Effects of artificial aeration, macrophyte species, and loading rate on removal efficiency in constructed wetland mesocosms treating fish farm wastewater

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Abstract: We studied the contribution of artificial aeration, loading rate, and macrophyte species on pollutant removal in horizontal subsurface flow constructed wetlands (HSSFCWs) treating reconstituted trout farm wastewater. Twelve 1 m^2 mesocosms located in a controlled greenhouse environment were used to test two species of macrophytes (*Phragmites australis*, *Typha angustifolia*), three loading rates (30, 60, and 90 $L \cdot m^{-2} \cdot d^{-1}$), and presence or absence of artificial aeration at the intermediate loading rate. There was no effect of any variable (macrophytes, loading, aeration) on total suspended solids (TSS) or chemical oxygen demand (COD) removal. Artificial aeration improved nitrogen removal while higher loading rates diminished removal of nitrogen and phosphorus. Macrophytes improved nitrogen and phosphorus removal, but this effect varied depending on loading rates and presence or absence of artificial aeration. We found no differences between *Phragmites* and *Typha* for treatment of trout fish farm wastewater. Under summer conditions, our results suggest that artificial aeration could be used to improve nitrogen removal by HSSFCWs.

Key words: horizontal subsurface flow constructed wetlands, artificial aeration, loading rates, *Phragmites australis*, *Typha angustifolia*, fish farm wastewater.

Résumé : Nous avons évalué l'effet de trois charges (30, 60 et 90 $L \cdot m^{-2} \cdot d^{-1}$), de deux espèces de macrophytes (*Phragmites australis* et *Typha latifolia*) et de la présence d'aération artificielle sur les performances épuratoires de marais filtrants à flux horizontal sous-surfacique traitant un effluent piscicole reconstitué. Pour y parvenir, douze mésocosmes (1 m^2) ont été utilisés dans une serre avec un environnement contrôlé. Aucune variable (macrophytes, charge, aération) n'a affecté l'épuration des matières en suspension (MES) et de la demande chimique en oxygène (DCO). La présence d'aération artificielle a favorisé l'enlèvement d'azote tandis que les charges plus élevées ont fait chuter l'épuration d'azote et de phosphore. La présence de macrophytes a favorisé l'enlèvement du phosphore et de l'azote, mais cet effet a varié selon la charge et la présence d'aération artificielle. *Typha* et *Phragmites* ont démontré des capacités similaires pour l'épuration d'un effluent piscicole. Nos résultats montrent que l'aération artificielle pourrait être utilisée dans les marais filtrants traitant des effluents piscicoles pour augmenter l'enlèvement d'azote durant la saison estivale.

Mots-clés : marais filtrants à flux horizontal sous-surfacique, aération artificielle, charge en effluent, *Phragmites australis*, *Typha angustifolia*, effluent piscicole.

Introduction

Constructed wetlands (CWs) represent an alternative to conventional methods of wastewater treatment for a wide range of effluents (Kadlec and Knight 1996). When adapting CWs to a new type of effluent, numerous factors having an influence on treatment performance have to be taken into account. These factors include the type of flow (Verhoeven and Meuleman 1999; Vymazal 2005), key design and effluent speciation (Leonard and Swanson 2001; Kadlec 2003; García et al. 2005), plant presence and management (Brix 1997; Karathanasis et al. 2003), climate and temperature (Manios et al. 2000; Kadlec and Reddy 2001). Over the last 7 years, we have been optimizing a two-stage system for fish farm wastewater treatment in cold climate (Comeau et al. 2001; Naylor et al. 2003; Ouellet-Plamondon et al. 2006). The first stage, designed for biochemical oxygen demand (BOD_5), nitrogen (N), and suspended solids (SS) removal, is a gravel-bed, horizontal flow constructed wetland with an artificial aeration system in the first part of the bed. The second stage, specifically aimed at phosphorus (P) removal, is an unplanted bed filled with highly adsorbent material.

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The optimization of the first-stage bed in cold climate necessitates investigation of several key factors. The present study reports results of a mesocosm experiment on the effect of artificial oxygenation, macrophyte species, and loading rate, three factors that may have a high influence on removal efficiency in the first-stage bed.

