© 2021 The Authors
472

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

Impact of microbial activity on the performance of planted and unplanted wetland at laboratory scale

Priyanka Jamwal* and Shahana Shirin

Centre for Environment and Development, Ashoka Trust for Research in Ecology and the Environment (ATREE), Jakkur 560064, Bangalore, India

*Corresponding author. E-mail: priyanka.jamwal@atree.org

Abstract

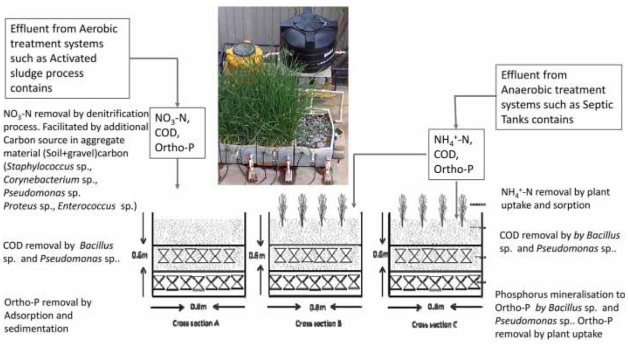
Three horizontal subsurface flow constructed wetland prototypes were set up to identify and understand the role of microflora in nutrient removal under diverse operating conditions. Out of three setups, one setup served as a control (without plants), and the rest were planted with *Typha domingensis*. The setups were operated at two different hydraulic loading rates (5 cm/day and 16 cm/day) for two months each. Among 27 bacteria species isolated, 80% of nitrate-reducing bacteria were observed in control, and 50–77% of nitrate-reducing bacteria were observed in the plant setups. Presence of diverse denitrifying bacteria and soil organic carbon contributed to high Nitrate-N removal in the control at both HLRs. Similar Ammonium-N (29%) and Ortho-P removal (30%) efficiency was observed at both HLRs in the control setup. Processes such as chemical sorption and adsorption dominated the Ammonium-N and Ortho-P removal in the control setup. High average Ammonium-N removal efficiency of 89% and 52% was observed in plant setups at 5 cm/day and 16 cm/day HLR. At low HLR, Ammonium-N removal in plant setups was dominated by nutrient uptake. In the plant setups, 35% and 15% Ortho-P removal efficiency was observed at low HLR (5 cm/day) and high HLR (16 cm/day) respectively. Hydraulic Retention Time (HRT) limited the uptake of ortho-P, thereby allowing mineralised phosphorus to escape the system without being absorbed by the plants.

Key words: HSSF-CW, hydraulic loading rates (HLR), microflora, *Typha domingensis*, wastewater treatment

Highlights

- At low HLR, Ammonium-N removal in CWs systems is dominated by plant uptake.
- Unplanted CW systems are efficient in removing Nitrate-N. Removal mechanism is dominated by the presence of diverse denitrifiers and organic carbon present in soil media.
- Ortho-P removal is dominated by physical processes in unplanted CW systems. In planted systems, Phosphorus mineralization leads to poor Ortho-P removal at high HLR.

Graphical Abstract



Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

INTRODUCTION

Wastewater treatment is necessary to address contamination of surface water bodies affected by the release of domestic and industrial effluents. Low cost and efficient wastewater treatment technology are crucial, especially in developing countries where resources to treat wastewater are limited (Iasur-Kruh *et al.* 2010).

The sewage generation estimated from class I and class II towns in India is 38,524 million litres per day (MLD), which accounts for 72% of the population. Out of the total wastewater generated, centralised wastewater treatment (CWWT) plants have capacity to treat 30% and the rest is discharged untreated into rivers, lakes and open storm water drains (Sharma & Sharma 2018). In Bengaluru city, 30% of the total sewage generated receives treatment. Many times, the effluents produced from CWWT systems fail to comply with the discharge standards set by the Central Pollution Control Board (CPCB). CWWT system rely on expensive equipment and require skilled personnel for operation and maintenance. Lack of underground drainage networks (UDGs) for sewage collection, skilled personnel to operate the treatment plants and frequent power cuts are listed as common reasons for the failure of CWWT systems to comply with the discharge standards (Jamwal *et al.* 2008, 2015). In addition, high operation and maintenance costs per year and high capital costs are the main barriers towards effective scaling up of these systems (Massoud *et al.* 2009; Capodaglio 2017).

Unlike CWWT plants, decentralized wastewater treatment systems (DWWTS) such as constructed wetlands (CWs) do not rely on a sophisticated operation and maintenance system and have a reasonably manageable drainage network. These systems use less energy and produce a limited amount of sludge compared to the CWWT systems (Singh *et al.* 2009). Effluent quality from CWs is less variable as the systems are resilient to shock loads and treated effluent can be reused for landscape irrigation, toilet flushing and so on (Philip *et al.* 2012). In addition to CWs, septic tanks and anaerobic digesters are some of the prevalent low-cost technologies deployed for decentralized wastewater treatment (Capodaglio 2017). Constructed wetlands are proven technologies that are efficient in treating industrial, municipal and agricultural wastewater with minimal energy inputs (Almuktar *et al.* 2018).

