Effects of different substrates in the mitigation of algae-induced high pH wastewaters in a pilot-scale free water surface wetland system

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ABSTRACT

Waste stabilization ponds (WSPs), as part of municipal wastewater treatment strategies, can exhibit variability in performance due to climatic conditions. Under elevated temperature and strong solar radiation, algal blooms and subsequent high pH effluents have often been observed. In this study, four substrates (gravel, peat, organic mulch, and topsoil) were evaluated for their ability to attenuate high pH effluents from a WSP. Synthetic wastewater with pH > 9.5, and low organic and nutrient loadings, was used to mimic algal-induced high pH effluents in 72 L rectangular bench-scale superficial constructed wetland configuration reactors. Peat exhibited the highest attenuation ability, where the effluent pH decreased substantially from 10.3 to 7.7, primarily due to its high organic contents. Peat also removed 53.7% of the influent total phosphorus, which could effectively limit algal growth. No statistically significant differences were discovered among gravel, topsoil, and organic mulch in terms of pH attenuation. Topsoil and organic mulch both have a relatively high alkalinity, making them ideal to maintain consistent pH levels. However, naturally high chemical oxygen demand levels in organic mulch raised concerns in the leaching of these compounds into the treated wastewater, making it less appealing for systems with low organic loading.

Key words | constructed wetland, nutrients, organics, pH, substrate, waste stabilization pond

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INTRODUCTION

In Canada, there are more than 3,500 wastewater treatment facilities, many of which apply biological treatment technologies such as the activated sludge process (Lotito *et al.* 2014), biofilm-based technologies such as trickling filters (Daigger & Boltz 2011), vegetation filters (Miguel *et al.* 2014), and pond systems such as oxidation ditches and waste stabilization ponds (WSPs) (Hosetti & Frost 1998). Among these technologies, WSP is considered to be the most sustainable technology for small communities that require low cost and low maintenance wastewater treatment facilities. They have the capability to effectively attenuate organic and nutrient concentrations, as well as pathogen levels in municipal wastewater (Maynard *et al.* 1999; Senzia *et al.* 2003; Reinoso *et al.* 2008).

However, due to the configuration of WSPs and limited control over these systems, performance concerns during certain parts of the year may arise (Kayombo *et al.* 2002). There is a synergistic balance between heterotrophic microorganisms and algae in a WSP system, where they work together to provide treatment for wastewater (Borde *et al.* 2003; Muñoz & Guieysse 2006). Heterotrophic microorganisms produce carbon dioxide via the metabolic respiration process, while algae utilize the carbon dioxide and produce oxygen via photosynthetic activity to replenish the dissolved oxygen. However, a shallow WSP basin encourages sunlight penetration throughout the water column depth, and temperature increases during the summer. Both the individual and the combined contributions of these two factors can impact the performance of the system. In this particular study, elevated temperature and high solar radiation in the summer encouraged the growth of algae in WSP. Although the presence of algae is common and expected in WSP, these particular conditions can trigger an algal bloom that disrupts the balance between algae and heterotrophic microorganisms in the WSP. Continuous consumption of dissolved carbon dioxide, a

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necessary requirement for photosynthesis, along with the limited replenishment of carbon dioxide from atmospheric diffusion and respiration from heterotrophic microorganisms, could give rise to increases in effluent pH level (>9.5) (Nishimura *et al.* 1984). This in turn allows for the continued proliferation of algae which, in turn, negatively impacts the performance of the WSP. Moreover, with dissolved carbon dioxide levels being relatively low, the water chemistry equilibrium will encourage the formation of carbonate and bicarbonate ions from carbon dioxide to maintain carbonate equilibrium, which further increases the pH level according to Equation (1).

$$H_2O + CO_{2(g)} \leftrightarrow H_2CO_3* \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad (1)$$

High pH level effluent could (i) shift the ammonium/ammonia equilibrium increasing the rate of ammonia formation and volatilization, (ii) alter the downstream ecological environment if the elevated pH water is discharged to the receiving environment, and (iii) not meet regulatory compliance ($pH = 6.5–9.5$), which is set by Environment Canada (Environment Canada 2012). The Amherstview Water Pollution Control Plant (WPCP) is a wastewater treatment facility in Southern Ontario, Canada, which experienced these high pH level events caused by algal blooms for a number of treatment seasons.

