pH, Redox, and Oxygen Microprofiles in Rhizosphere of Bulrush (*Scirpus validus*) in a Constructed Wetland Treating Municipal Wastewater

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Abstract: Microenvironmental studies regarding plant oxygen release in a wastewater environment are important to understand the principles of constructed wetlands for wastewater treatment. pH, oxidation reduction potential (ORP), and dissolved oxygen (DO) microprofiles for the lateral and main roots of the bulrush (*Scirpus validus*) in a vertical flow constructed wetland fed with municipal wastewater were measured using microelectrode techniques. pH was found to be low (6.91–6.98) near the lateral root surface, indicating possible nitrification or H^+ extrusion. The ORP at the lateral root surface was between +250 and +317 mV and gradually reached the bulk solution ORP (+14 to −54 mV) at a radial distance of ~4,750 μm. DO values at the lateral root surface varied from 0.64–2.04 mg L^{-1} as bulk biochemical oxygen demand (BOD) changed from 24 to 1,267 mg L^{-1}. DO at the lateral root surface and the thickness of the oxygen layer around the root marginally increased with an increase in bulk BOD, while ORP at the lateral and main root surface decreased. pH and DO values did not change near the main root and had the bulk solution values. The results of this study provide insights into root-induced microenvironments and would be helpful for the quantification of the total amount of oxygen contributed by plants in constructed wetlands. © 2004 Wiley Periodicals, Inc.

Keywords: constructed wetland; microelectrode; pH; ORP; DO; rhizosphere; wastewater treatment; bulrush

INTRODUCTION

Plants are the most prominent components of a constructed wetland ecosystem. There are a number of important roles that plants play in a constructed wetland (Kadlec and Knight, 1996; Reed et al., 1995). It is generally agreed that plants have aesthetic and ecological appeals: they help in odor and insect control and in wastewater treatment (Campbell and Ogden, 1999). Plants provide a renewable support medium for microbial growth, a matrix to retain solids, and assist in plug flow hydraulics (Hiley, 1995). Root-induced changes can be many; prominent among them are changes in rhizosphere dissolved oxygen (DO), pH, redox potential (ORP), and exudation of low-molecular weight organic compounds (Marschner et al., 1987). However, the role of wetland plants in wastewater treatment is not fully investigated and understood.

Some wetland plants transfer oxygen through the roots to the immediate external environment (Colmer, 2003; Armstrong et al., 2000; Christensen et al., 1994; Sorrel and Armstrong, 1994; Armstrong and Beckett, 1987; Armstrong, 1979, 1978). However, this may not be adequate for the oxidative degradation of wastewater organics. Radial oxygen loss (ROL) from roots has been measured for some wetland plants (Colmer, 2003; Armstrong et al., 2000; Christensen et al., 1994; Sorrel and Armstrong, 1994) pH has been measured near roots of various plants (Gallany and Schumacher, 1993). However, most such studies are done under controlled conditions and in nutrient culture (e.g., agar). Extrapolation of these results to represent the harsh field conditions of a constructed treatment wetland may be difficult.

On the other hand, there is practically no information on the measurements of pH, ORP, and DO microprofiles and ROL in actual constructed treatment wetlands. While microscale studies on ORP and pH in wetland root-zones were almost ignored, estimates on ROL were made based on indirect inferences drawn from BOD and NH_4^+-N removal, rather than actual field measurements (Gersberg et al., 1991). In such estimates plant uptake of NH_4^+-N was either ignored or considered to be nominal. Also, pollutant removal mechanisms in wetlands were considered to be the same as in a normal wastewater treatment plant (Kadlec and Knight, 1996). Such bold assumptions are not apparently correct. Published estimates of plant oxygen release ranged from 5–45 g O_2/day-m^2 of wetland surface area (note: not the plant root area or the gas–liquid interface area) (Reed et al., 1995). The US EPA (2000) reported the figure to

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be between 0 and 3 g O_2/m^2 of wetland surface area/day. Difficulty in measurement/estimation of oxygen concentration in the rhizosphere has been a major reason for such widely disparate estimates (Kadlec and Knight, 1996; Liehr et al., 2000). Therefore, it is imperative to quantify the available oxygen to understand the contribution of plants toward actual wastewater treatment (US EPA, 2000; Campbell and Ogden, 1999; Reed et al., 1995).

The major objective of this study was to use microelectrode techniques to measure pH, ORP, and DO microprofiles near different roots (viz., main root and lateral root) of *Scirpus validus* in a vertical flow constructed wetland under different BOD loading conditions. The basic hypothesis of the present study is that the wetland plant will release some amount of oxygen through their roots to oxygenate the immediate surrounding of the roots. The degree of this oxygenation depends on the degree of oxygen stress (i.e., oxygen demand present). In other words, the root oxygen flux would increase when there is more oxygen demand in the immediate root surroundings. To stimulate actual field conditions for treating municipal wastewater, the vertical flow constructed wetland was fed with wastewater with different BOD loading rates. In this way, bulk DO concentrations and ORP conditions in the wetland could be manipulated and the corresponding root-induced microenvironments could be recorded. It is expected that the results from the present study will provide insight into root-induced microenvironments and would represent actual field conditions after reasonable extrapolation.

MATERIALS AND METHODS

Reactors and Plants

Two reactors (122 cm high, 20 cm OD, 19 cm ID, made of acrylic plastic pipes, Fig. 1a) were operated as vertical flow constructed wetlands (VFCWs) at room temperature (22–25°C). The reactors were filled (114 cm) with washed pea-size gravel (passing through US Sieve No. 4 and retained on US Sieve No. 8) that are conventionally used in subsurface flow constructed wetlands. The porosity of the media was 0.35. Total working volume for each reactor was 0.015 m^3. The top end of the pipe was kept open and an outlet pipe was provided at the lower end. Sampling ports were provided every 15 cm along the height of the reactors. A 2.5-cm diameter nipple was screwed into each sampling port. The nipple was fitted with a cork stopper through which a pipe (12 mm OD, 10 mm ID) was fitted permanently for the insertion of the microelectrode assembly (Fig. 1b). A syringe needle was also inserted into the cork stopper for the collection of bulk wastewater samples. Near each sampling port a spacer made from two perforated acrylic plastic sheets was used to ensure an empty space (~2.5 cm in height and void of pebbles) in the column for ease of insertion of microelectrodes.

