≤ the detection limit of the DO microelectrodes. ORP values were not converted to ORP_7 as pH was not measured simultaneously. The length over which there was a measurable oxygen concentration has been taken as the active root length. The active root length of the present lateral = 50.24 μm. All the laterals of 7 brown main roots were examined. The average active root length calculated based on all these measurements = 40.40 ± 15.11 μm.

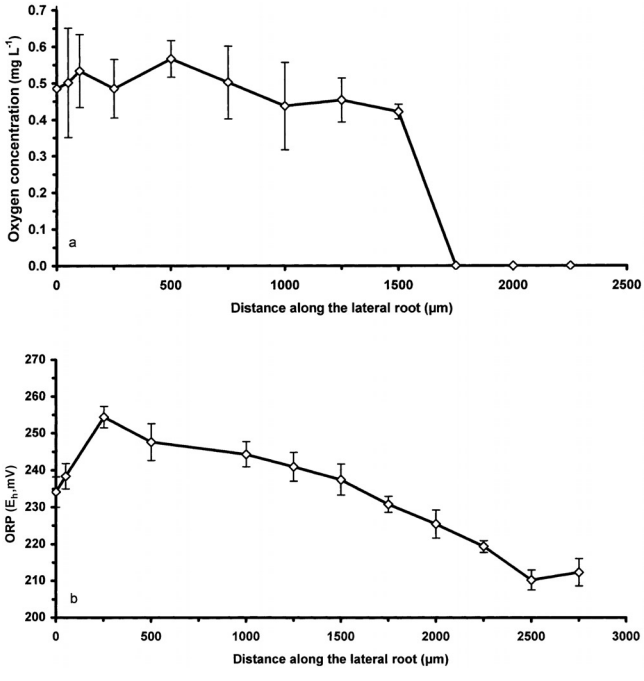

Figure 5. (a) DO, and (b) ORP typical microprofiles along the axis (from the tip side of the root, see Fig. 2) of an active lateral root of a white main root. Error (± 1 standard deviation, *SD*), calculated from five consecutive stable readings) is within the size of the data symbol, except where shown. The DO value was assumed to be zero if the reading at a particular point was ≤ the detection limit of the DO microelectrodes. ORP values were not converted to ORP_7 as pH was not measured simultaneously. The length over which there was a measurable oxygen concentration has been taken as the active root length. The active root length of the present lateral = 1500.36 μm. All the laterals of five white main roots were examined. The average active root length calculated based on all these measurements = 1676.05 ± 115.39 μm.

(Model: Laborlux S, Leitz Wetzlar, Germany) was used to determine root diameters.

RESULTS

Two Distinct Types of Roots and DO Profiles of Their Laterals

In our earlier work (Bezbaruah and Zhang, 2004), we thought that bulrush has only one type of main roots. However, during the measurements for the active root length using the present reactor, it was discovered that there are two distinct types of main roots in the bulrush rhizosphere that contain active laterals. One type of the main roots was brown in color (called "brown root" hereafter), and the other was relatively white (called "white root" hereafter); the laterals associated with them called "brown laterals" and "white laterals," respectively, hereafter. Brown main roots were usually short ($\approx$2.5–10 cm) but they were the most abundant ones. White main roots were usually longer ($\approx$7.5–18 cm); they were younger roots as compared to the brown ones as the white roots were observed to be associated with younger shoots or concentrated near actively growing shoots.