Similar to WSPs, constructed wetlands (CWs) are also considered to be a green and sustainable passive treatment technology that mainly attenuates wastewater through biological processes. One of the merits of CWs, compared to WSPs, is that they are more resilient to fluctuations under natural environmental conditions due to the complexity of their ecosystems (Pietro & Ivanoff 2015). The components of a typical CW include inlet and outlet structures, substrates, rooting media, and vegetation. Both the individual and the combination of these components are important in terms of treatment performance (Kadlec & Wallace 2008). As such, they could contribute a greater system capacity to buffer the effects of temporary high temperatures and/or high solar radiation, which would minimize the overall impact in terms of affecting effluent quality in CWs compared to WSPs.

CWs have also been shown to attenuate acidic or alkaline wastewater to neutral pH levels. Acidic wastewater from a food processing plant at Connell, Washington (Kadlec *et al.* 1997), and alkaline wastewater at Estevan, Saskatchewan (Pries 1996), have both been attenuated to neutral before being discharged to the environment by free water surface (FWS) CWs.

Substrates are vital components of CWs in terms of contaminants and nutrient transportation and removal. They provide a surface area to allow sustained biofilm to grow, which eventually enhances microbial processes. In recent years, alternative substrates (e.g., mulch, zeolite, and volcanic ash) have attracted more and more attention because of their high specific surface area and increased nutrient retention capacity compared to the traditional substrates such as gravel. Some studies have explored alternative substrates that have the potential to be applied in different CW systems (Gray *et al.* 2000; Aslam *et al.* 2007; Mateus *et al.* 2012; Bai *et al.* 2014; Herrera-Melián *et al.* 2014; Liu *et al.* 2014). However, most of these studies only focused on the effects of the alternative substrates on organic matter and nutrient removal from municipal or industrial wastewaters in subsurface flow CWs. There is very little information about the effects of these substrates on FWS CWs. Most importantly, very few studies focused on the effects of substrates on system pH, especially for algal-induced high pH wastewaters.

As such, this study aims to provide a comparative evaluation of four locally available substrates (gravel, topsoil, peat, and organic mulch) that are considered for application in a pilot-scale FWS CW for the polishing of secondary effluents at the Amherstview WPCP. The main objective of this study was to compare their treatment performance, in terms of pH attenuation as well as organic and nutrient removal, under a FWS CW operational configuration using superficial bench-scale reactors without the presence of vegetation. The assessment was mainly based on their geophysical properties without the confounding effects of vegetation. This study was also designed to identify the most suitable substrates for the further on-site pilot-scale studies.

MATERIAL AND METHODS

Preparation of synthetic wastewater

A synthetic wastewater was prepared every 2 days and used as an influent for all experimental wetland systems. The synthetic wastewater was prepared using (g in 1 L tap water): 0.00050 g $C_6H_{12}O_6$, 0.01008 g $NaHCO_3$, 0.00047 g $NaNO_3$, 0.00009 g NH_4Cl, 0.00004 g KH_2PO_4, 0.01339 g $MgCl_2{\cdot}6H_2O$, 0.01651 g $CaCl_2{\cdot}2H_2O$, 0.00005 g $FeSO_4{\cdot}7H_2O$, 0.00003 g $MnSO_4{\cdot}H_2O$, and 0.70000 g NaOH. All chemicals used were ACS reagent grade or analytical reagent grade supplied by Fisher Scientific. The prepared synthetic wastewater was stored in a 300 L Nalgene feed tank, prior to being dosed into the experimental system.

The synthetic wastewater was generally designed to mimic the secondary effluent at the Amherstview WPCP, with relatively low organic and nutrient concentrations. Sodium hydroxide and sodium bicarbonate were mainly used to buffer the solution to maintain the relatively constant pH in the synthetic wastewater. A pH of 10.5 was used to simulate the high pH events at the Amherstview WPCP.

Configuration of bench-scale wetland systems

Ten bench-scale wetland reactors were manufactured using Plexiglass, and were fabricated and supplied by Kingston Plate and Window Glass in Kingston, Ontario. The length, width, and height of each wetland reactor was 600 mm, 200 mm, and 800 mm, respectively. The experimental system was configured to mimic a FWS CW without the presence of vegetation. A ¼″ (6.25 mm) hole was drilled into each reactor side panel (0.2 m × 0.8 m) and equipped with a ¼″ male to female PVC ball valve to control the flow. The heights of the inlets and outlets of the reactors were 250 mm and 600 mm, respectively. The substrates were added to the bottom of each reactor to a depth of 0.2 m. The synthetic wastewater was simultaneously pumped into six reactors from the feed tank using a Masterflex L/S peristaltic pump with a 12-channel, eight-roller cartridge pump head that can hold up to six cartridges at the same time. Masterflex Tygon E-Lab pump tubing was used to connect the reactors to the feed tank.