Bulrush (*Scirpus validus*) was transferred from a field wetland to one reactor in June 2000; the other reactor was kept as the control (with no plant). Bulrush was selected for the study because it is one of the most widely used plants in constructed wetlands, it has vigorous growth and rooting habits, and is also widely available. Artificial light sources (2, 120 W plant lights, GE, Cleveland, OH) were used with a timing device (12/12 h light/dark cycle) to stimulate photosynthesis in the plant. The reactors were wrapped in black plastic to simulate real root-zone conditions (no illumination) and to prevent algal growth. The reactors were fed with supernatant of primary settled municipal wastewater (Table I) at a hydraulic retention time (HRT) of ~10 days (flow rate = ~1.5 L d^{-1}) since June 2000. The water level was maintained ~5 cm below the top surface of the reactor (Fig. 1a). The reactors had been in operation for $1\frac{1}{2}$ years before measurements were started. The plant had grown well and formed new shoots and roots. Therefore, it was believed that the plant had fully developed. Measurements in the reactors started in March 2002.

Microelectrodes

pH, ORP, DO, and Ag/AgCl reference microelectrodes were constructed with uncleaned and untreated single barrel borosilicate glass tubes (1.50 mm OD and 0.86 mm ID) (Sutter Instruments, Novato, CA, B 150-86-15). Micropipettes were pulled using a Sutter Instrument micropipette puller (Model P-30) using a heating index of 999 and a pulling index of 500. The tips of the pulled micropipettes were broken with a pair of forceps to achieve an outer tip diameter of 3–10 μm. Any pipette with a tip size >10 μm was not used. The glass micropipettes were then filled with a low melting point alloy (LMA), Belmont 2451 (44.7% Bi, 22.6% Pb, 19.1% In, 8.3% Sn, and 5.3% Cd, Belmont Metal, Brooklyn, NY) to the tip as per the method described by Pang and Zhang (1998). The other end of the LMA was then melted and an insulated copper wire was pushed in for electrical connections. A recess of the required length was then created in the LMA-filled micropipette (Bezbaruah and Zhang, 2002a), which was then used to make pH, ORP, and DO microelectrodes as follows.

For pH microelectrodes, the recessed portion (5–10 μm tip) was first filled with platinum using the electroplating method (Pang and Zhang, 1998). The pH microelectrode was constructed by anodic electrodeposition of iridium oxide on the platinum metal (Bezbaruah and Zhang, 2002b). A current of 0.03–0.13 pA (current density of 0.0002–0.0004 mA cm^{-2}) at 0.3–0.5 V was used for ~20 min. The working life of the pH microelectrode was 4–8 weeks (initial slope ~–60 mV/pH), and the response time ($t_{80,}$ i.e., 80% response time) was <5 sec in the 0–12 pH range. For ORP microelectrodes (5–10 μm tip), platinum was slowly deposited (Pang and Zhang, 1998) onto the surface of the LMA to fill the recess completely. The plating current used was 0.3–0.6 μA at 0.5–0.8 V, and the plating time varied between 15 and 20 min. The average

working life of the ORP microelectrode was 4–6 weeks with a response time (t_{80}) <5 sec. The ORP microelectrodes were very stable and worked very well in wastewater environment (Bezbaruah, 2002). To make DO microelectrodes, gold was electroplated onto the surface of the LMA on the recessed end (3–5 μm tip) (Zhang, 1994). The plating current used was 21–22 pA at 0.5–0.7 V, and the plating time varied between 15 and 20 min. The working life of the DO microelectrode was 2–4 weeks, and its response time (t_{90}) was <5 sec in the 0–9 mg DO L^{-1} range. Similar microelectrodes were used by others in biofilm (i.e., a thin layer of microbes attached on a substratum) and plant rhizosphere studies (Armstrong et al., 2000; Zhang, 1994). For Ag/AgCl reference microelectrode (Zhang, 1994), the pulled micropipette (5–10 μm tip) was filled with 3 M KCl saturated with AgCl and mixed with 2% agar as the solidifying agent. A 0.127 mm silver wire (Aldrich, Milwaukee, WI; 26,555-1) coated with AgCl was