Horizontal subsurface flow constructed wetlands (HSSFCW) are widely used in cold climates to treat various types of wastewater (Kadlec and Knight 1996; Mander and Jenssen 2002). However, oxygen availability in the substrate is often low, resulting in incomplete nitrification, the most limiting factor in N removal in HSSFCW (Kuschk et al. 2003; Tanner and Kadlec 2003). Preceding HSSFCW with a vertical-flow pre-treatment filter improves oxygen availability (Mæhlum and Stålnacke 1999) but vertical beds require more careful construction and operation, especially under cold climate conditions with freezing temperatures reaching well below zero in winter. Artificial aeration in the first part of the SSFCW matrix of the wetland has been used as an alternative solution to enhance treatment performance (Davies and Bart 1990; Cottingham et al. 1999; Higgins 2003). In addition to enhancing nitrogen removal by favouring nitrification, artificial aeration prevents partially degraded organic matter from accumulating in the bed matrix (Davies and Bart 1990; Cottingham et al. 1999). However, injecting air in the first part of the SSFCW matrix requires energy input and is generally not regarded as desirable when building an extensive treatment system. Nevertheless, in fish farms, aeration is already widely used in fish basins to keep a high oxygen level, so that artificial aeration is readily available to use for constructed wetlands at a low capital cost. In a mesocosm experiment, we previously showed that artificial aeration enhances N removal in fish farm effluent under a low loading rate, but its contribution under higher, more realistic loading rates remains to be demonstrated (Ouellet-Plamondon et al. 2006).

Presence of macrophytes is known to enhance treatment performances in constructed wetlands (Karathanasis et al. 2003), especially for N removal (Brix 1997). This enhancement may vary depending on macrophyte species (Tanner 1996; Coleman et al. 2001; Picard et al. 2005). The positive effect of macrophytes on treatment performances is attributed to an enhancement of oxygen availability and the release of organic compounds in the filtration matrix stimulating microbiological activity (e.g., nitrification) and higher P sorption via higher redox potentials (Brix 1997). In the context of using artificial aeration in the HSSFCW matrix, the role of macrophytes in further improving oxygen availability may become insignificant, so that the contribution of macrophytes in pollutant removal needs to be re-assessed.

To achieve best treatment performances in HSSFCWs, typical organic loads between 8 and 12 g $BOD_5 \cdot m^{-2} \cdot d^{-1}$ are recommended to treat secondary domestic wastewater in temperate conditions, and this is generally associated with a SS load of 1 to 10 $g \cdot m^{-2} \cdot d^{-1}$ (Kadlec and Knight 1996). Excessive loading rates result in higher pollutant loads and shorter retention times, which can lead to organic matter accumulation, a reduction of effective void space, and lower removal efficiencies, especially for BOD_5, N, and P (Tanner et al. 1995*a*, 1995*b*, 1998). Thus, evaluating the relation between loading rate and treatment efficiency for a particular CW design and type of wastewater is necessary in order to find the optimal charge that will maximize pollutant removal, maintain satisfactory treatment efficiencies, and minimize construction costs.

The objective of our mesocosm experiment was to evaluate the individual and cumulative effects of three main factors on pollutant removal efficiency: three different loading rates, presence of two species of macrophytes (*Phragmites australis* and *Typha angustifolia*), and presence or absence of artificial aeration at the intermediate loading rate. While the experiment was designed for a specific application and effluent type, its results may provide insights for other comparable HSSFCW applications.

Materials and methods

Experimental system

Twelve 3-year old sub-surface flow mesocosm treatment units (1.25 m long, 0.8 m wide, and 0.3 m deep) were used, which were located in a controlled greenhouse environment, at the Botanical garden of Montreal (Ouellet-Plamondon et al. 2006). The mesocosm units had been operated for 3 years. Graduated buckets were installed at the end of each mesocosm to collect and measure the quantity of treated effluent on a daily basis. Two 1500 L refrigerated bulk tanks were used to store a reconstituted effluent from a fish farm, while peristaltic pumps distributed the effluent at the chosen loading rate (Table 1). The mesocosms were filled with river gravel (granitic, Ø10–15 mm), with a narrow section at the inlet filled with large gravel (granitic, Ø30–40 mm) to facilitate water distribution. Water table level was kept 4 cm under the substrate surface.