Substrates

Four locally available substrate materials: gravel, peat, organic mulch, and topsoil, were applied in this experiment as substrates that could potentially attenuate high pH effluents. All these substrates were purchased from The Home Depot in Kingston, Canada. Prior to the experiment, all the commercial substrates were washed in deionized water to remove any dirt and undesired materials. Sand was used in two reactors to serve as controls, and duplicate systems were used for each substrate. The porosity of each substrate was measured by pouring water in a 1 L graduated cylinder with fully loaded substrate in it. The porosity was determined by the volume of water required to fill the void spaces in the substrates divided by the volume of the substrates (1 L) (Equation (2)). The physical properties of the substrates are presented in Table 1 while the substrates are shown in Figure 1.

Table 1 | Density and porosity of substrates applied in this experiment

Properties	Gravel	Peat	Topsoil	Organic mulch
Density (kg/m^3)	1,545	800	1,520	505
Porosity	0.44	0.31	0.50	0.76

Porosity is from 0 to 1. 0 indicates the lowest porosity and 1 indicates the highest porosity.

$$\phi = \frac{V_v}{V_T} \tag{2}$$

ø: Porosity (dimensionless)
V_v: Volume of void space in substrate (L)
V_T: Total or bulk volume of substrate (L)

Operation of reactors

The overall experimental period for each substrate was 4 weeks for gravel, peat, organic mulch and topsoil. Sand was used during the entire experiment period to serve as a control. The substrate reactors and control reactors were run in duplicate. At the beginning of the experiment, deionized water was fed to the reactors using a peristaltic pump to allow the substrates to become hydrated and settle. After the settling period of 2 days, the deionized tap water was drained and feeding of the reactors with the synthetic wastewater was initiated. For each experiment, the peristaltic pump was operated at 50 rpm (6.9 L/d) during the start-up phase to gradually fill the reactors and minimize disturbance of the substrates. When the free water level reached the desired height (600 mm), the pump rate was increased to 140 rpm (19.2 L/d) to accommodate the design hydraulic retention time (HRT) for the experiment of 2.5 days, which also corresponded with the anticipated pilot-scale FWS CW at the Amherstview WPCP. All reactors were operated under continuous flow conditions and at 25°C. The operational parameters of this experiment are summarized in Table 2. The substrates were first allowed to acclimatize to the synthetic wastewater for a 1-week period before any sampling and analysis were initiated. Then, 2 weeks of intensive sampling and analysis were followed for each substrate to assess their performance and attenuating capacity.

Figure 1 | Substrate material: gravel (top left), peat (top right), organic mulch (bottom left), topsoil (bottom right).

Table 2 | Operational parameters for the experimental reactors

Parameters	HRT	Flow rate	Organic loading rate	Nitrogen loading rate	Phosphorus loading rate
	2.5 d	19.2 L/d	0.33 g/d	0.028 g/d	0.008 g/d

Sampling and analysis

Over a 2-week monitoring period, wastewater samples were collected on a daily basis from the outlet of each wetland reactor, as well as the feeding tank. Twelve sets of data were collected for pH, redox potential (E_h), alkalinity, ammonium, nitrite, and nitrate. Eight sets of data were collected for total phosphorus (TP) and total nitrogen (TN), and five sets of data were collected for chemical oxygen demand (COD).

pH and E_h were analyzed using a Fisher Scientific accumet XL60 benchtop meter with Fisher Scientific pH and E_h probes. Ammonium and nitrate were measured using the same benchtop meter with Fisher Scientific ammonium and nitrate probes. Nitrite and alkalinity were determined using Thermo Scientific Orion

AQUAfast Colorimetric Tablet Reagents and a HACH DR 2800 spectrophotometer based on USEPA approved methods. The TN and TP analyses were conducted using a Digital Reactor Block 200 and HACH DR 2400 spectrophotometer based on USEPA approved standard procedure for wastewater analysis. The COD analyses were performed based on *Standard Methods* (APHA 2012).