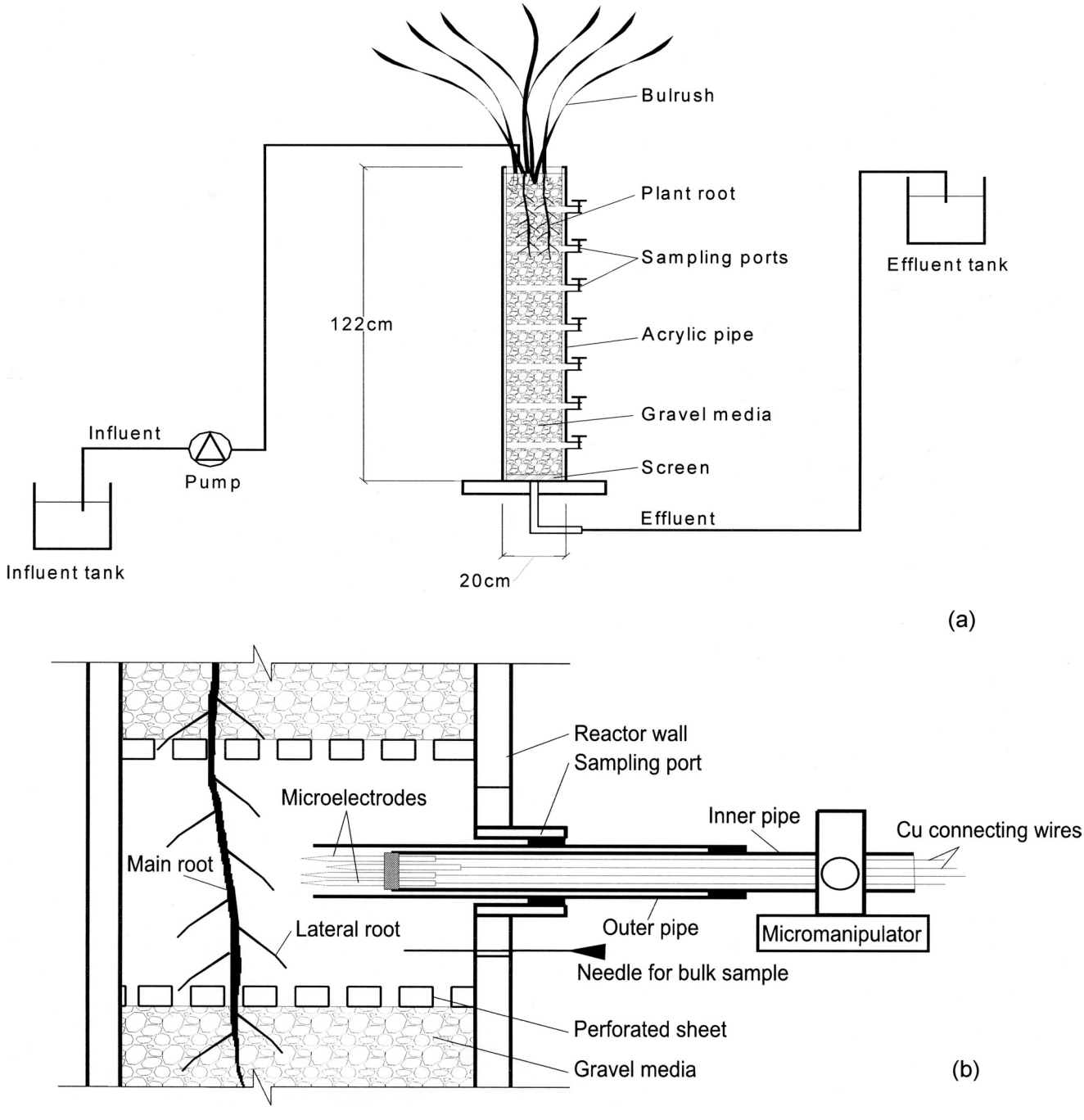

Figure 1. **a:** Schematic of the vertical flow constructed wetland (VFCW) reactor used in the study. The average bulrush (*Scirpus validus*) leaf biomass during the study was 25.79 g. The control unit was similar but without the plant. **b:** Details of the first sampling port from the top of the reactor (not to scale). The measurements were taken at this sampling port.

Table I. Feed and bulk wastewater characteristics.[a]

BOD_5 (mg L^{-1})			NH_4^+-N (mg L^{-1})			pH			ORP (E_h) (mV, SHE)		
	Bulk[c]			Bulk			Bulk			Bulk	
Feed[b]	R[d]	C[d]	Feed	R	C	Feed	R	C	Feed	R	C
47	24	38	21.6	18.5	22.2	7.29	7.10	7.24	+33.7	+3.1	−0.1
128	89	105	20.1	19.8	21.6	7.25	7.12	7.23	+6.3	+13.9	+9.7
242	215	225	18.7	18.2	22.4	7.21	7.05	7.22	−65.0	−54.1	−58.4
568	489	503	21.2	20.7	23.8	7.29	7.13	7.23	−58.6	−45.2	−47.0
731	687	675	19.5	19.2	25.4	7.20	7.05	7.21	−43.8	−37.5	−42.2
1439	1267	1309	20.3	19.7	22.8	7.23	7.12	7.24	−153.2	−46.8	−149.1

[a] DO in feed and bulk solution was always below the detection limit of the microelectrode.
[b] Feed means the primary settled municipal wastewater with/without BOD manipulation.
[c] Bulk means the wastewater sampled near the root zone in the planted reactor or from the first sampling port of the control unit.
[d] R = planted reactor, and C = control unit.

inserted into the pipette. The average working life of the reference microelectrode was 2–4 weeks.

All four microelectrodes (pH, ORP, DO, and reference) were tied together and fitted to a glass pipe (6 mm OD, 5 mm ID) and glued to the pipe with water resistant glue (Devcon, ITW Performance Polymers, Riviera Beach, FL). While pH, ORP, and DO microelectrode tips were put at the same level, the reference microelectrode was pulled back about 5 mm to make it easier to maneuver (Fig. 1b). It should be noted, however, that the reference microelectrode could have been inserted anywhere in the system, not necessarily very near to the other microelectrodes. The copper connecting wires were taken through the other end of the pipe. The smaller glass pipe was then put inside a bigger glass pipe (8 mm OD, 7 mm ID) so as to protect the microelectrode tips from breakage during insertion into the reactor. The bodies of these microelectrodes were colored using different permanent markers for easy identification while in the root zone. That formed the microelectrode assembly (Fig. 1b). A plastic pipe and a metal clamp were used in the main sampling port to prevent water leakage. Plumber's putty was used between the outer and inner pipes of the microelectrode assembly to ensure easy maneuvering of the inner pipe by the micromanipulator while preventing water leakage.

The microelectrodes were calibrated relative to the Ag/AgCl microelectrode. The pH microelectrode was first calibrated using standard pH buffers of 4, 7, and 10. Before insertion into the reactor the pH microelectrode was calibrated in wastewater samples of different pH values (Bezbaruah, 2002). The calibration in wastewater was necessary to do away with the redox effect on the microelectrode. The "wastewater calibration curve" was used for data interpretation. The ORP microelectrode was calibrated as per the ASTM (1981) method using quinhydrone saturated pH buffer of 4 and 7. It was also checked using quinhydrone saturated 0.05 M potassium hydrogen phthalate (Zhang and Pang, 1999). The DO microelectrode was calibrated in 0.85% saline solution bubbled with gas containing either 0, 10, or 21% oxygen. The microelectrodes were also calibrated after the measurements were over and the average values were used in interpreting the data. The performance and characteristics of all these microelectrodes were consistent with those reported by others (Yu and Bishop, 2001; Zhang, 1994; Zhang and Pang, 1999; VanHoudt and Lewandowski, 1992; Linsenmeier and Yancey, 1987; Revsbech et al., 1983).