Pollutant concentration in fish farm effluents varies according to farm operation, design, and pre-treatment (Cripps and Bergheim 2000). We used a flow-through freshwater trout (*Salvelinus fontinalis*) fish farm located in St-Damien (Québec, Canada) as a model for our experiment (Comeau et al. 2001). On average, fish raised at this farm daily ingested 1.2% of their total weight in summer and 0.4% in winter, which resulted in the retention of 40% of P (or 4.5 kg of P produced per tonne of fish) and the release of 1.19 g BOD_5, 1.07 g TSS, 0.39 g TN, and 0.04 g TP per fish per day (Ouellet 1998). As a result, pollutants were evacuated in two distinct pathways: a highly diluted effluent directly rejected in a stream, and a more concentrated effluent mixed with the decanted sludge of the farm and stored in a silo acting as an anaerobic digestor. The overflow of the silo contains pollutant concentration suitable for treatment by constructed wetlands and is the form of fish farm wastewater that serves as a model in our study. The reconstituted effluent for the mesocosm experiment was made with fish farm sludge collected in the silo, analysed for its pollutant concentration, and brought and stored at –20 °C at the Botanical garden. Every week, we diluted decanted fish farm sludge and added fish sauce (rich in chemical oxygen demand (COD)) to reach concentrations of 113 ± 33 mg TSS/L and 230 ± 80 mg COD/L.

Table 1. Hydraulic charge, pollutant loads, and retention times for each loading rate used in the study and recommended for HSSFCW treating domestic wastewater.

	Hydraulic charge	Retention time[b]	TSS[c]	COD[c]	TKN[c]	TP[c]
	$L \cdot m^{-2} \cdot d^{-1}$	d	$g \cdot m^{-2} \cdot d^{-1}$	$g \cdot m^{-2} \cdot d^{-1}$	$g \cdot m^{-2} \cdot d^{-1}$	$g \cdot m^{-2} \cdot d^{-1}$
Recommended[a]	80	5 to 10	7	14 to 21	<6	
30 L[c]	30	3.42	3.4±1.0	6.9±2.4	1.2±0.3	0.4±0.1
60 L[c]	60	1.71	6.8±2.1	13.8±4.8	2.3±0.5	0.8±0.1
90 L[c]	90	1.14	10.2±3.1	20.7±7.2	3.5±0.8	1.2±0.1

[a]Based on Kadlec and Knight (1996) and IWA (2000).
[b]Retention times were estimated using the method detailed by Chazarenc et al. (2003).
[c]Experimental loading values are averages of the 21 days monitored in this study.

To better mimic the silo overflow, we added urea ($CO(NH_2)_2$) and KH_2PO_4 to increase total Kjeldahl nitrogen (TKN) and total phosphorus (TP) concentrations to 40 ± 10 mg TKN/L and 13 ± 3 mg TP/L, respectively.

Three sets of three mesocosms were fed with 30, 60, and 90 $L \cdot m^{-2} \cdot d^{-1}$ of effluent, the intermediate loading giving a pollutant load approximately equal to the one recommended for HSSFCWs for domestic wastewater treatment (Kadlec and Knight 1996; IWA 2000), but with a shorter retention time (Table 1). An additional set of three mesocosms with a 60 $L \cdot m^{-2} \cdot d^{-1}$ loading rate was aerated with a diaphragm air pump diffusing a continuous air flow of 2 ± 1 $L \cdot min^{-1}$ in a horizontal 20 cm diameter circle at the bottom of the entrance of the beds. In each set, one bed was planted with *Phragmites australis*, one bed was planted with *Typha angustifolia*, and one bed was left unplanted. The beds were planted on May 2002 (Ouellet-Plamondon et al. 2006), and their aboveground portion were harvested at the end of each growing season, in November 2002 and 2003. Plants were thus in their third growing season during the experiment (summer 2004). Alimentation with the reconstituted effluent described here started at the end of May 2004.