Statistical analysis

In order to compare the pH attenuation capacity of the different substrates in the wetland systems, parametric and non-parametric statistical analyses were performed using Microsoft EXCEL and XLSTAT.

Various statistical normality tests (Shapiro-Wilk, Anderson-Darling, Lilliefors, and Jarque-Bera) were performed to verify whether the distribution of data approximated normality, which was accepted when $\alpha = 0.05$. When data approximated normality, the data were analyzed through one-way analysis of variance (ANOVA) to illustrate the statistically significant difference ($p < 0.05$) of the means, where if a significant difference ($p < 0.05$) was observed among four comparable different substrates, multiple post-hoc comparison analysis including Tukey (honest significant difference), Fisher (least significant difference), and Bonferroni tests, were performed to determine the statistically significant difference between groups. When the data did not approximate normality, a non-parametric statistical analysis, Mann-Whitney test ($p < 0.05$), was performed instead of ANOVA to compare pH attenuation, nitrogen, organic output, and removal efficiency between groups.

RESULTS

Overall performance

Table 3 summarizes the mean effluent pollutant concentrations and percentage removal in each of the experimental reactors with different substrates. In terms of pH attenuation, peat exhibited the highest capability for pH attenuation over one HRT cycle and maintained this pH level throughout the experimental period. Only gravel and topsoil demonstrated positive percentage removal of COD, while the organic substrates, peat and organic mulch, showed a tendency to increase the COD concentration in the reactors. Only organic mulch exhibited the potential to exceed the wastewater effluent COD concentration guidelines of 25 mg/L, which would be above the regulatory discharge guidelines set by Environment Canada (Environment Canada 2012). In terms of nutrient removal, although the influent synthetic wastewater had relatively low nutrient concentrations (TN = 1.49 mg/L, TP = 0.41 mg/L), the substrates are able to contribute to further treatment; the only exception was the effluent TN concentration of peat.

pH attenuation

Figure 2 illustrates the pH variation with time for each of the substrates. Peat demonstrated the highest potential to lower system pH, which started above 10.5 initially and was reduced to below 8 after two HRT cycles. After one HRT cycle (2.5 days), the pH in the peat reactor dropped from 9.87 to 8.40, and dropped further to 7.76 and remained at that level until the end of the experiment.

Table 3 | Mean pollutant removal performance across the wetland reactors. (Standard deviation of pollutant concentration is indicated in brackets)

			Gravel		Peat		Topsoil		Organic mulch	
Parameters	**Unit**	**INF**	**EFF**	**RF**	**EFF**	**RF**	**EFF**	**RF**	**EFF**	**RF**
pH	N/A	10.37 (0.20)	9.21 (0.11)	N/A	7.76 (0.11)	N/A	9.27 (0.17)	N/A	9.12 (0.21)	N/A
E_h	mv	91.4 (5.9)	123.9 (10.1)	N/A	143.1 (23.9)	N/A	125.1 (15.3)	N/A	121.9 (10.5)	N/A
Alkalinity	mg/L	128.0 (50.0)	47.5 (5.0)	62.9	52.2 (10.0)	56.9	102.3 (33.6)	20.1	105.1 (35.9)	17.9
COD	mg/L	17.38 (7.47)	10.50 (1.98)	39.6	19.00 (1.85)	NEG	13.63 (4.27)	21.6	28.00 (11.22)	NEG
Ammonium	mg/L	0.17 (0.08)	0.07 (0.03)	58.8	0.13 (0.04)	23.5	0.05 (0.01)	70.6	0.04 (0.01)	76.5
Nitrate	mg/L	3.02 (0.78)	2.53 (0.69)	16.2	4.13 (1.03)	NEG	2.02 (0.84)	33.1	4.00 (2.45)	NEG
Nitrite	mg/L	0.009 (0.003)	0.004 (0.002)	55.6	0.014 (0.010)	NEG	0.027 (0.022)	NEG	0.044 (0.015)	NEG
TN	mg/L	1.49 (0.27)	1.30 (0.48)	12.8	1.64 (0.21)	NEG	1.00 (0.15)	32.9	1.00 (0.24)	32.9
TP	mg/L	0.41 (0.19)	0.11 (0.02)	73.1	0.19 (0.05)	53.7	0.15 (0.05)	63.4	0.20 (0.03)	51.2

INF, influent concentration; EFF, effluent concentration; RF, removal efficiency; N/A, not applicable; NEG, negative removal efficiency.