In this study, no measurable DO on the surface of any two main roots was detected by the DO microelectrodes, but both brown and white laterals had measurable DO. Replicate measurements confirmed reliability (reproducibility of microprofiles) and the appropriateness of the method used in this study for DO and ORP measurements (Bezbaruah, 2002). Figure 3 shows the typical DO profiles of these laterals measured at the bulk BOD_5 of 76 mg L^{-1} in the reactor shown in Figure 1 at an HRT of 10 days. The thickness of the oxygen layer varied markedly for the two types of laterals, that is, $\approx$240 μm and $\approx$25 μm for the white and brown laterals, respectively. A comparison between the results of the present study and those previously reported (Bezbaruah and Zhang, 2004) indicates that the main roots investigated in our earlier work were white roots. The brown

Table II. Amount of oxygen released by bulrush (*Scirpus validus*) per unit wetland surface area.[a]

Bulk BOD_5 mg L^{-1}	ROL[c] ng cm^{-2} min^{-1}	Average root diameter (d_r) mm	Average active root length (L_a) μm	Average active lateral roots per main root (N_a)	Average main root per shoot (N_r)	Average number of shoots (N_p) m^{-2}	O_2 released by plants (ORWP) mg m^{-2} d^{-1}	
							Based on average parameter values	Range[d]
Part A								
76 (B)[b]	61.09	0.1	40.40 ± 15.11	5.6 ± 1.9	109.5 ± 14.5	194.0 ± 22.7	0.66	0.21–1.55
76 (W)[b]	67.77	0.1	1676.05 ± 115.39	6.2 ± 1.6	5.3 ± 0.9	194.0 ± 22.7	1.64	0.83–2.88
Part B								
24 (B)	23.19	0.1	40.40 ± 15.11	5.6 ± 1.9	109.5 ± 14.5	194.0 ± 22.7	0.25	0.08–0.59
24 (W)	37.27	0.1	1676.05 ± 115.39	6.2 ± 1.6	5.3 ± 0.9	194.0 ± 22.7	0.90	0.46–1.58
89 (B)	66.28	0.1	40.40 ± 15.11	5.6 ± 1.9	109.5 ± 14.5	194.0 ± 22.7	0.72	0.23–1.68
89 (W)	74.59	0.1	1676.05 ± 115.39	6.2 ± 1.6	5.3 ± 0.9	194.0 ± 22.7	1.80	0.91–3.17
215 (B)	95.28	0.1	40.40 ± 15.11	5.6 ± 1.9	109.5 ± 14.5	194.0 ± 22.7	1.04	0.33–2.41
215 (W)	119.09	0.1	1676.05 ± 115.39	6.2 ± 1.6	5.3 ± 0.9	194.0 ± 22.7	2.89	1.46–5.08
489 (B)	122.29	0.1	40.40 ± 15.11	5.6 ± 1.9	109.5 ± 14.5	194.0 ± 22.7	1.33	0.42–3.09
489 (W)	146.09	0.1	1676.05 ± 115.39	6.2 ± 1.6	5.3 ± 0.9	194.0 ± 22.7	3.53	1.79–6.20
687 (B)	133.47	0.1	40.40 ± 15.11	5.6 ± 1.9	109.5 ± 14.5	194.0 ± 22.7	1.45	0.46–3.38
687 (W)	143.77	0.1	1676.05 ± 115.39	6.2 ± 1.6	5.3 ± 0.9	194.0 ± 22.7	3.47	1.76–6.11
1267 (B)	153.59	0.1	40.40 ± 15.11	5.6 ± 1.9	109.5 ± 14.5	194.0 ± 22.7	1.67	0.53–3.89
1267 (W)	163.11	0.1	1676.05 ± 115.39	6.2 ± 1.6	5.3 ± 0.9	194.0 ± 22.7	3.94	2.00–6.93

Sample calculation (as per Eq. (1) for ROL and Eq. (2) for ORWP) (Values from Fig. 3a are used for ROL; average parameter values from Part A of this Table are used for ORWP).

$ROL = [60 \cdot 2.267 \times 10^{-5} \cdot (0.44 \times 10^{-4} - 0.33 \times 10^{-4})]/[0.005 \cdot \ln(0.00714/0.005)] = 6.109 \times 10^{-8}$ g cm^{-2} min^{-1} (for Brown lateral root).