Nutrient uptake by wetland plants plays a significant role in reducing nutrients in the wastewater (Wu *et al.* 2019). Plants improve wastewater quality by absorbing nutrients such as phosphorus, nitrogen and other elements during their growth cycle (Almuktar *et al.* 2018). Depending on the species, inflow quality and nutrient loading rate, plants are capable of removing 3% to 47% of nitrogen and 3% to 60% of phosphorus from wastewater (Gottschall *et al.* 2007). *Typha* species are one of the emergent plants extensively used in the constructed wetlands. It has a dense root system that provides oxygen to the rhizosphere region, which helps in maintaining the contaminant removal efficiency of CWs (Shehzadi *et al.* 2014). Contrary to this, a few studies reported the microbial degradation process as a major contributor to nutrient removal in constructed wetlands (Iasur-Kruh *et al.* 2010; Gorgoglione & Torretta 2018; Saeed *et al.* 2019). Unplanted constructed wetlands have also shown comparable nutrient removal efficiencies. Biofilm growth on aggregate material and microbial degradation contribute to organic matter and nutrient removal in unplanted systems (Coleman *et al.* 2001). Microorganisms enhance the transformation and mineralization of the contaminants and contribute to the nutrient removal in wastewater (Wang *et al.* 2016a). Also, bacteria help in degradation and reduction of organic and inorganic compounds such as Ammonium-N and Nitrate-N by utilizing carbon and other inorganic species for their metabolic pathway (Llanos-Lizcano *et al.* 2019).

Knowledge regarding the type of microflora and their contribution to contaminant removal helps with designing efficient constructed wetland systems (Rajan *et al.* 2018). In addition, understanding the role of microorganisms and plants in constructed wetland and their response in diverse operational conditions is crucial to optimize the biological wastewater treatment (Lee *et al.* 2009). Here, we conducted laboratory experiments to identify the microflora and understand their

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

contribution to contaminant removal under diverse operational conditions in planted and unplanted constructed wetland systems.

METHODOLOGY

Constructed wetland setup

The laboratory-scale horizontal subsurface flow constructed wetland (HSSF-CW) prototypes were setup at Ashoka Trust for Research in Ecology and the Environment (ATREE), Bangalore. Each setup was constructed using three plastic rectangular open tanks of dimension 1 m × 0.6 m × 0.6 m (length × width × height). Out of three containers, one served as a control setup (without plants) and the other two served as experimental setups (planted). Each setup was filled with three layers of aggregate material (0.2 m height). The bottom layer comprised terracotta pieces, the second layer comprised a mixture of terracotta and soil and the third (topmost) layer comprised soil, respectively (Figure 1). Finally, to finish the look, gravel pieces were added above the inflow pipes of the three systems (Figure 2). The gravel aggregate material remained dry throughout the test period.

Terracotta is a lightweight porous material that provides high organic removal efficiency when used as a substrate in a constructed wetland for wastewater treatment (Jamwal *et al.* 2019). The soil used in