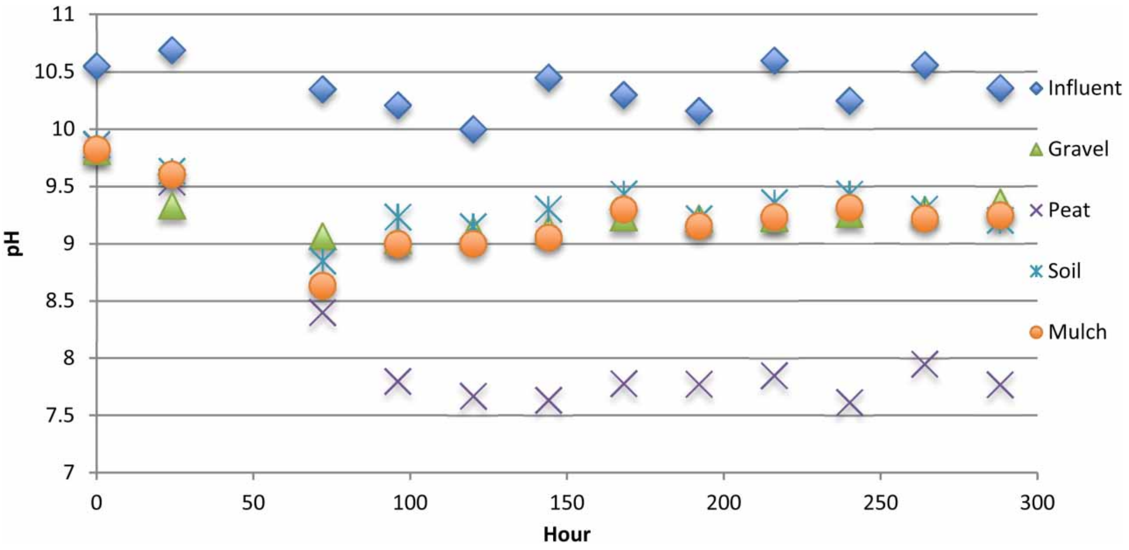

Figure 2 | Trends in pH levels with different substrates over time.

The reactors to which gravel, organic mulch, and topsoil were applied exhibited average effluent pH levels of 9.21, 9.12, and 9.27, respectively, throughout the experiment. These results demonstrated that pH levels below 9.50 could be consistently achieved within two HRT cycles, which would allow for the polished effluent to be discharged to the receiving environment. The result from the ANOVA analysis rejected the null hypothesis ($p < 0.0001$) that the mean effluent pH of all substrates were all equal. However, the ANOVA analysis can only indicate whether there is a significant difference between the mean effluent pH levels within the four groups. It cannot identify which groups have statistically significant differences. Therefore, post-hoc pairwise comparison analyses (Tukey pairwise comparison test, Fisher test, and Bonferroni test) were performed to identify the statistically significant difference between groups. The three tests uniformly demonstrated that the pH attenuation performance of peat was significantly different from the other substrates, while there was no statistical difference among gravel, organic mulch, and topsoil, as shown in Table 4.

Table 4 | Statistical analysis comparing pH attenuation ability of different substrates. (Significant statistical difference ($p < 0.05$) is indicated in bold numbers)

	Peat	Gravel	Organic mulch	Topsoil
Peat	N/A	< **0.0001**	< **0.0001**	< **0.0001**
Gravel	< **0.0001**	N/A	0.999	0.981
Organic mulch	< **0.0001**	0.999	N/A	0.933
Topsoil	< **0.0001**	0.981	0.933	N/A

Alkalinity

Alkalinity is the measure of the capacity of water or any solution to neutralize or 'buffer' strong acids. This measurement of acid neutralizing capacity is important in terms of understanding how an aqueous environment can resist sudden changes in pH. In natural waters, this capacity is mostly attributed to basic ions such as bicarbonate, carbonate, hydroxide ions, as well as trace metals that can be neutralized by acid (Snoeyink & Jenkins 1980). Since the synthetic wastewater that was supplied in this experiment contained very little trace metals, it was assumed that the total alkalinity could be attributed to bicarbonate, carbonate, and hydroxide ions, as shown in Equation (3).