Treatment performance

Data were collected from June to August 2004, during three periods of seven consecutive days (June 21–27, July 14–20, and August 5–11), with macrophytes being fully active. The following variables were measured daily according to Standard Methods (APHA et al. 2005): TSS (method # 2540 D), COD (method # 5220 D), TKN (method # 4500-N_{org} D using the Quickchem method # 10-107-06-2-D from Lachat Instruments), and TP (method # 4500-P B and 4500-P E). Evapotranspiration was estimated by measuring the total inflow and outflow. Removal efficiency calculations were based on mass balance:

$$[1] \qquad R = \left(1 - \frac{E_v E_c}{P_v P_c}\right) 100$$

where R is the removal efficiency, E_v is the treated effluent volume, E_c is the treated effluent concentration, P_v is the polluted influent volume, and P_c is the polluted influent concentration. Total daily pollutant mass removal rates ($g \cdot m^{-2} \cdot d^{-1}$) were also calculated.

Two sets of analyses of variance (ANOVA) followed by multiple comparisons of means according to Tukey's method were performed to test differences between treatments according to macrophytes, loading rate, and aeration for the whole period. The first set of analyses contained all (six) of the 60 $L \cdot m^{-2} \cdot d^{-1}$ units and tested the effect of artificial aeration, plant type, and sampling periods by including these parameters as factors in the ANOVA (aeration analyses; 17 or 18 samples included per variable). The second set of analyses, consisting of all (nine) non-aerated units, tested the effect of loading rates, plant type, and sampling periods by including these parameters as factors in the ANOVA (loading rate analyses; 14 to 16 samples included per variable).

All analyses were performed using SAS software (SAS Institute Inc., version 8e, 2001) and were considered significant at the 0.05 level. Non-parametric ranking transformations where used in the few cases when variables did not meet normality assumptions or did not have homogeneous variance. Statistical analyses are based on comparison of units with temporal replication rather than strict treatment replication, which raises the need for prudence in the interpretation of interactions between factors (Hurlbert 1984). Although removal efficiencies varied according to sampling periods, the statistical design of the ANOVAs allowed us to quantify the amount of variation due to temporal variation and to test the effect of the main three factors, regardless of the effect of sampling periods on pollutant removal.

Results and discussion

TSS and COD removal

For all treatments, TSS and COD removal efficiencies ranged from 76% to 91% and 83% to 92%, respectively. These high efficiencies in all treatments are probably due to the easily degraded settled sludge we used to create the reconstituted effluent. Also, our monitoring occurred in summer only, the period when HSSFCW are usually more efficient (Werker et al. 2002). There was no significant effect of artificial aeration, loading rates, and macrophyte species on TSS and COD removal on a percentage basis (results not shown), while mass removal decreased with smaller loads (Table 2). Thus, under summer conditions, one can add more TSS and COD within a certain range to a HSSFCW without diminishing removal efficiency,

Table 2. Mass removal of TSS and COD in relation to loading rates. Significance refers to ANOVA results and letters in parenthesis refer to Tukey's tests results.

		Mass removal ($g \cdot m^{-2} \cdot d^{-1}$)		
Pollutant	Significance	90 $L \cdot m^{-2} \cdot d^{-1}$	60 $L \cdot m^{-2} \cdot d^{-1}$	30 $L \cdot m^{-2} \cdot d^{-1}$
TSS	$p < 0.001$	9.9 (A)	6.1 (B)	3.2 (C)
COD	$p < 0.01$	18.7 (a)	13.2 (a)	6.5 (b)

Fig. 1. Relation between TKN removal efficiency (mean ± standard deviation), artificial aeration (df = 1, F = 38.14, p = 0.0252) and macrophyte species (df = 2, F = 12.79, p = 0.0183). Small letters refer to the results of Tukey's tests and are only related within one macrophyte (e.g., "b" in unplanted is different of "a" in unplanted but not similar to "b" in *Typha*).