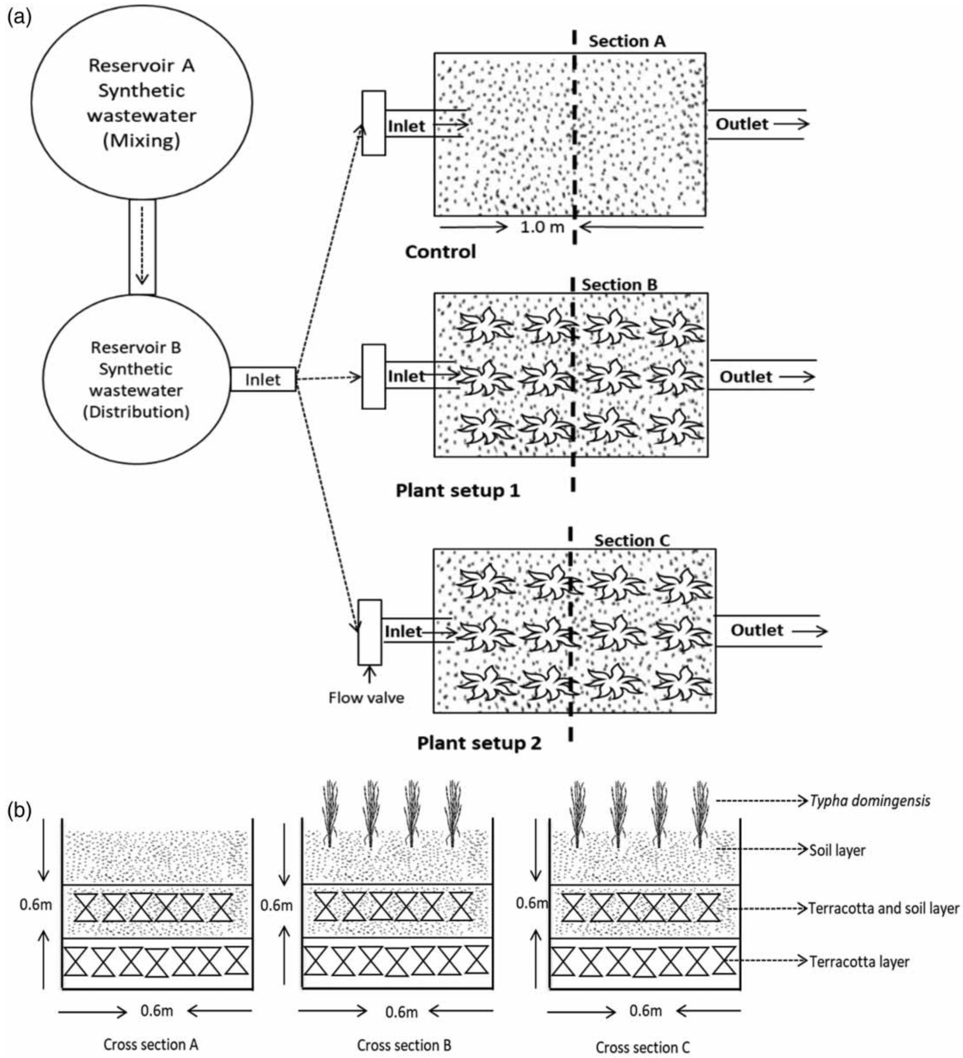


Figure 1 | (a) Flow diagram of the laboratory setups, (b) cross-section view of the setups.

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

Figure 2 | View of constructed wetland setups at ATREE.

CW setups was obtained from the Jakkur Lake, Bangalore, which is sandy clay loam soil with medium infiltration rate (Suma & Srinivasa 2015). The seeds of emergent plant *Typha domingensis* were washed thoroughly after soaking for 24 hours. The seeds were added at 3 cm depth of the wet soil of the two plant setups. The plants' density was maintained at 34 plants/m^2 (McMullen 2018).

Synthetic wastewater was fed to the setups through two overhead tanks. Reservoir A (300 L) was used for mixing and storing synthetic wastewater. Reservoir B (200 L) was used for distribution of synthetic wastewater to three setups. The synthetic wastewater was prepared in the laboratory using urea, regular granular sugar, NH_4Cl, K_2HPO_4, fertilizer (N, P, K, S, Mg, Cu, Fe and Mn) and partially treated sludge (Weerakoon *et al.* 2013). The partially treated sludge was obtained from the Sewage Treatment Plant in Jakkur, Bangalore, India.

Constructed wetlands/green infrastructure are generally used as a polishing/tertiary treatment system to remove nutrients and organic matter before the effluent is discharged into surface water bodies. The composition of synthetic wastewater mimics the secondary treated wastewater with COD levels <200 mg/l (theoretical) and Nitrate-N (mg/L) 40.4 ± 9.7, Ammonium-N (mg/L) 10.4 ± 3.1, Total nitrogen (mg/L) 55.1 ± 11.9, Ortho-P (mg/L) 7.6 ± 0.4. The well mixed synthetic wastewater was added daily to Reservoir A. The volume of wastewater added depends on the hydraulic loading rate (HLR). Table 1 presents the quantity of the compounds used for the preparation of

Table 1 | Composition of synthetic wastewater

Compounds	Quantity/100 L
Urea	2.4 g
Sugar	8.0 g
NH_4Cl	0.4 g
K_2HPO_4	0.004 g
Fertilizer	10.025 g
Partially treated sludge	260 ml

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

synthetic wastewater. The theoretical COD, Total Nitrogen and Total Phosphorus in synthetic wastewater were 158.5 mg of Oxygen/L, 25.6 mg/L and 19.0 mg/L respectively.

Operation and maintenance of setups

The nutrient removal efficiency of the synthetic wastewater was investigated at a high flow rate (100 L/day) corresponding to 16 cm/day hydraulic loading rate (HLR) and low flow rate (30 L/day) corresponding to 5 cm/day HLR. The system was operated for two months at each HLR. The HLR designs were based on the previous laboratory-based studies focused on understanding the pollutant removal efficiencies of constructed wetland systems (with/without plants) operated at different HLR. The HLR ranged from 2.5 cm/day to 30 cm/day (Weerakoon *et al.* 2013). This study is an extension to published work to assess the impact of aggregate material on pollutant removal efficiency at different HLRs (Jamwal Phillips & Karlsrud 2019; Jamwal Biswas & Phillips 2020). Each set of experiments (at constant HLR) was run for two months, after which they were harvested and a new set of experiments was launched at different HLR. The experiments were started with 5 cm/day of HLR. The plants matured at two months, after which they were harvested and the system was set to operate at 16 cm/day HLR. To keep the analysis simple, data from four months of operation is presented.

Synthetic wastewater was added to reservoir A, which is connected to reservoir B with a check valve installed on the feeder pipe (Figure 1). The check valve ensured delivery of constant and approximately equal flows into the three setups. The inflow was adjusted using a control valve mounted on the inlet pipe. Hydraulic retention time (HRT) for each setup was calculated considering the pore volume. The average porosity estimated for control, plant setup 1 and plant setup 2 were 12, 10 and 15% respectively. The pore volume for each setup was determined at the beginning of the study by pouring a known amount of water into the setup filled with aggregate material.