$ORWP = O_2$ released by brown roots + O_2 released by white roots = $\{1440 \cdot \pi \cdot (61.09 \cdot 10^{-9}) \cdot (40.40 \cdot 10^{-4}) \cdot (0.1 \cdot 10^{-1}) \cdot (5.6 \cdot 109.5) \cdot 194 \cdot 0.5\} + \{1440 \cdot \pi \cdot (67.77 \cdot 10^{-9}) \cdot (1676.05 \cdot 10^{-4}) \cdot (0.1 \cdot 10^{-1}) \cdot (6.2 \cdot 5.3) \cdot 194 \cdot 0.5\} = \{0.66 \cdot 10^{-3}\} + \{1.64 \cdot 10^{-3}\}$ g O_2 m^{-2} d^{-1} = $2.30 \cdot 10^{-3}$ g O_2 m^{-2} d^{-1} = 2.30 mg O_2 m^{-2} d^{-1}.

[a]Based on Eq. (1). $D_w = 2.267 \times 10^{-5}$ cm^2 s^{-1}. Based on measured/calculated ROL, active lateral root lengths and number of active laterals, number of main roots, number of shoots. $f = 0.5$ based on the assumption that there is a 12-hour day and 12-hour night cycle.

[b]'B' and 'W' mean brown and white main roots, respectively (or the laterals roots associated with them).

[c]ROLs for laterals of brown main roots in Part B are projected values based on the assumption that the brown roots exhibit similar ROL characteristics as in the white roots in the vertical flow wetland (Bezbaruah and Zhang, 2004). (See explanation about Part B in the main paper under "Plant Oxygen Release Extrapolated for ROLs at Different Bulk BOD_5.") A plot for bulk BOD_5 versus ROL for the white laterals in the vertical wetland gives the best fit equation as ROL = 32.876 · Ln (BOD_5) − 66.203 (R^2 = 0.9778). An equation (ROL = 32.876 · Ln (BOD_5) − 81.287, with R^2 = 1) is derived to project ROL values for the brown laterals at various bulk BOD_5; the known ROL = 61.09 ng cm^{-2} min^{-1} at bulk BOD_5 = 76 mg L^{-1} is used to find out the constant (−81.287).

[d]Total oxygen release is the summation of oxygen released by brown and white laterals. The total minimum and maximum oxygen releases are similarly calculated.

roots were not capable of penetrating 15 cm depth in the vertical wetland, and hence, were not visible in the measurement zone. The DO profiles of the laterals of white main roots from this study are more or less the same as those obtained from the lab-scale vertical flow wetland with the similar bulk BOD_5 (e.g., 89 mg L^{-1}) (Bezbaruah and Zhang, 2004). However, due to the different flow conditions, the thickness of the DO layer obtained from this study is smaller than that obtained in the vertical flow wetland.

Active and Main Root Count, Plant Density, and ORWP

The active root count was done using DO and ORP microelectrodes for seven brown and five white main roots (Table I). While each brown main root contained up to 15 lateral roots, only ≈ 6 lateral roots were active with an average active length of 40.40 μm. Figure 4 shows the typical DO and ORP microprofiles along the axis of a brown lateral root. On the active surface of brown laterals and within the active root zone [i.e., where there was some measurable amount of DO (>0.05 mg L^{-1})], the DO values varied between 0.26 and 0.55 mg L^{-1}; the ORP (not ORP_7) values were in the range of +224.3 to +253.7 mV. It appeared that only the tip portion of the brown laterals were active with respect to oxygen release.

While each white main root contained 10–20 lateral roots, only ≈ 6 of them were found to be active and giving out oxygen (Table I). It was observed that the white laterals nearer the growing tip of the main white root were active. In most cases, almost the whole length of these white laterals was found to be active. The average active root length of white lateral was found to be 1676.05 μm. Typical DO and ORP microprofiles for a white lateral are given in Figure 5. On the active surface of white laterals and within the active root length, the DO values ranged from 0.22 to 0.68 mg L^{-1} while the ORP (not ORP_7) fluctuated between +225.1 and +275.1 mV.