$$\text{TotalAlkalinity} = 2\left[\text{CO}_3^{2-}\right] + \left[\text{HCO}_3^-\right] + \left[\text{OH}^-\right] - \left[\text{H}^+\right] \tag{3}$$

Table 3 summarizes the mean effluent alkalinity from each of the four substrates. The mean effluent alkalinity for organic mulch and topsoil was 105.1 mg/L (2.10 meq/L) and 102.3 mg/L (2.05 meq/L), while the mean effluent alkalinity for peat and gravel was 52.2 mg/L (1.04 meq/L) and 47.5 mg/L (0.95 meq/L), respectively. The ANOVA analysis confirmed that a significant difference ($p < 0.0001$) could be noted for the mean effluent alkalinities. The Tukey pairwise comparison test indicated significant differences between the reactor with mulch and gravel, mulch and peat, topsoil and gravel, and topsoil and peat, while there was no significant difference between mulch and topsoil.

As can be surmised from Equation (3), the total alkalinity of the solution will depend on both system pH and carbonate concentrations. Solutions with lower pH levels will tend to exhibit lower total alkalinities when similar concentrations of carbonate and bicarbonate ions are present. Peat demonstrated the highest pH attenuation capacity. Therefore, its low effluent pH level might contribute to its low alkalinity concentration. However, gravel had a significantly lower total alkalinity compared to organic mulch and topsoil, which exhibited similar mean effluent pH levels. This result indicated that there are different pH attenuation mechanisms that occur with different substrates.

Peat, organic mulch, and topsoil, often have a large organic content. Among all organic matters, humus substances (HSs) are the major source of natural organic matter in the soil as well as in geological organic deposits such as peat. HSs include humic acids (HAs) and fulvic acids (FAs). The HAs and FAs in the peat can be released under alkaline conditions in aqueous environments (Su & Puls 2007). These acids react with the hydroxide ions directly to lower the pH without interfering with the inorganic carbonate species in the system.

In the case of gravel, calcite precipitation can contribute to the consumption of alkalinity, which encourages the drop of pH level in the reactor. Calcium, more specifically calcium carbonate, is a common substance found in the majority of gravel materials, often in the form of minerals such as calcite and aragonite. The amount of calcium in gravel depends on the composition of the rock fragment itself, and its potential dissolution on the surface area of the material exposed to the solution. Mayes & Younger (2006) conducted a study to assess the rates of buffering across a natural wetland receiving steel slag drainage in northeast England. The effluent pH was consistently below 9, with calcite precipitation rates (9.88 $g/m^2/d$) up to two folds of peak values (3.66 to 4.35 $g/m^2/d$) in natural waters (Dreybrodt *et al.* 1992). This theory is supported by the low alkalinity concentrations observed in the reactor and the composition of gravel.

Nitrogen removal

The influent nitrogen concentrations were considered to be relatively low in this experiment compared to other studies (Saeed & Sun 2011; Bai *et al.* 2014). However, even at this low level concentration, the substrates were still able to contribute to the removal of nitrogen in experimental reactors. Table 3 summarizes the mean effluent and percentage removal of multiple nitrogen species. Organic mulch, topsoil, and gravel demonstrated high ammonium percentage removal, 76.5%, 70.6%, and 58.8%, respectively, whereas peat only had a percentage removal of 23.5%. Most of the ammonium in the wastewater is transformed to ammonia, and removed through mass transfer from the water surface to the atmosphere (Equation (4)). This process is mainly dependent on the system pH, as a high pH environment ($pH > 9.3$) encourages the ammonium volatilization process (Białowiec *et al.* 2011). Other than that, the ammonium can be also converted to nitrate or nitrite through nitrification process. During the nitrification process, hydrogen ions are produced, according to Equations (5) and (6), which can then lower the pH level of the system. FWS CW favored the oxygen diffusion, and the high E_h (nitrification → 100 mv to 350 mv) indicated that nitrification might be favored in these reactors and enhanced the pH attenuation.