Table 2 presents the mass loading rates at two different flow rates for control, plant setup 1 and plant setup 2.

Table 2 | Mass loading rate, HLR and HRT of control and plant setups

					Average mass loading rate ($g/m^2/day$)				
Setup units	**Desired Inflow rate (L/day)**	**Actual average Inflow rate (L/day)**	**HLR (cm/day)**	**HRT (Hrs)**	**NO_3-N**	**NH_4-N**	**PO_4-P**	**Total COD**	**Overhead tank refilling frequency**
Control	30	33	5	27	2.20	0.57	0.42	4.83	Once in two days
Setup 1		34	6	41	2.30	0.59	0.43	5.05	
Setup 2		31	5	30	2.08	0.54	0.39	4.56	
Control	100	95	16	8	4.38	3.22	1.33	9.56	
Setup 1		98	16	6	4.50	3.31	1.36	9.83	Once a day
Setup 2		98	16	10	4.48	3.30	1.36	9.78	

Soil sample collection and microflora analysis

For plant setups, soil samples (20 g) were collected from the rhizosphere region of the soil layer (3.8 cm deep) and for the control setup, samples were collected from the soil layer. The samples were collected in a sterile bag from the four corners and centre of each setup. The samples from each setup were mixed thoroughly to create a composite soil sample. Serial dilution and plating on Nutrient agar (NA) and Reasoner's 2A agar (R_2A) was carried out for each composite soil sample (a mixture of 5 soil samples from each setup). NA is a general-purpose nutrient medium used for cultivation and growth of a broad range of non-fastidious bacteria. Nutrient agar has been successfully used to grow nitrifier bacteria such as *Nitrosomonas* sp. (Kramer 2016). Whereas R_2A media is used for isolation of slow growing aerobic and facultative heterotrophic bacteria. The low nutrient

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

in R_2A helps the growth of slow growing and stressed bacteria. Among the common conventional nutrient media, R_2A is best for cultivation of diverse strains in the soil sample including potentially new species (Hu *et al.* 2007; Pham & Kim 2016). The methodology used here is a general method deployed to find all the microorganisms present in soil.

10 g composite soil samples from each setup were dispensed into the buffer solution (0.85% NaCl solution). The diluted sample was kept on a shaker for 20–30 minutes to obtain a uniform suspension. Fivefold serial dilution was prepared for each soil sample. The samples were plated by pour plate in NA and spread plate in R_2A. The Petri plates were incubated at 28 °C for 24–48 hours. After the incubation, the colony counter was used for the enumeration of the bacteria. The dry weight was measured by weighing soil after drying it in a hot air oven overnight at 105 °C. The colony-forming units (CFU) were calculated using Equation (1) (Aneja 2007).

$$\text{CFU/g dry weight} = \frac{\text{Plate count} \times \text{Dilution factor}}{\text{Dry weight of soil} \times \text{Volume of sample plated}} \tag{1}$$

The distinct colonies from the agar plates were subcultured in NA and R_2A agar. Ten colonies from the control and eight colonies from plant setup 1 and nine colonies from plant setup 2 were subcultured. Morphological tests like gram staining were done to identify the morphology of the bacteria. Biochemical tests were carried out for the identification of isolated bacteria from the soil in the genus level. This includes catalase test, oxidase test, indole test, methyl red test, Voges-Proskauer test, citrate utilization test, Hydrogen sulfide production test, carbohydrate fermentation test, gelatin hydrolysis test, casein hydrolysis test, litmus test and nitrate reduction test (Aneja 2007).

Water sample collection and analysis

Water samples were collected at the inlet (I) and the outlets of the plant setup 1 (S1), plant setup 2 (S2) and control (C). The samples were collected in a sterile borosilicate glass and plastic bottles (LDPE, medical-grade USP Class VI autoclavable) and were tested for physical parameters; that is, temperature and conductivity (once in two days); chemical parameters; that is, pH (once in two days), dissolved oxygen (DO) (fortnightly once), organic matter (total COD) (two times a week), Ortho-Phosphates (Ortho-P) (once in two days), Nitrate-N (once in two days), Total Nitrogen (TN) (weekly once) and Ammonium-N (once in two days); biological parameter; that is, *E. coli* (Fortnightly once). All the parameters were tested following the methods described in APHA Standard methods for the examination of water and wastewater (APHA 2005). Conductivity, pH and temperature were measured using a YSI Pro 1030 sensor (YSI, Yellow springs the USA) and Nitrate-N was measured using a Hach Nitrate Pocket Colorimeter (Hach Company, Loveland, USA). Ammonium-N, TN, Ortho-P was analyzed photometrically (Merck KGaA, Darmstadt, Germany). Samples were tested for total Chemical Oxygen Demand (COD) by following the methods described in APHA standard methods. *E. coli* was detected and enumerated by Colilert 18 Quanti tray/2000 method (IDEXX Laboratories, Westbrook, USA).