Counting for main roots was done for bulrush (*Scirpus validus*) collected from the Hanson Lakes Wetland on two occasions. Initially, main root counts were made before the experiments to find out the active roots were performed. While doing the first set of main root counts, it was thought that all the main roots would have lateral roots with the similar ROL characteristics. The average number of main roots per shoot (stem) was found to be 120. When it was discovered that there are two distinct types of main roots in the bulrush rhizosphere, viz., brown and white main roots, a new bush of bulrush was collected from the Hanson Lakes wetland, and the two types of main roots (and the laterals associated with them) were separately counted. As shown in Table II, the average number of main roots per shoot (stem) was found to be 115 (109.5 ± 14.5 brown and 5.3 ± 0.9 white). The white roots made up an average 5% of total roots in a bulrush plant.

The plant shoot (stem) density of bulrush (*Scirpus validus*) was found to be 194.0 ± 22.7 shoots per m^2 of the wetland surface area based on measurements at the Hanson Lakes constructed wetland; however, the plant shoot density may change from wetland to wetland. Using Eq. (2), the amount of oxygen given out by plant roots per m^2 (of wetland surface area) at the bulk BOD_5 of 76 mg L^{-1} is found to be 1.04–4.43 mg O_2 $m^{-2}d^{-1}$.

DISCUSSION

Methodologies and Their Appropriateness

As indicated in our earlier work, the major factors that may affect plant oxygen release and its DO microprofiles include: the plant itself (e.g., species, health), its growth conditions (e.g., ORP and BOD of bulk solution, availability of nutrients, biofilm, and media in the rhizosphere), and the conditions under which the measurements are made (e.g., the HRT, time of the day). Bezbaruah and Zhang (2004) have discussed the major factors that may affect plant oxygen release and its DO microprofiles. The factors that may affect the ORWP include all parameters used for the calculation of DO or ORP microprofiles, e.g., ROL, d_r, N_a, N_r, and N_p [see Eq. (2) for definitions]. The reliability and possible errors in measurement of the parameters used Eq. (2) are discussed in the following paragraphs.

There might have been some error in oxygen measurement in the reactor shown in Figure 1; as a result of this we might have measured less oxygen than what plant roots actually release to the immediate root environment. There is a strong possibility that the pure nitrogen had created a gradient in the leaf base and oxygen transfer into the root was hampered. However, it definitively cannot be said that there was an adverse impact and hence, the amount of oxygen released was much lower in this reactor. It is likely that the nitrogen stream would result in a substantial lowering of oxygen transport to the roots if this was dependent mainly upon diffusion from the aerial parts. However, if there were a significant pressure flow through the stems, the effect would be reduced. It is not known whether the *Scirpus* has a significant pressure flow or not. It is also possible that the primary settled wastewater acted as a stronger sink and the gradient between outside air and root cells was strong enough to compete with the nitrogen gas. Furthermore, the *Scirpus* has a lot of intercellular air spaces to facilitate possible oxygen transport to the roots. It is important to note that the *Scirpus* is not a widely studied species; we decided to study it because of its extensive use in constructed wetlands.

Another factor for low oxygen release may be inherent to the plant's defense mechanism against phytotoxins. Earlier studies with wetland plants have shown that roots are lignified (or root porosity reduced) when exposed to a toxic environment (Armstrong and Armstrong, 2001; Colmer, 2003b; Ederli et al., 2004). We exposed our plant roots to primary settled wastewater, which contained many elements in the reduced form. Normally, a plant root is expected to

oxidize its immediate environment, but if toxicity is overwhelming then the roots get lignified and thus avoid direct contact with the toxins. The possible lignification might be the reason for the small active root length (e.g., 40.40 $\pm$ 15.11 μm for the brown laterals and 1676.05 $\pm$ 115.39 μm for the white laterals), and hence the reduced amount of oxygen. It is also possible that the laterals roots released oxygen over longer lengths, but we could not measure the oxygen that was below the detection limit of the oxygen microelectrode.