$$NH_4^+ + OH^- \leftrightarrow NH_{3(g)} + H_2O \tag{4}$$

Peat and organic mulch exhibited high effluent nitrate concentrations (4.13 mg/L and 4.00 mg/L) compared to gravel and topsoil (2.53 mg/L and 2.02 mg/L), and they both received negative nitrate percentage removal at influent concentration of 3.02 mg/L. One of the reasons might be the ammonium has been converted to nitrate. However, in this study, the influent ammonium concentration was quite low (0.17 mg/L); therefore, nitrate leaching from the peat and organic mulch is indicated here. Since the influent nitrite concentration is extremely low (0.009 mg/L), no further nitrite reduction is expected. The results justified our anticipation that the effluent nitrite concentrations were actually higher than the influent, when peat, organic mulch, and topsoil were applied to the reactors. Surprisingly, gravel further reduced the nitrite concentration to only 0.004 mg/L. The low percentage removal of nitrate and nitrite also affected the removal of TN. The maximum percentage removal of TN was only 30%, which was achieved by organic mulch and topsoil. Peat even exhibited negative removal of TN. The low percentage removal of nitrate, nitrite, and TN observed in this experiment may be due to the oxidation state in the reactors. The formula describing the denitrification reaction, which is the primary mechanism for the removal of nitrate, nitrite, and TN in CWs, is shown in Equation (7). A readily available carbon source and a low-oxygen condition are the primary conditions required for denitrification to take place in wetlands. It is likely that the low-oxygen condition was not sustained in the reactors throughout the experiment. Denitrification typically occurs under anaerobic conditions, where an E_h range of 50 mv to −50 mv allows denitrifiers to thrive. However, the reactors exhibited an average E_h of approximately 120 mv, which might have inhibited the growth of denitrifiers resulting in low denitrification rates. These reactors were designed to simulate FWS CW conditions, in which aerobic conditions would be promoted that could hinder the removal of nitrogen.

These findings were not consistent with other studies in which high ammonium and TN removals were reported. Saeed & Sun (2011) applied organic mulch in a vertical flow CW reactor and demonstrated that organic mulch with high organic carbon concentrations, which serve as an additional carbon source, could enhance the percentage removal of both ammonium and TN to 99.6% and 97.8%, respectively, at influent ammonium and TN concentration of 18.4 mg/L and 22.3 mg/L, respectively. Bai *et al.* (2014) investigated the feasibility of reusing drinking water treatment residuals with organic matter content of 57.65 mg/g as a substrate in CWs to treat secondary effluent at short HRT (1–3d). However, the result from our study corresponded to a study conducted by Li *et al.* (2015), who also investigated the removal of low concentration nutrients in CWs by incorporating zeolite and calcium silicate hydrate substrate. The maximum percentage removal of ammonium was 82% at an influent concentration of 2 mg/L, whereas the maximum percentage removal of TN was 30% at an influent concentration of 12 mg/L (Li *et al.* 2015).

Overall, all substrates effectively removed ammonium, whereas none of the substrates exhibited high percentage removals of nitrate and nitrite in this study. Gravel showed moderate ammonium removal efficiencies (58.8%), as well as for nitrate (16.2%), nitrite (55.6%), and TN (12.8%), and it is the only substrate that demonstrated positive percentage removal for all nitrogen species in this experiment. Peat showed similar nitrate accumulation as was observed for the organic mulch. Low percentage removal of ammonium efficiency coupled with very low nitrite concentration indicates that there might be external nitrates sources from the substrate. The negative percentage removal of TN suggested that nitrogen might be leached from the peat.

$$NH_4^+ + 1.5O_2 \rightarrow H^+ + H_2O + NO_2^- \tag{5}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \tag{6}$$