Measurement Methods

The measurements were taken in the first sampling point from the top of the reactor (Fig. 1b). Many of the plant roots reached the first sampling point from the top (i.e., roots were at least 15 cm). Some roots penetrated beyond that sampling point (i.e., >15 cm in length) and even beyond the second sampling point (i.e., >30 cm in length). However, they were not accessible because they were mostly attached to the wall of the reactor. Potential (mV) and current (pA) measurements were done using a chemical microsensor (Model II, Diamond General, Ann Arbor, MI) connected to a ground line separated from the building grounding. For measurement of the microprofiles, an individual root was identified using a magnification glass placed outside the reactor near the sampling port. A 60 W ordinary electric light was used on the opposite side for proper illumination of the root zone while identifying the root. Initially, the microelectrode assembly was positioned manually near the root. The inner pipe of the microelectrode assembly (Fig. 1b) was then fitted to a 3D micromanipulator (Model MPC-100, Sutter Instruments). The micromanipulator can be operated either in fine (0.04 μm) or coarse (0.20 μm) mode in all three directions. To ensure that the microelectrode initially touched the surface of the root, the micromanipulator was operated so that the tip of the microelectrode pushed the root slightly (observed through the magnification glass). That was an extremely delicate step, and precaution was taken to ensure that the microelectrode tip was not broken. The tip of the

microelectrode was then withdrawn slightly using the micromanipulator so that the root moves back to the original position. The root surface touched by the tip of the microelectrode was defined as the starting point (0, 0) of the microprofiles.

In the case of pH and ORP, microelectrodes were moved in steps of 50 μm until about 3,000 μm and then in steps of either 100 or 250 μm. A total of ~80 readings were taken for each pH and ORP microprofile. In the case of DO measurements, the microelectrode was moved away from the root surface in 10-μm steps until the DO dropped to that of bulk wastewater, and then it was either moved in steps of 50, 100, or 250 μm. A total of ~180 readings were taken for each DO microprofile. To ensure reproducibility of the results, the microprofiles were measured again by tracing the original path towards the root surface. However, the microprofiles reported here are ones obtained as the microelectrodes were moved away from the root surface.

Analytical Methods

Samples from the feed tank and bulk wastewater in the root zone were collected after every set of experiments for microprofile measurements and analyzed for soluble chemical oxygen demand (SCOD), BOD_5, NH_4^+-N, DO, temperature, total suspended solids (TSS), ORP, and pH. All parameters were analyzed as per the Standard Methods (APHA et al., 1995). Macroelectrodes were used to measure pH (Orion, Cambridge, MA; P/N 910600), ORP (Orion, P/N 9778BN) and NH_4^+-N (Orion, P/N 95-12 with Orion pH adjusting solution) in conjunction with Fisher Accumen 925 meters. The pH macroelectrode was calibrated before each day's measurement using the two-point calibration method. The ORP macroelectrode was checked using quinhydrone saturated pH solutions (pH 4 and 7). NH_4^+-N calibration was done routinely using solutions of known concentrations and checked by the method of standard addition; the slope of the macroelectrode was checked before each day's measurement using 1 ml and 10 ml of 1 M NH_4Cl as per the manufacturer's instructions. For quality control purposes NH_4^+ values (in samples) were occasionally measured using the Dionex DX500 HPLC/IC system with an IonPac CG12A (4 × 50 mm) as the precolumn and an IonPac CS12A (4 × 250 mm) as the separating column; a conductivity detector (CD20) and SRS (CSRS-II 4-mm) with a current of 100 mA were used. NH_4^+ values so determined were compared with those obtained using the macroelectrode. DO and temperature were measured using a YSI 5100 DO meter with a YSI 5010 probe. TSS, BOD_5, and SCOD were measured as per Methods 2540 D, 5210 B, and 5220 C of the Standard Methods (APHA et al., 1995), respectively. The flow rate of each reactor was measured at the beginning and the end of each feed condition and averaged; HRT was calculated from the average flow rate.

Plant leaf biomass (dry weight) was measured by drying sample leaves at 80°C for 48 h and weighing them after desiccation. Before drying, the diameters of the base and the lengths of the cylindrical leaves were measured. Diameters and lengths of other leaves of the plant in the reactor were then measured, and dry weights were calculated based on these measurements.

Experimental Plan

All measurements were made under dynamic BOD loading conditions. Initially, measurements were made with BOD of the primary settled wastewater only. The BOD was then changed to a new higher value and measurements were taken after 2–3 days (1.57 days is the theoretical time required for the wastewater with the new BOD to completely replace the old wastewater up to the level where microprofile measurements were done). Once measurements were completed the feed was changed back to the primary settled wastewater; the reactor was then allowed to run for at least 2 days. This was to ensure that there was no persistent toxicity for the plant due to the increased BOD. The BOD was then changed to a still higher value and measurements were taken after 2–3 days. The same procedure was always repeated throughout the experiment. The feed BOD was manipulated either by adding glucose (for BODs of 242–1,439 mg L^{-1}) or deionized water (for BOD of 47 mg L^{-1}). In the case of dilution, NH_4Cl was added to maintain the NH_4^+-N level as in the primary settled wastewater.

The reactors were run with feed BOD concentrations of 47, 128, 242, 568, 731, and 1439 mg L^{-1}. BOD_5, NH_4^+-N, pH, and ORP of the feed and the bulk solutions are shown in Table I. pH, ORP, and DO microprofiles were measured in two types of roots, viz., main root and lateral root (see Fig. 1b), and in the control unit. Root hairs were not present in our system; this may be because of the highly reductive wastewater present (Hiley, 1995).

RESULTS

Establishing Measurement Methods

In the present experiment, a Faraday cage was not used during the measurements, as the reactors were too big to be put inside the existing Faraday cage; the microelectrodes were not shielded coaxially either. The readings fluctuated a lot when there was human movement near the reactor. To do away with such fluctuations (noise), the reading for each position of the microelectrode (inside the reactor) was recorded from a far enough distance. The microelectrodes were calibrated both inside the Faraday cage and outside it (on the platform used for the two reactors). The data indicate that there is little difference in the calibration data for pH, ORP, and DO microelectrodes when the Faraday cage was not used. The maximum standard deviations

(SDs) in the signals from pH, ORP, and DO microelectrodes (calibrated outside the Faraday cage) were 5.20 mV, 5.29 mV, and 4.02 pA, respectively (Table II). The curves generated from the calibration data collected outside the Faraday cage have R^2 values equal to those obtained for the curves from data obtained during calibration inside the cage.