The present study used plants and feed similar to those used in a conventional treatment wetland. The plants used in this study were obtained from the field wetland and had $\approx 7-8$ weeks for adapting to the new environment. Care was taken to avoid overlapping of roots while the microprofiles were mapped. In the case of the main roots, a glass or metal rod was used to make sure the other main roots were not in the immediate vicinity. In the case of laterals roots we used a microscope to observe the roots. We did not have the problem of overlapping of roots. However, in such microscale measurements such an error is a possibility.

The inherent limitations of our methods are that our reactor was void of media, which would affect the pattern of flow and biofilm growth in the rhizosphere and the surrounding areas. For example, in field wetlands, the thickness of the DO layer (i.e., the diffusive boundary layer) might be ≈ 5 times thinner (Bezbaruah and Zhang, 2004) than that obtained in our reactors, which would result in a ROL higher than that obtained from this study. However, as discussed by Bezbaruah and Zhang (2004), it is very difficult, if not impossible, to quantify such differences. Therefore, further studies, such as, measuring DO and ORP concentration profiles and counting active roots and their associated length in field wetlands, are needed.

The average diameter of main roots (d_r) was determined based on microscopic observation. The method is easy and should be error free. The present methods for determining the length of each active root and the numbers of active laterals per main root (N_a) are completely new. We believe that using the DO or ORP microelectrodes to determine whether there is a measurable DO on the root surface may not carry much error. However, the two methods still could be wrong statistically due to our limited measurements, that is, only 5 or 7 main roots were examined out of possibly thousands of main roots in the plant (Table I). In addition, the selection of these main roots was not based on any statistical rule; rather it was based on convenience of measurement in the existing experimental set-up. It is not known whether all the roots will behave the same way. However, it was observed during the study that not all roots were active with respect to oxygen release.

The method to count the main roots per shoot (N_r) may be assumed correct other than the possible loss of some main roots during the transfer process from the field to the laboratory, which was minimized as far as possible. In the laboratory, although the wetland media attached to the roots was removed manually, main roots were usually not damaged; a few laterals might have been damaged. However, only the average numbers of active laterals (N_a), rather than the total numbers of laterals, would affect the calculation of the *ORWP*. Therefore, damage of laterals may not affect the results. To count the main roots, the cutting points (on the rhizome) were decided based on the number of shoots selected, i.e., the part of the rhizome to which the selected shoots were attached was cut. It should be pointed out that no special care was needed to decide at what point the cutting should be done. Although an extra length of the rhizome may change the count of the roots of a sub-bush substantially (e.g., a positive internal error), the root count for the next sub-bush (e.g., a negative internal error) would compensate for the error. Therefore, the final total number of roots of the whole bush should not change at all because the internal errors would cancel each other.

The present reactor was run inside the laboratory at room temperature and with artificial light. We didn't run our experiment under different temperature conditions. Temperature may have a pronounced effect on the amount of oxygen release. As a general rule, the amount of oxygen will increase with a decrease in temperature. Also the amount of oxygen release might have been different if the experiment was carried out in a field wetland. However, our present instrumentation didn't allow us to do the experiment outside in the field.

We used a small number of plants and related measurements to extrapolate the amount of oxygen released by wetland plant per square meter of wetland root surface. The scaling factor is a major issue that needs careful evaluation and justification. After careful evaluation, we designed our experiment so as to cover all major parameters that may contribute towards the total amount of oxygen release. However, under the scope of our present research it was not possible to examine whether scaling-up the result from the small number of roots was justified and correct. Under similar growing and loading conditions all roots of the wetland plant should behave in a similar way as far as root oxygen release is concerned. Given the fact the all major parameters that may affect the amount of plant oxygen in a constructed wetland had been taken into account and the samples taken represented the wetland environment, we can assume the present scale-up is logical. There may be slight changes in radial oxygen loss (ROL) characteristics, depending on the location the plants in the wetland (i.e., BOD concentration in the wastewater to which plants are exposed); however, that should not affect our present scaling-up as our results represent an average condition.