The contaminant removal efficiency and Mass Loading Rate (MLR) of Nitrate-N, Ortho-P, Ammonium-N and total COD was estimated using Equations (2) and (3) respectively. Considering shorter HRTs, inflow was assumed to be equal to outflow, hence the MRR removal efficiency (Equation (5)) was similar to contaminant removal efficiency (Equation (2)).

$$\text{Removal efficiency (\%)} = \frac{C_i - C_o}{C_i} \times 100 \tag{2}$$

$$\text{Mass Loading Rate (g/m}^2\text{/day)} = C \times \text{HLR} \tag{3}$$

$$\text{Mass Removal Rate (MRR) (g/m}^2\text{/day)} = (MLR_i - MLR_o) \tag{4}$$

$$\text{Mass Removal Rate (\%)} = \frac{(MLR_i - MLR_o)}{MLR_i} \times 100 \tag{5}$$

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

where C_i is the concentration of contaminant in the influent and C_o is the concentration of contaminant in the effluent. MLR_i ($g/m^2/day$) is the mass loading rate at the inlet and MLR_o ($g/m^2/day$) is the mass loading rate at the outlet.

HLR is the hydraulic loading rate (cm/day).

Considering that, the mass loading rate is a function of flow rate and pore volume, different values of MLRs were obtained at 5 cm/day and 16 cm/day HLR. Also at similar HLR, slight variations in MLR were observed, which could be attributed to difference between the inflows and pore volume of the three setups (Table 2).

Statistical analysis

Descriptive statistics of effluent quality were run through IBM SPSS Statistics 23.0 (Armonk, NY: IBM Corp.). An independent sample t-test was used to test the significance of the difference between the (a) quality of influent and effluent of the control, plant setup 1 and plant setup 2, (b) removal efficiency between control and plant setups and (c) contaminant load reduction at 5 cm/day and 16 cm/ day HLR.

RESULTS AND DISCUSSION

Microflora identification

Similar number of colonies (5 log CFU/g) were observed in R_2A agar for both control and plant setup, whereas, in NA, a greater number of colonies (5 log CFU/g) were observed in the control compared to the plant setups (4 log CFU/g). This could be attributed to the presence of anaerobic bacteria in the control setup that thrived and grew inside the NA (pour plate method). In addition to aerobic and facultative bacteria, NA pour plates are known to support the growth of anaerobic bacteria. Few studies have reported significant increase in anaerobic bacterial communities in unplanted systems compared to constructed wetlands planted with *Typha angustifolia* (Gaballah *et al.* 2020). In the case of plant setups, radial oxygen leakage (ROL) leads to oxygenated conditions resulting in an environment dominated by aerobic and facultative bacteria. Typha species are known for the release of oxygen to the soil through the deep root system, thereby inhibiting the development of anaerobic bacteria (Samsó & García 2013).

Three *Staphylococcus* spp. and three *Pseudomonas* spp., one *Bacillus* sp., one *Corynebacterium* sp., one *Enterococcus* sp., and one *Proteus* sp. were isolated from the control. Three *Pseudomonas* spp., two *Bacillus* spp., one *Streptococcus* sp., one *Lactobacillus* sp., and one *Corynebacterium* sp. were isolated from setup 1. Four *Bacillus* spp., two *Staphylococcus* spp., one *Pseudomonas* sp., one *Lactobacillus* sp., and one *E. coli* were isolated from setup 2 (Appendix). These bacterial species are known to degrade organic matter and nutrients present in soil and groundwater. They tend to thrive in variable environmental conditions and helps with nutrient cycling. A study conducted by (Vaish *et al.* 2018), reported the presence of *Staphylococcus* spp. in high pH condition (up to 9.5) in garden soil, sewage, groundwater and the human gut. *Staphylococcus* spp. present in soil carries the NarG gene that is responsible for the conversion of Nitrate-N to nitrogen gas (Philippot *et al.* 2002). *Pseudomonas* spp. are abundantly found in the rhizosphere region and are known to carry out solubilization and mineralization of insoluble phosphorus (Rodríguez & Fraga 1999). Also due to their diverse metabolic activity, they are capable of carrying out bioremediation of organic pollutants (Kahlon 2016). *Bacillus* spp. play a significant role in degrading organic compounds in the constructed wetland (He *et al.* 2016). They promote release of ortho phosphorus in soil by producing organic acid and acid phosphatases (Saeid *et al.* 2018). *Corynebacterium* spp. are facultative anaerobic bacteria which are widespread in soil and water (Betts 2006). *Corynebacterium* spp. also contributes to the reduction of Nitrate-N to nitrogen gas in water and soil under anoxic conditions.

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

Proteus spp. are proteolytic bacteria and considered as a fecal indicator in wastewater. They have high metabolic activities and contribute to organic matter reduction in water and soil (Drzewiecka 2016). *Streptococcus* spp. are fecal indicator bacteria that persist longer in the environment and are found in soil, water, food and dairy products (Gerba 2015). *Lactobacillus* spp. (Lactic Acid Bacteria) are found in soil and help with removing odor in the sewage treatment process and also help with composting of organic wastes (Higa & Kinjo 1989). *Escherichia coli* thrive under extreme conditions and persist for an extended period in tropical, subtropical and temperate conditions. The naturally occurring *Escherichia coli* strain promotes plant growth and nutrient uptake in soil (Nautiyal *et al.* 2010).