When recording a reading at a particular location, the reading was allowed to stabilize for long enough such that the variation between any two successive readings was less than 10%. To ensure that the measured data in the rhizosphere are representative, we recorded five consecutive positive stable readings and used the average value to map each point of the microprofile. The maximum SDs of pH, ORP, and DO readings thus measured were 0.02 (pH unit), 2.76 mV, and 0.23 mg L^{-1}, respectively (Fig. 2). Such small SDs in the measured readings indicate that the measurements, although taken outside the Faraday cage, were more or less stable.

To our knowledge, no information has been reported on measurements of DO, pH, and ORP microprofiles in the wetland plant rhizosphere in the presence of actual wastewater. The calibration/measurement techniques and associated SDs in the measured values indicate that our techniques are good enough. The error bars and data points, however, are not specifically shown in the figures in the following presentation (Figs. 3–5) to maintain clarify.

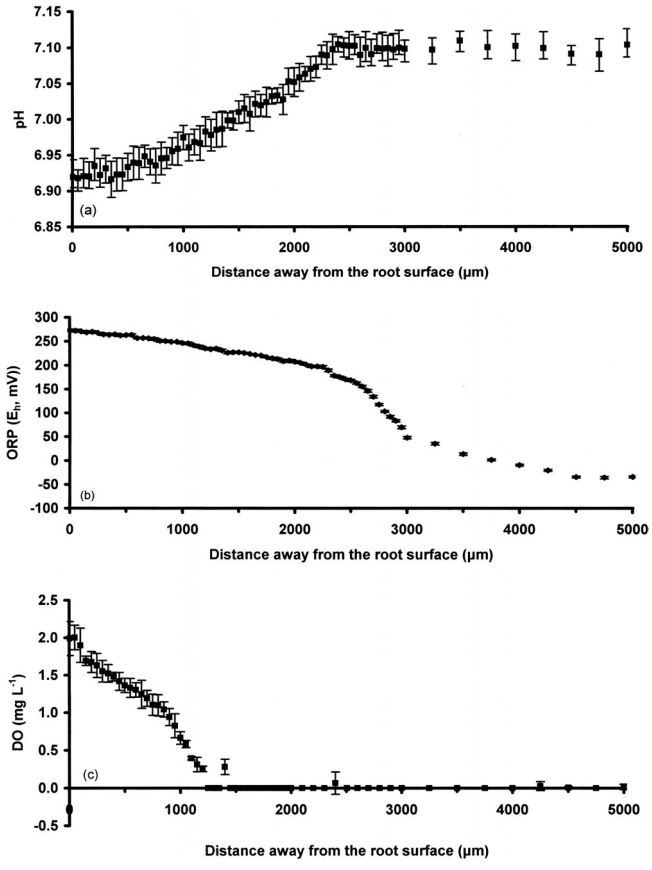

Figure 2. Microprofiles near the lateral root for bulk BOD of 687 mg L^{-1}. Error (±1 SD), calculated from five consecutive stable readings, is within the size of the data symbol, except where shown. **a:** For pH, SD = 0.01–0.02. **b:** For ORP, SD = 0.07–2.76 mV. ORPs have not been corrected to pH 7. **c:** For DO, SD = 0.00–0.23 mg L^{-1}.

pH Microprofiles

The pH was found to be low (6.91–6.98) at the surface of the lateral root relative to the bulk solution. Values gradually increased to the bulk solution pH (7.04–7.12) at 1,850–2,750 μm in the radial direction away from the root surface (Fig. 3a). Similar microprofiles were obtained under all BOD loading conditions and there was no noticeable change in the microprofile with an increase in BOD loading. In the case of the main root and the control unit, no change in pH in the radial direction could be observed under any BOD loading condition. Another important observation is that the bulk solution pH in the planted reactor was always lower (by 0.11–0.17 units) than that in the control unit under all BOD loading conditions.

Root-induced changes in rhizosphere pH have important consequences for plant nutrition (Marschner, 1995). Plants take up significant amounts of sparingly soluble nutrients from the rhizosphere by using their ability to acidify the rhizosphere (Rao et al., 2002). The decreased pH near the lateral root can be interpreted to be the result of nitrification in oxic (i.e., with DO) microsite near the roots. Nitrification in the immediate oxic surrounding of wetland plant roots was either observed or predicted by others (Reddy et al., 1989; Risgaard-Petersen and Jensen, 1997). In maize plants grown in a soil of pH 6.0, the rhizosphere pH decreased to ~4.0 when NH_4^+ was supplied (Marschner et al., 1987). In rice field soil, pH was found to decrease from 6.5 in the soil to 4.0 at the root surface (Begg et al., 1994). Reddy et al. (1989) and Arth and Frenzel (2000) suggested that when NH_4^+ is present near a root, part of it is

Table II. Calibration of microelectrodes inside and outside the Faraday cage.[a]

Calibration conditions	Calibration curve	R^2	Max. SD[b]
pH: Inside FC[c]	$y = -55.160\ x + 689.85$	1.00	0.30 mV
Outside FC[c,d]	$y = -55.273\ x + 680.29$	1.00	5.20 mV
ORP: Inside FC	$y = -58.973\ x + 661.65$	1.00	0.34 mV
Outside FC[d]	$y = -58.913\ x + 660.11$	1.00	5.29 mV
DO: Inside FC	$y = 5.9685\ x$	0.99	1.00 pA
Outside FC[d]	$y = 6.0198\ x$	0.99	4.02 pA

[a]Each of the data points used for the regression of calibration curves is the average of five consecutive stable readings. This exercise was necessary to find out possible noises present in our microelectrode readings, as the reactors could not be put inside the existing Faraday cage (FC).

[b]Maximum standard deviation. Any five consecutive stable readings generate an average and a standard deviation, the maximum standard deviation is reported here.