In light of above discussion, it can be said that the results of this study are the closest possible estimation that one can get under the present experimental conditions and instrumentation used. The results reported in this article should be viewed in the background of these possible errors, and the fact that this is one of first endeavors to quantify wetland plant oxygen release based on micro-scale measurements of all the possible parameters that may affect oxygen release. However, with the change of instrumentation and

measurement techniques in the future one should be able to come up with a better estimation of plant oxygen release.

Plant Oxygen Release Extrapolated for Radial Oxygen Loss at Different Bulk Biochemical Oxygen Demands

Bezbaruah and Zhang (2004) demonstrated that the amount of oxygen released by the roots varies with the amount of oxygen stress (BOD) present in the immediate root environment. Using Eq. (1) to calculate the ROLs with the DO microprofiles reported by Bezbaruah and Zhang (2004), it is found that the ROL increased from ≈ 37 to ≈ 163 ng O_2 cm^{-2} root surface min^{-1} when the bulk BOD increased from 24 to 1267 mg L^{-1}, respectively, for white lateral roots. It should be noted that oxygen at the outer radius of the diffusive zone was convected away by the streaming effluent. Therefore, we used the thickness of the oxygen diffusive zone to calculate r_r [e.g., 71.4 μm = 50 μm (root radius) + 21.4 μm (oxygen diffusive zone thickness)] and the corresponding DO at the r_r as the C_r (e.g., 0.33 mg L^{-1} in Fig. 3a) in Eq. (1) for ROL calculation.

It is not known why the boundary layer of the white laterals is much thicker than that of the brown laterals. The laterals of the brown main roots were found to exhibit very high ROL even though the measured oxygen concentration on the root surface was small. This is because the thickness of the oxygen layer determines the r_r value in Eq. (1). The DO concentrations on or near the two types of the laterals are usually of the same order of magnitude while the thickness of the oxygen layer ($= r_r - r_s$) had 1 or 2 orders of magnitude difference. Therefore, the DO gradient of brown laterals is steeper than that of white laterals. It is also important to note that the value of oxygen diffusion coefficient (D_w) for the bulk wastewater in Eq. (1) was assumed to be the same as that for water (2.267×10^{-5} cm^2 S^{-1} at 23°C); however, the ROL value may slightly change if the actual diffusion coefficient for wastewater is used.

Dissolved oxygen profiles for the brown and white laterals were mapped only at 76 mg BOD_5 L^{-1} in the present study. ORWP calculations based on these observations are shown in Table II (Part A). Total oxygen release (i.e., from both white and brown laterals) by *Scirpus validus* roots is between 1.04 (= 0.21 + 0.83) and 4.43 (= 1.55 + 2.88) mg O_2 m^{-2} (of wetland surface area) d^{-1} at a bulk BOD_5 of 76 mg L^{-1} (in the present reactor, Part A in Table II). Although we feel confident about the data obtained from the present reactor, it may be more reasonable to use the DO microprofiles of white laterals obtained from the vertical wetland (Bezbaruah and Zhang, 2004) to calculate ORWP. This is because the vertical flow wetland used similar media as in the field wetlands and was run for >1.5 years; no root damage had occurred during the measurements of the DO microprofiles as the reactor provided an opportunity to handle the roots delicately. Since DO microprofiles of brown laterals could not be mapped in the vertical flow wetland (because brown laterals were not long enough and were inaccessible with our measurement tools), we assumed that root oxygen release from the brown roots would increase with an increase in bulk BOD from 24 to 1267 mg L^{-1}. After necessary projection (see the footnotes in Table II) the maximum possible oxygen released by plant roots is found to be only 10.82 (3.89 from brown laterals and 6.93 from white laterals) mg O_2 m^{-2} (of wetland surface area) d^{-1} when bulk BOD_5 was 1267 mg L^{-1} (Table II, Part B).