Table 4 presents the bacterial species and percent diversity that contributed to the transformation of organic carbon, Ammonium-N, Nitrate-N and Ortho-P in experimental setups. As compared to planted setups, control setup exhibited the highest diversity of Nitrate-N reducing bacterial species (80%) and lowest diversity of organic carbon oxidising species (40%). This could be attributed to anaerobic conditions responsible for inhibiting the growth of aerobic bacteria in the control setup. None of the bacterial species identified contributed to removal/transformation of Ammonium-N in the control setup. Ammonium-N removal in plant setups is mainly dominated by plant uptake (Wu *et al.* 2019). Maximum percentage of Ortho-P transforming bacterial species were observed in plant setup 2 followed by plant setup 1 and the control setup. The lowest percentage of Ortho-P transforming bacterial species could be attributed to anaerobic conditions that inhibited the growth of *Bacillus* spp. and *Pseudomonas* spp. in the control setup.

Performance evaluation

The contaminant removal efficiency of the three setups was estimated using Equation (2). Table 5 presents the contaminant levels at the inlet and outlets and removal efficiency of control and plant setups. The theoretical COD estimated is greater than the actual COD measured in the influents. Given that synthetic wastewater stayed in the reservoirs, degradation of organic matter and nutrients within the storage tanks might have led to this difference (Table 2). Similar differences were reported in the studies conducted by other researchers (Weerakoon *et al.* 2013; Jamwal *et al.* 2019). For example, one of the studies reported BOD_5 levels in the inflows as 25 mg/l compared to the theoretical BOD_5 levels of 44.7 mg/l (Weerakoon *et al.* 2013).

Nutrient reduction and transformation

At both HLRs (5 cm/day and 16 cm/day), a significant reduction in the Ammonium-N levels was observed at the outlet of the control and the plant setups ($p < 0.05$). Physical processes such as volatilisation and sorption to the filter media are reported to contribute to Ammonium-N removal. Ammonium-N volatilisation occurs at high pH (>8). This might not have contributed as lower pH levels were observed in the all the setups (Table 5). Sorption to the filter media and soil could be an important factor contributing to the removal of Ammonium-N. In the control setup, similar removal efficiency was observed at both HLRs indicating dependence of Ammonium-N removal on the availability of sorption sites in the filter media rather than the HRT. Laboratory experiments conducted by various researchers have attributed 40%–60% Ammonium-N reduction to sorption on gravel media (Riley *et al.* 2005; Hedström 2006).

High average Ammonium-N removal efficiency of 89% and 52% was observed in plant setup as compared to control at 5 cm/day and 16 cm/day respectively (Figure 3). Both physical and biological processes contributed to Ammonium-N removal in plant setup. At low HLR, an additional 60% Ammonium-N removal could be attributed to plant uptake. Whereas at high HLR, the contribution of plants to Ammonium-N removal dropped to 20%, suggesting that retention time limits the

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

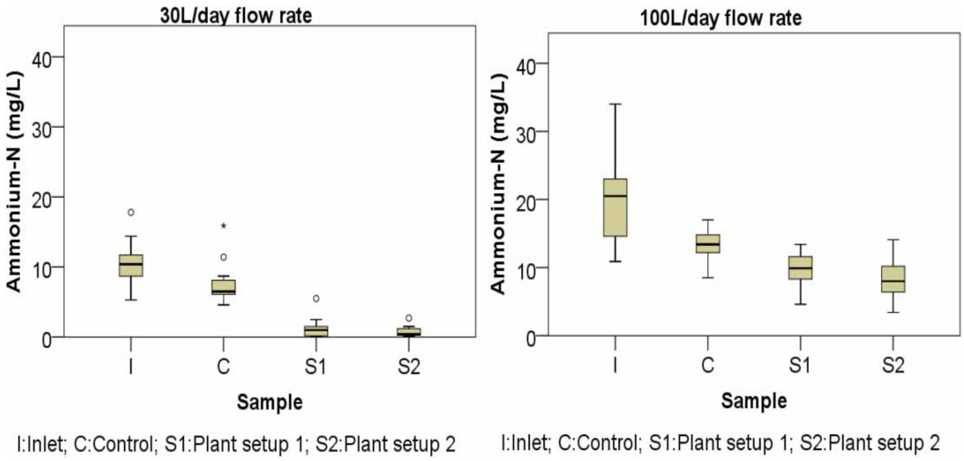


Figure 3 | Ammonium-N levels at the inlet and outlet of control and plant setups.

uptake of Ammonium-N by plants. Previous studies suggest that both nutrient uptake by plants and transformation by nitrifying bacterial species contribute to Ammonium-N removal in planted CW systems. Plants require less energy for absorption and uptake Ammonium-N than Nitrate-N and thus have been reported to contribute significantly to Ammonium-N removal (Wu *et al.* 2019). In a similar study, 83% Ammonium-N removal efficiency was observed in a constructed wetland planted with *Thalia geniculate* as compared to an unplanted constructed wetland where 59% reduction was observed (Llanos-Lizcano *et al.* 2019).