$$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \tag{7}$$

Phosphorus removal

The results in Table 3 indicated that the percentage removal of TP was higher in gravel compared to the organic substrates throughout the experimental period. Although the peat and organic mulch demonstrated reasonable percentage removal of TP (53.7% and 51.2%), gravel (73.1%) showed the best performance in terms of TP removal in this study. This result was contradictory to a number of studies that have demonstrated that organic substrates generally exhibit higher phosphorus retention capacities due to their chemical properties (Lüderitz & Gerlach 2002; Herrera-Melián *et al.* 2014). Peat and organic mulch have been reported to contain substantial HSs, which have been noted to play an important role in terms of TP removal via adsorption (Saeed & Sun 2011). However, the alkaline condition in our experiments might contribute to this result. Adsorption and precipitation are two well-known mechanisms that govern the phosphorus removal in wetland systems. Inorganic phosphorus tends to adsorb on hydrous oxides of Fe, Al, or Ca and may precipitate as insoluble Fe-P ($FePO_4$ or $Fe_3(PO_4)_2$) and Al-P ($AlPO_4$) under acid conditions and Ca-P ($Ca_5(OH)(PO_4)_3$) under alkaline conditions (Vymazal 2004). Therefore, in this study, the mineral-based substrate gravel could have provided sufficient Ca that could form Ca-P precipitates under alkaline conditions to remove P from the water column compared to the organic substrates. Blanco *et al.* (2016) also studied phosphorus removal by utilizing basic oxygen furnace steel slag as a substrate for CWs. In batch experiments, 84–99% of phosphorus removal was achieved, while 95% of phosphorus removal was reached under continuous flow conditions. They concluded that the main phosphorus removal mechanism was likely Ca-P precipitation which depends on Ca^{2+} and OH^- release after dissolution of $Ca(OH)_2$ in water. In terms of adsorption, in our study, the HRT is relatively short. Therefore, the time required to establish the sorption equilibrium plays an important part in phosphorus removal. Lantzke *et al.* (1998) reported that gravel sorption equilibrium could be established fairly quickly (within 48–150 hours) compared to organic substrates. As such, relatively short experimental period and lower HRT may favor phosphorus removal by mineral-based substrates compared with organic-based substrates.

Other phosphorus removal mechanisms tend to occur more slowly than adsorption, for example, microbial phosphorus removal and plant uptake in wetland applications (Lantzke *et al.* 1998). Vymazal (2004) concluded that phosphorus removal through plant uptake only represented no more than 17% of TP removal in wetlands. In addition, the organic substrates we applied in this experiment may contain higher phosphorus background concentration.

Organic compounds removal

COD is considered to be the representative of organic carbon concentration in wetland reactors. The ANOVA result showed that there was no statistically significant difference between influent and effluent from all substrates. Since the influent COD was relatively low (17.38 mg/L) due to the synthetic wastewater used in this particular experiment, and the fact that it already met regulatory compliance (25 mg/L), the introduction of organic substrates may have negative influence on the effluent COD concentrations. Peat and organic mulch both showed negative percentage removal of COD as anticipated, and organic mulch even had a mean effluent COD concentration at 28 mg/L, which exceeded regulatory compliance. Herrera-Melián *et al.* (2014) evaluated another organic-based substrate (palm tree mulch) in CW applications. Although the best percent removal of COD was 77%, COD concentrations were only reduced to 264 mg/L. The results using organic mulch in this study also corresponded to the results presented by Saeed & Sun (2011) where high effluent COD concentrations were observed when organic substrates were applied. They indicated that this was likely due to organic carbon leaching from the organic substrates.

On the other hand, the reactors containing the mineral-based substrate, gravel, were observed to achieve further COD (39.5%) removals. Compared with organic-based substrates, gravel typically does not contain organic content, and therefore leaching organics is not an issue when gravel is applied. This was further supported by the result of this study in which gravel was the only substrate applied that exhibited positive percent removals of COD.

CONCLUSION

Overall, all substrates in this study demonstrated their ability to attenuate high pH effluents to a level below 9.5, which is in compliance with wastewater discharge guidelines, set by Environment Canada. Among these substrates, peat showed superior pH attenuation ability and was able to effectively reduce the effluent pH level to 7.7 without sacrificing the water quality. The released organic acid was the main driving force of pH attenuation due to the low alkalinity in the effluents. Organic mulch was also able to attenuate the pH level below discharge guidelines as well. However, the pH was only reduced to around 9.5. It also showed a tendency to leach organics to the effluents, which indicated the organics released by organic mulch were not sufficiently acidic to completely neutralize the pH compared to peat. Topsoil demonstrated the best overall performance in terms of both organics and nutrient removal, and the effluents were well in compliance with discharge limits. Therefore, peat and topsoil are considered to be the potential substrates for the further on-site pilot-scale studies at the Amherstview WPCP.

FUTURE WORK

Based on this work, although peat and topsoil have demonstrated potential to attenuate high pH wastewater without sacrificing high quality effluent, long-term evaluation of these substrates is still required to gain better understanding of the stability of their treatment performance. Therefore, a 3-month, long-term evaluation experiment is proposed to further examine the substrates peat and topsoil. Different organic loadings and HRT as well as shock loading test will be applied to further evaluate the pH attenuation ability of these substrates.

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