[c]In standard pH buffers.

[d]Done on the platform where the reactors were kept.

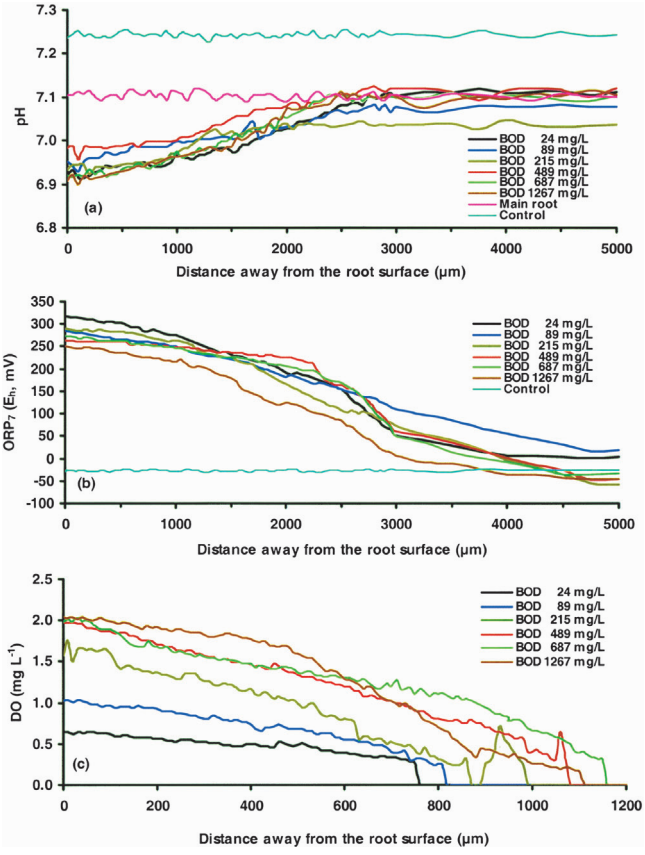

Figure 3. pH (**a**), ORP_7 (corrected to pH 7) (**b**), and DO microprofiles (**c**) near lateral roots for different bulk BODs. To maintain clarity, the pH microprofile for the main roots and the control unit, and ORP_7 microprofile for the control, are shown only under 687 mg L^{-1} bulk BOD (other microprofiles followed similar trends). Values < the detection limit of the DO microelectrode is equated to zero.

directly taken up by plants and the other part is oxidized to NO_3^-. The NO_3^- is either used by plants or diffused into the adjacent anaerobic zone, where it is denitrified. The presence of *Lobelia dortmanna* was found to profoundly influence nitrification within sediment (Risgaard-Petersen and Jensen, 1997).

Rhizosphere acidification may arise from the root uptake of cations (viz., NH_4^+) (Rao et al., 2002) coupled with root exudation of organic acids and amino acids (Marschner et al., 1987) and release of CO_2 from the root (Rao et al., 2002). The exudation of organic acid may also be due to the deficiency of nutrients (in wastewater) (Rao et al., 2002). In the present NH_4^+-N dominated wastewater, bulrush roots might have mostly taken up NH_4^+ ions as the source of nitrogen. In order to maintain electroneutrality at the wastewater–root interface, H^+ ions are excreted by roots in quantities that are stoichiometrically equal to NH_4^+ uptake (Haynes, 1990). Arth and Frenzel (2000) used multichannel electrodes to prove that there exists a competition between wetland plant roots and nitrifiers for NH_4^+. Even though nitrification will be oxygen-limited, both plant uptake and nitrification might have contributed to lower pH near the root surface. A decrease in the rhizosphere pH can occur in response to deficiencies of PO_4^{2-}, Zn, and Fe (Haynes, 1990). While PO_4^{2-} deficiency can be ruled out for the present municipal wastewater fed system, Zn and Fe deficiencies are possible.

It is difficult to decide whether the decrease in pH near the lateral root is because of nitrification or H^+/organic acid exudation by roots or both. Further studies would be necessary to draw any conclusive inference.

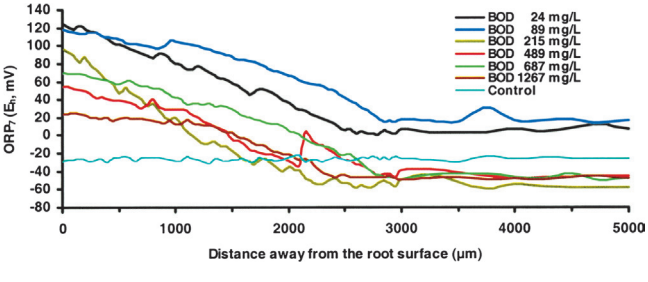

Figure 4. ORP_7 (corrected to pH 7) microprofiles near main roots for different bulk BODs. To maintain clarity, the control unit microprofile is shown only for 687 mg L^{-1} bulk BOD (other microprofiles followed a similar trend).

ORP Microprofiles

The ORP values obtained from measurements are converted to ORP at pH 7 (ORP_7) for ease of comparison using Eq.1 (Zhang and Pang, 1999):

$$ORP_7 = ORP_o + Slope \cdot (pH_o - 7) \qquad (1)$$

where ORP_7 = OPR adjusted to pH 7; ORP_o = original ORP measured; pH_o = pH measured at the same point of ORP measurement, and $Slope$ = 59 mV/pH unit (Zhang and Pang, 1999). In this study, ORP was measured with respect to a Ag/AgCl reference microelectrode. However, to make it universal, all ORP values are reported with respect to the standard hydrogen electrode (E_h). The ORP values ob-

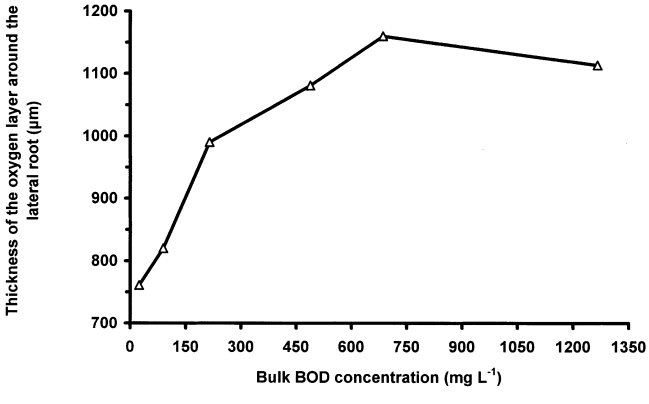

Figure 5. The thickness of the oxygen layer around the lateral roots as a function of bulk BOD. The average diameter of the lateral roots = 0.1 mm.

tained from the microelectrode (with respect to Ag/AgCl) are converted to ORP with respect to a standard hydrogen electrode (SHE) by adding 222 mV (Yu and Ji, 1993). ORP referred to here always means ORP (E_h) at pH 7 unless otherwise mentioned.