The nitrifying bacterial species are chemotrophic and grow when the organic matter in the wastewater drops significantly (Gajewska *et al.* 2020). The growth of nitrifying bacteria such as *Nitrospirae* is inhibited by (a) root exudates (organic carbon) released by the plants; (b) low concentration of Ammonium-N in the rhizosphere region; and (c) low oxygen levels within the treatment zone of the constructed wetland (Table 5). Given the presence of either of the three conditions, the growth of nitrifying bacteria was limited in the control as well as plant setups. Therefore, physical process and plant uptake dominated the Ammonium-N removal in control and plant setups respectively.

At both HLRs (5 cm/day and 16 cm/day), significant reduction in Nitrate-N was observed at the outlet of control and the plant setups ($p < 0.05$) (Figure 4). Unlike Ammonium-N removal efficiencies, no significant difference was observed in the Nitrate-N removal efficiency between control and plant setups ($p > 0.05$) (see Table 5). At 5 cm/day HLR, the Nitrate-N removal efficiency of control, plant

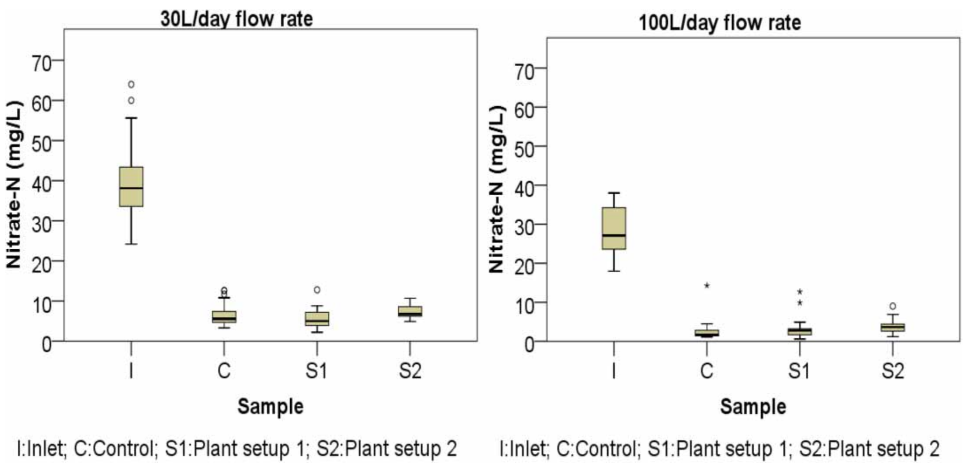


Figure 4 | Nitrate-N levels at the inlet and outlet of control and plant setups.

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

setup 1 and plant setup 2 were 82, 85 and 81% respectively. At 16 cm/day HLR, the Nitrate-N removal efficiency for control, plant setup 1 and plant setup 2 were 90, 87 and 85% respectively.

Presence of conditions favourable for the growth of denitrifying bacteria contributed to Nitrate-N removal in the control setup. Whereas radial oxygen leakage (ROL) from the roots of plants inhibited the growth of denitrifying bacteria and contributed to low bacterial diversity and denitrification rates in the plant setups (Table 3). In the control setup, 80% of the bacteria showed positive results for the Nitrate-N reduction test compared to 50 and 77% in plant setup 1 and plant setup 2 (see Table 4). Previous studies reported the effectiveness of denitrifying bacteria in removing nitrates in marsh ponds and unplanted wetlands (Dong & Reddy 2010; García *et al.* 2010). Absence of O_2, redox potential, temperature, pH, and moisture content are some of the environmental factors that influence denitrification rate (García *et al.* 2010).

Table 3 | Colony-forming units in control and plant setups under two media types

Sample	Media	CFU/g of soil
Control	NA	2.08×10^5
Setup 1	NA	1.90×10^4
Setup 2	NA	5.39×10^4
Control	R_2A	1.18×10^5
Setup 1	R_2A	1.97×10^5
Setup 2	R_2A	3.73×10^5

Table 4 | Bacterial species diversity and their role in removing contaminants from the wastewater

	Bacterial species putatively responsible for the removal of/transformation of			
Setup	Organic Carbon (% diversity)	Oxidation of Ammonium-N (% diversity)	Nitrate-N (% diversity)	Ortho-P (% diversity)
Control	• *Bacillus* sp. • *Pseudomonas* sp. (40%)	Limited microbial influence in control setup	• *Staphylococcus* sp. • *Corynebacterium* sp. • *Pseudomonas* sp. • *Proteus* sp. • *Enterococcus* sp. (80%)	• *Bacillus* sp. • *Pseudomonas* sp.(40%)
Plant setup-1	• *Bacillus* sp. • *Pseudomonas* sp. (62%)	Removal dominated by physical processes	• *Bacillus* sp. • *Pseudomonas* sp. • *Corynebacterium* sp. (50%)	• *Bacillus* sp. • *Pseudomonas* sp. (62%)
Plant setup-2	• *Bacillus* sp. • *Pseudomonas* sp. (66%)		• *Bacillus* sp. • *E. coli* • *Staphylococcus* sp. • *Pseudomonas* sp. (77%)	• *Bacillus* sp. • *Pseudomonas* sp. (66%)