Comparison with Previous Studies

In the past, the researchers used different methods and assumptions to arrive at a value that might possibly represent the amount of oxygen contribution by wetland plants (Table III). Reed et al. (1995) and the U.S. EPA (2000) summarized work by others and reported plant oxygen contribution (per unit wetland surface area) as 5–45 g O_2 m^{-2} (of wetland surface area) d^{-1} and 0–3 g O_2 m^{-2} (of wetland surface area) d^{-1}, respectively. Armstrong et al. (1990) and Brix and Schierup (1990) estimated oxygen release (per unit wetland surface area) by *Phragmites spp*. to be 5–12 g O2 m^{-2} (of wetland surface area) $d^{-1}$and ≈ 0.02 g O_2 m^{-2} (of wetland surface area) d^{-1}, respectively. Estimates on ORWP were also made based on indirect inferences drawn from BOD and NH_4^+-N removal (Gersberg et al., 1991) as ≈ 7.2 g O_2 m^{-2} (of wetland surface area) d^{-1}. Armstrong et al. (1990) reported a release of 5–12 g O_2 m^{-2} (of wetland surface area) d^{-1} for hydroponic *Phragmites* with the assumed root size and the root density. Burgoon (1993) reported 28.6 g O_2 m^{-2} (of wetland surface area) d^{-1} ORWP for a bulrush-gravel laboratory scale system based BOD removal. However, in such estimates plant uptake of NH_4^+-N was either ignored or considered to be nominal. The mechanisms of pollutant removal in wetlands were also considered to be the same as in a normal wastewater treatment plant (Kadlec and Knight, 1996).

The phenomenon of ROL and results from earlier reported experiments have led to the assumption that significant aerobic micro-sites exist in all wetland systems. It is widely believed that constructed wetlands have both aerobic and anaerobic treatment zones. However, the U.S. EPA suggests that plant oxygen release in a constructed wetland be ignored so far as meeting wastewater oxygen demand is concerned (U.S. EPA, 2000). The possible reason for such a measure may be the variable nature of radial oxygen loss measured by researchers. The difficulty in measurement/estimation of oxygen in the rhizosphere has been a major reason for such widely disparate estimates (Kadlec and Knight, 1996; Liehr et al., 2000).

The value obtained from the present study [1.04–4.43 mg O_2 m^{-2} (of wetland surface area) d^{-1} (see Part A of Table II)] is much lower than those reported by others (Table III). While our results may be the reflection of the aforementioned possible errors (e.g., the reactor design, scale-up factor, etc.), the methodology developed in this

Table III. Plant oxygen release reported by various researchers (including the present study).

Source	Plant and media	Amount of oxygen released by plants g O_2 m^{-2} (of wetland surface) d^{-1}	Based on	Remark
U.S. EPA (2000)	–	0–3.0	Literature review	
Reed et al. (1995)	–	5–45	Literature review	
Burgoon (1993)	Bulrush; plastic media	0–10.3	BOD and NH_4^+-N removal	Assumes 4.5 g and 1.5 g O_2 per g of N and BOD, respectively. As reported by Kadlec and Knight (1996).
	Bulrush; gravel	28.6	BOD removal	
Gersberg et al. (1991)	Bulrush; gravel	7.2	NH_4^+-N and BOD_5 removal in a 66 m^2 wetland	Assumes 4.5 g O_2 utilized per g of NH_4^+-N, and 1.0 g per g BOD_5.
Armstrong et al. (1990)	*Phragmites*; hydroponic	5–12	Water reoxygenation	Lab study. Extrapolated with assumed root size and density distribution. As reported by Kadlec and Knight (1996) and Liehr et al. (2000).
Brix and Schierup (1990)	*Phragmites*; soil	0.02	Gaseous O_2 uptake	Field study.
Bavor et al. (1988)	Cattial; gravel	0.8	NH_4^+-N removal	Field study. Assumes 4.5 g O_2 utilized per g of NH_4^+-N, and 1.5 g per g BOD. As reported by Kadlec and Knight (1996).
	Bulrush; gravel	0.8	NH_4^+-N removal	
	Phragmites; gravel	0.8	BOD removal	
Present research	Bulrush; gravel	$1.04 \times 10^{-3} - 4.43 \times 10^{-3}$	ROL from roots and number of active roots	Field and lab study. Based on actual measurement of roots releasing O_2.