At the root surface, the ORP of the lateral roots were between +250 and +317 mV. The ORP gradually decreased to the bulk solution ORP (+14 to −54 mV) at ~4,750 μm from the root surface (Fig. 3b). The increased ORP at the root surface should be due to the presence of oxygen. In the case of the main root, the ORPs were in the range of +124 to +24 mV at the root surface and achieved the bulk solution ORP (+14 to −54 mV) at ~2,800 μm from the root surface (Fig. 4). It appears that the ORPs at the surface of the lateral and the main root decreased with an increase in the bulk BOD. The ORP microprofiles obtained for the main root cannot be explained with relation to DO, as there was no increased oxygen concentration near the main root (see "DO Microprofiles," below). However, it is possible that there was still some oxygen near the surface of the main roots, but below the detection limit (~0.05 mg L^{-1}) of the microelectrodes. That may be the reason for the relatively higher ORPs near the surface of the main roots. The ORP microprofile in the control unit was always flat and showed the bulk solution ORP throughout. The ORP of the bulk solution was found to be in the same range in both the planted and the control units.

E_h (= ORP vs. a standard hydrogen electrode) was observed to vary between +200 mV and +700 mV in the root zone of different wetland plants by others (Zhu and Sikora, 1995; Steinberg and Coonrod, 1994). Wetland plants maintain a higher ORP near the roots despite the general trend of reduced ORP in flooded roots (Armstrong, 1978). The high redox potentials in the rhizosphere (Marschner et al., 1987) of submerged plants are passive examples of oxidation power of roots. This is apparently caused by oxygen released from the roots and is necessary for immobilization of excessive levels of soluble Mn and Fe in the rhizosphere (Marschner et al., 1987). Steinberg and Coonrod (1994) have found that redox potential in the root zone of *Juncus alpinus* grown in nutrient solution varied between +400 to +700 mV. The ORPs in our reactor varied between +250 mV and +317 mV at the lateral root surface. These results, although lower than those reported earlier, seem to be reasonable, as our measurements were in a wastewater environment.

DO Microprofiles

Plants need oxygen for root respiration and some amount of oxygen is leaked by the plants into the immediate surroundings of the roots to create an ideal oxic microsite around individual young roots. This ROL and consequent creation of an oxic environment around roots are considered an important adaptation by plants to counter the ill effects of phytotoxins present in an anaerobic environment (Armstrong et al., 2000). Therefore, the basic hypothesis in the present study is that the wetland plant will release some amount of oxygen through their roots to oxygenate the immediate surroundings of the roots. The degree of this oxygenation depends on the degree of oxygen stress (i.e., oxygen demand present). In other words, the root oxygen flux would increase when there is more oxygen demand in the immediate root surroundings.

In this study, a DO (or oxygen) layer around the root is defined as the radial distance from the root surface to the point where the DO equals the DO in the bulk solution (or the DO is below the detection limit of the microelectrode). Measurements in the present reactors indicate that the lateral roots had a prominent DO layer around it which varied between 760 and 1,160 μm under different BOD loading conditions (Fig. 3c). The thickness of the oxygen layer around the lateral root increased marginally (Fig. 5) with an increase in the bulk BOD except when the bulk BOD was 1,267 mg L^{-1}. DO at the root surface slightly increased with an increase in the bulk BOD. For the initial bulk BOD of 24 mg L^{-1}, the DO concentration at the lateral root surface was 0.64 mg L^{-1}, which increased to 2.04 mg L^{-1} when the bulk BOD was 1,267 mg L^{-1}. The DO microprofiles for the main root and the control, and that beyond 750–1200 μm for the lateral root coincided with the x-axis except for occasional small peaks possibly indicating the presence of other roots. There might have been some oxygen near the surface of the main roots, but that might be below the detection limit of our microelectrode. The DO microprofiles for the lateral roots under various BOD loads (Fig. 3c) indicate that with an increase in oxygen demand in the bulk solution (an external oxygen sink) the oxygen concentration near the roots increased, and this supports our hypothesis.

In this study, feeding wastewater with different BOD concentrations resulted in the same DO concentration (i.e., ~0.0 or below the detection limit of the microelectrode) in bulk solution but different root-induced DO layers (i.e., different microenvironments). This result indicates the importance of measurements of microscale environments around roots; any measurement of DO and BOD in the bulk solution may be misleading if the role of plants is the major focus. With an increase in bulk BOD, some increase in net root oxygen release may be expected, but this increase should normally reflect a steeper oxygen gradient. In the present study, an increase in oxygen concentration at the root surface was also observed with an increase in bulk BOD. It might be possible that higher bulk BOD made parts of the root microenvironment very anaerobic, forcing the roots to adopt special defense mechanisms. The plant roots might have developed more aerenchyma and simultaneously might have reduced respiratory oxygen demand in some parts of the roots. Each of these could lead to increases in the internal root oxygen concentrations. This would explain the higher root surface oxygen concentrations recorded in the present study.

The thickness of the oxygen layer surrounding the plant root and the DO concentrations at the surface of lateral

roots measured during the present study are consistent with previously reported values. Other researchers reported oxygen concentrations ~1.63 mg L^{-1} (Armstrong et al., 2000) and 0.64–14.40 mg L^{-1} (20–450 μM, the higher value is suggestive of pure oxygen in the gas phase) (Christensen et al., 1994) at the root surface of *Phragmites australis* and *Littorella uniflora*, respectively. The thickness of the oxygen layer surrounding the *L. uniflora* roots varied from ~0.5–5 mm away from the root surface (Christensen et al., 1994).