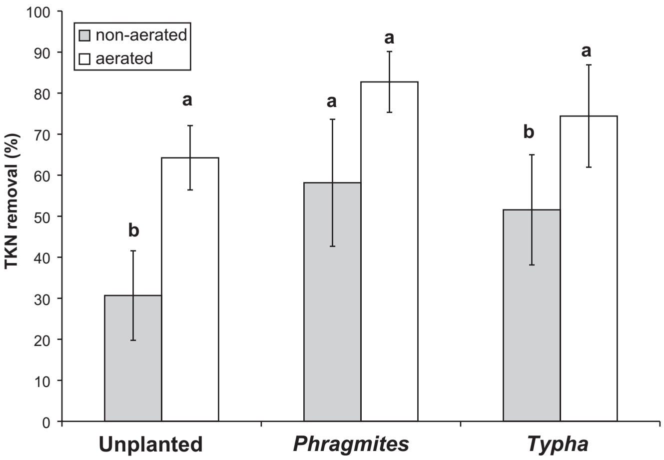
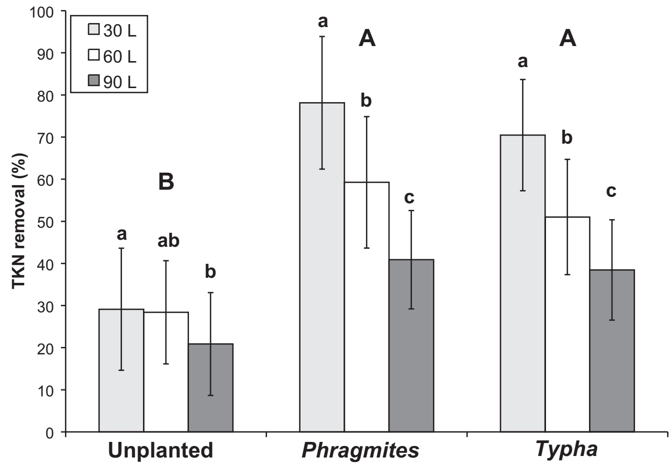


Fig. 2. Relation between TKN removal efficiency, loading rates (df = 2, F = 50.60, p = 0.0014) and macrophyte species (df = 2, F = 25.56, p = 0.0053). Capital letters refer to the differences between macrophytes species while small letters refer to differences between loading rates and are only related within one macrophyte.



up to a certain point where clogging problems may start to arise (Tanner et al. 1998).

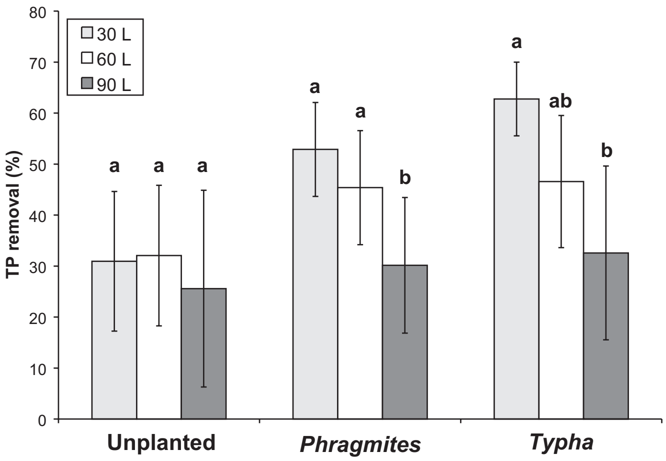
TKN removal

Artificial aeration significantly improved TKN removal in *Typha* and unplanted mesocosms (Fig. 1). Also, artificial aeration improved TKN removal in *Phragmites* mesocosms, but this increase was not statistically significant (Fig. 1). Overall pattern of artificial aeration effect on TKN mass removal was similar, with aerated units removing significantly more TKN than non-aerated units: 1.86 ± 0.55 $g \cdot m^{-2} \cdot d^{-1}$ (aerated) compared to 1.17 ± 0.49 $g \cdot m^{-2} \cdot d^{-1}$ (non-aerated).