study, once updated, can be used for direct measurement of oxygen release of constructed wetland plants used for wastewater treatment. Therefore, our study is complementary to the existing methods (e.g., mass balance methods). Typically, subsurface flow constructed wetlands treat primary settled wastewater (e.g., bulk $BOD_5 < 100$ mg L^{-1}). Therefore, the ORWP obtained from this study, that is, 1.04–4.43 mg O_2 released per m^2 (of wetland surface area) per day, may be taken as a typical value. For wetlands treating other types of wastewater (with different BOD and redox characteristics), the ORWP may be different. This is because the development of roots in aquatic environments depends on the reducing conditions existing in the bulk wastewater (White, 1995). Although our method has its own limitations, it is free from the inherent limitations associated with the mass balance method used by others. The findings of the present study indicate that wetland plants do not contribute a significant amount of oxygen towards wastewater degradation, and therefore, justify the U.S. EPA's argument that plants do not release enough oxygen that would be sufficient to meet the oxygen demand present in wastewater.

The phenomena of plant oxygen release should always be seen in light of the fact that the basic purpose of plant oxygen release is for the detoxification of the root environment by decreasing the concentration of reduced ions (e.g., Fe^{2+}, Mn^{2+}, and S^{2-}; Marschner, 1995). Chen et al. (1980) reported that plant roots vigorously oxidize reduced species (Fe^{2+}) at the rhizosphere in an effort to improve the immediate root environment. It should also be noted that there may be temporary proliferation of aerobic organisms in the rhizosphere stimulated by the presence of phytotoxic substances (Armstrong and Armstrong, 2001).

SUMMARY

The major findings of this study can be summarized as follows:

1. There are two distinct types of adventitious or main roots in bulrush (*Scirpus validus*), laterals of which are active in regard to oxygen release to their immediate wastewater environment. The brown roots are apparently older and matured while the white ones are younger roots. The brown main roots were found to constitute $\approx 95\%$ of total main roots present.
2. The ROL from the lateral roots of the white main roots was found to be ≈ 68 ng cm^{-2} (of root surface) min^{-1} and that for the laterals of the brown main roots was ≈ 61 ng cm^{-2} (of root surface) min^{-1} when the bulk BOD_5 was 76 mg L^{-1}.
3. The active lateral root length (i.e., root length over which ROL could be measured) was ≈ 40 and ≈ 1676 μm in the laterals of the brown and the white main roots, respectively.
4. For primary settled municipal wastewater (bulk BOD_5 = 76 mg L^{-1}), the amount of oxygen released by bulrush was found to be 1.04–4.43 mg O_2 m^{-2} (of wetland surface area) d^{-1}, of which the white roots contributed $\approx 71\%$. The ORWP is projected to vary from 0.54 to 10.82 mg O_2 m^{-2} (of wetland surface area) d^{-1} as the bulk BOD_5 increased from 24 to 1267 mg L^{-1}.

Even though it has been said that plants do not contribute a significant amount of oxygen for wastewater treatment in a constructed wetland (U.S. EPA, 2000), there has been no conclusive proof so far. This study has demonstrated experimentally for the first time that wetland plants do not give out enough oxygen to their immediate root environment. This study has also established a method for quantification of oxygen release from wetland plants. Based on the results presented in this article, it can be conclusively said that constructed wetlands should be designed as an anaerobic or an aerobic–anaerobic hybrid system rather than as an aerobic system.

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