A higher rate of oxygen release was reported in *Juncus ingens* when titanium (III) citrate buffer was used as the external oxygen sink as compared to the rate without any oxygen sink (Sorrel and Armstrong, 1994). However, plants may have some physiological limits related to oxygen transfer and, hence, the oxygen concentration at the root surfaces cannot go on increasing indefinitely with an increase in the bulk BOD, as observed here (Fig. 3c).

DISCUSSION

In the past, several studies (Armstrong et al., 2000; Christensen et al., 1994; Sorrel and Armstrong, 1994) were conducted to understand the science of oxygen transport and loss in plant roots grown in wetland sediment. Armstrong et al. (2000) used DO microelectrodes with tip diameters of 12–18 μm to measure oxygen profiles along the roots laid horizontally in a Perspex rack, 2–3 mm below the surface of a fluid with 0.05% (w/v) nutrient agar. During the measurements, oxygen-free nitrogen was continuously streamed over the surface of the medium to impose an external oxygen sink on the roots, to encourage plant's oxygen release, and to ensure that the root obtained its oxygen only via the shoot. Christensen et al. (1994) used DO microelectrodes (35 μm tip diameter) to measure oxygen microprofiles for *L. uniflora* roots under dark and light conditions. The growth medium used was 0.9% nutrient agar, and freshly prepared amorphous FeS was added to the agar layer below the rhizosphere as the oxygen sink. Similarly, titanium (III) citrate buffer was used by others as an oxygen sink in studying oxygen release from *J. ingens* using a macroelectrode (Orion 90-02) (Sorrel and Armstrong, 1994). The test conditions in those studies mimicked the oxygen demand in waterlogged soil to some extent and the measurement methods were more or less appropriate. But the results from those studies might not be an approximation of the real field conditions in a constructed treatment wetland. In addition, the redox system present in the nutrient agar (Armstrong et al., 2000; Christensen et al., 1994; Sorrel and Armstrong, 1994) or the buffer (Sorrel and Armstrong, 1994) is not representative of what is present in an actual treatment wetland treating municipal wastewater. For example, use of FeS as the only oxygen sink (Christensen et al., 1994) in a layer below the rhizosphere may not be a true representation of the field situation. It is also important to note that redox systems in the works reported by others were generated by a single chemical compound (e.g., titanium citrate buffer and FeS), whereas the redox system in actual wastewater is due to a complex mix of various inorganic and organic compounds.

The results obtained during the present study may be the closest possible estimations that we can get using our existing reactors and measurement systems given the limitations in instrumentation and noise reduction. We used a plant, media, and feed similar to those used in a conventional treatment wetland. The reactors were run for more than $1^1/_2$ years, which is long enough for the wetlands to mature. The plant had 28 active leaves (total leaf biomass = 25.79 g) when the experiments were conducted. It was, however, observed that the leaves of bulrush grown in our reactor had smaller diameters than their natural counterparts; otherwise, the health of the plant was good.

However, our sampling zone was devoid of media, which might have affected the flow pattern and the biofilm growth in the rhizosphere and in the surrounding areas. Some amount of turbulence might have been present in the measurement area and must have affected the microprofiles; however, this aspect was not evaluated experimentally during this study, as the microelectrodes were not sturdy enough to penetrate the gravel media. Nevertheless, the results of this study are still useful, or at least are the starting points, for the evaluation of microscale environments in the rhizosphere of wetland plants. For example, we can estimate the relationship between the mass transfer coefficient (e.g., for DO) in our system ($K_{w/o\ media}$) and that in a system with a sampling zone filled with media ($K_{w/\ media}$) using the following equation (Cussler, 1984):

$$K = 1.17(d * v/v)^{-0.42} * (v/D)^{-0.67} \qquad (2)$$

where K = mass transfer coefficient; d = particle diameter; v = superficial velocity; D = diffusivity; and v = kinematic viscosity. The equation was developed for any packed beds and for the same parameter (e.g., DO). The v, D, and v can be assumed. Given that the geometric diameter of our media was 3.4 mm [$(4.76*2.38)^{1/2}$] and the inner diameter of our packed-bed was 19 cm, we can determine that $K_{w/o\ media} = 0.185*K_{w/media}$. Furthermore, we can assume that the mass transfer equation can be described with:

$$j = K(C_{surface} - C_{bulk}) = D * (C_{surface} - C_{bulk})/L \qquad (3)$$

where j = mass flux of parameter of interest (e.g., DO), $C_{surface}$ and C_{bulk} = parameter concentration on root surface or in bulk solution, and L = thickness of concentration profile. Then, if we further assume that the concentration difference in the two systems are the same, we find that the thickness of the concentration profile in our system ($L_{w/o\ media}$) is equal to $5.4*L_{w/media}$. Taking the DO profiles as an example, we then estimate that

if the sampling port is filled with media, the DO layer thickness around the lateral roots would be between 141 and 215 μm (0.185∗760 or 1,160) under different BOD loading conditions.

Biofilm was observed in the rhizosphere in the sampling zone of our system; we believe that it might be different from biofilms grown in the rhizosphere filled with media. Such differences in biofilm growth and distributions in rhizosphere might result in pH, ORP, and DO microprofiles different from the ones reported here. However, it is difficult to estimate the degree of such differences and, hence, we cannot propose any correction for such discrepancies at this stage.

SUMMARY

Wetland plants radiate some amount of oxygen from their roots to the immediate root surroundings to create a layer of oxygen around the growing roots. However, the extent of this oxygen layer has been found to be very limited (~1 mm, Figs. 3c, 5) in this study. Although we found that DO at the root surface increased nominally with an increase in oxygen demand of the bulk solution (oxygen sink), this DO concentration does not increase indefinitely. Nevertheless, the results of this study will be helpful for the quantification of the total amount of oxygen contributed by plants. This is of utmost importance, as this would help in making the plant population an engineering design parameter for the design of constructed treatment wetlands (Gersberg et al., 1991).

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