Total Kjeldahl nitrogen removal efficiency decreased at higher loading rates, and this relation was affected by macrophyte treatment. Indeed, the decline was more pronounced in planted units, even though removal still remained higher than in any unplanted units (Fig. 2). Total Kjeldahl nitrogen removal saturated at the highest loading rate, and removal efficiency decreased to a point where mass removal for the highest (1.18 ± 0.63 $g \cdot m^{-2} \cdot d^{-1}$) and intermediate loading rates (1.09 ± 0.51 $g \cdot m^{-2} \cdot d^{-1}$) were approximately equal. These mass removal rates, including those in aerated units, were within or slightly over the high range of published annual mass rates for nitrogen, ranging from 0.15 to 1.4 $g \cdot m^{-2} \cdot d^{-1}$ (Tanner et al. 1995*b*). The fact that our experiment was run during summer, the most favourable season for plant and microbial activity, was probably responsible for our high TKN removal efficiency. The saturation observed at the highest loading rate was probably due to a lack of available oxygen, which can limit nitrification (Kuschk et al. 2003), or to other variables (e.g., short-circuiting) not specifically monitored during this experiment. Artificial aeration may have improved TKN removal at high loading rates as it did at the intermediate one, if there is a sufficient carbon source to ensure optimal denitrification (Spieles and Mitsch 2000), but this would remain to be tested. In our case, the annual harvesting of aerial parts of macrophytes could limit internal carbon loading and accumulation, and consequently limit denitrification even if oxygen is added to stimulate nitrification.

Planted units were always more efficient than unplanted ones and this improvement was always statistically significant except in one case (comparison of aerated units between *Typha*, 74% mean removal, and unplanted, 64% mean removal). Macrophytes are thought to enhance TKN removal mainly by increasing nitrification rates via higher oxygen concentrations and increased redox potentials in the root zone (García et al. 2003; Wießner et al. 2005). However, the superiority of *Phragmites* over unplanted units in presence of artificial aeration suggests a minor but significant role of direct uptake or any other process mediated by the presence of plants. Therefore, our results show that macrophytes may still contribute to nitrogen removal efficiency even when artificial aeration is used, suggesting that

Fig. 3. Relation between TP removal efficiency, loading rates (df = 2, $F = 7.34$, $p = 0.0458$) and macrophyte species (df = 2, $F = 11.50$, $p = 0.0220$). See Fig. 1 for details on letter interpretation.



macrophyte contribution to nitrogen removal is more than solely through enhanced rhizosphere oxygenation.

TP removal

Total phosphorus removal efficiency, which ranged between 35% and 40% among treatments, was not affected by artificial aeration. Presence of macrophytes significantly enhanced TP removal but this beneficial effect of plants decreased with increasing loading rates, whereas unplanted units had low removal efficiencies at all loading rates (Fig. 3).

In planted constructed wetlands, phosphorus removal is usually more important in the first year of operation, mainly because of adsorption to the substrate and plant establishment. However, in mature wetlands, phosphorus is mainly removed via adsorption, sedimentation, and cationic exchange processes, while plant uptake plays a minor role (Kadlec and Knight 1996). Cases of rhizosphere oxidation promoting phosphorus adsorption to the substrate next to *Phragmites* roots have been reported (Wathugala et al. 1987), but we found no signs of increased removal efficiency in aerated unplanted units. Total phosphorus removal being dependent on retention times (Tanner et al. 1999), the higher TP removal in planted units may be attributed to higher evapotranspiration rates compared to unplanted units, which leads to higher retention times in summer (Chazarenc et al. 2003).

Conclusion

Presence of macrophytes had no effect on TSS or COD removal, but it promoted TKN and TP removal. The positive effect of macrophytes was generally still significant when artificial aeration was used, suggesting that the role of plants goes beyond the sole addition of oxygen near the root zone. No statistically significant difference was found between *Typha* and *Phragmites*, indicating that species selection should depend on other criteria than efficiency, such as species availability, cost, and risks of invasiveness in neighbouring ecosystems (Grandtner 1999).

In our mesocosms experiment, artificial aeration significantly enhanced nitrogen removal at an intermediate loading rate, whereas it had no effect on TSS, COD, and TP removal. Artificial aeration may thus be an acceptable alternative to improve TKN removal in constructed wetlands, especially where it can be readily available at a low capital cost, such as in fish farms. However, the effectiveness of artificial aeration at higher loading rates would still remain to be tested.

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