Denitrification is the process by which Nitrate-N is converted to nitrogen gas. The facultative anaerobic bacteria carry out denitrification. They use Nitrate-N (and nitrite) as an electron acceptor under anoxic condition, utilise organic carbon, and convert it to nitrogen gas. Studies reported that a COD/TN ratio ~5 is optimal for effective removal of TN via the nitrification and denitrification process. A few studies reported 40–50% TN removal efficiency at low COD/TN ratio of 2 (Collison & Grismer

2013; Zhu *et al.* 2014; Wang *et al.* 2016b). An average COD/TN ratio observed in inflows was approximately two, which was further enhanced by the presence of organic carbon in the soil layer. This might have contributed to high Nitrate-N removal efficiency at 16 cm/day HLRs in all three setups.

Ortho-P reduction

Figure 5 presents the Ortho-P levels at the inlet and outlet of the control and plant setups. At both HLRs, significant reductions ($p < 0.05$) in the Ortho-P levels were observed at the outlet of the three setups. Similar removal efficiency (30%) was observed in the control at both HLRs, whereas lower removal efficiencies were observed at high HLR in the plant setups. In the plant setups, higher Ortho-P removal efficiency was observed (35%) compared to the control (30%) at low HLR (5 cm/day) ($p < 0.05$). Ortho-P removal in unplanted CW is dominated by chemical adsorption of soluble reactive phosphorus (SRP) to the soil/aggregate material (Yang *et al.* 2001). Whereas, in plant setups, in addition to chemical adsorption, higher retention times (5 cm/day) allowed for uptake of the reactive Ortho-P by plants, therefore contributing to high removal efficiencies in plant setups. Higher HRT allows the phosphorus to interact/react with the microorganisms, substrate and plants in a constructed wetland. Studies have reported a linear relationship between phosphorus removal efficiency and HRT in the planted CW (Quan *et al.* 2016).

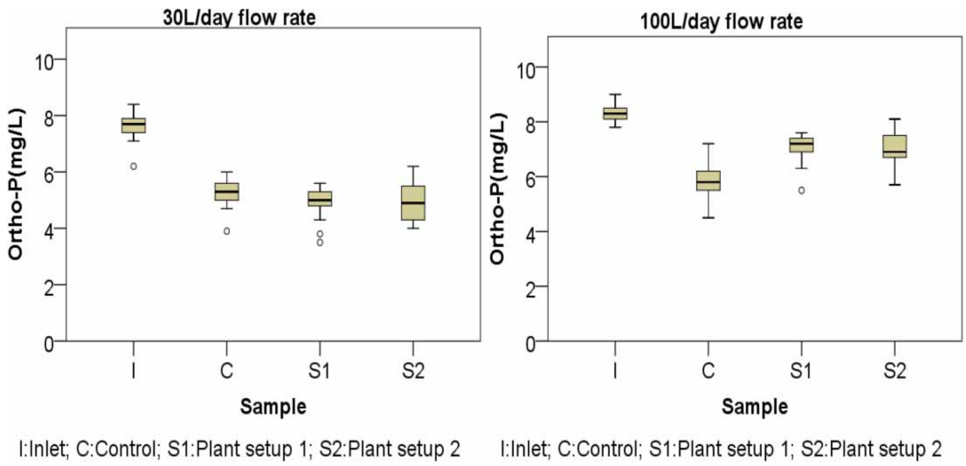


Figure 5 | Ortho-P levels at the inlet and outlet of control and plant setups.

At higher HLR (16 cm/day), greater Ortho-P removal efficiency was observed in the control unit (30%) as compared to plant setup 1 (15%) and plant setup 2 (16%) ($p < 0.05$). Chemical adsorption to aggregate material and soil particles contributed to the removal of Ortho-P in the control (Ghosh & Gopal 2010). Also, the low percentage of phosphate-solubilizing bacteria (40%) prevented phosphorus mineralisation, thereby controlling Ortho-P levels in the outlet of the control setup. Whereas, in plant setups, inorganic P-solubilizing bacteria (IPSB) like *Bacillus* spp. and *Pseudomonas* spp. solubilized the inorganic form of phosphorus to Ortho-P and made it available for plant uptake. Organic P-mineralizing bacteria (OPMB) such as *Bacillus cereus* and *Bacillus megaterium* are also known for making Ortho-P available to plants in agricultural soil. Both IPSB and OPMB encourage conversion of inorganic and organic phosphorus to Ortho-P (Cao *et al.* 2018). The inorganic phosphate compounds such as dicalcium phosphate, tricalcium phosphate, hydroxyapatite and rock phosphate present in the rhizosphere region of the soil are converted into Ortho-P (Rodríguez & Fraga 1999). Whilst high microbial diversity has contributed to Ortho-P levels, high flow rates and low HRT (16 cm/day) allowed Ortho-P to escape without being absorbed by the plants thereby contributing to lower Othro-P removal in plant setups.

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

Organic matter reduction

Figure 6 presents the total COD levels at the inlet and outlet of the control and plant setups. At both flow rates, a significant reduction in total COD levels was observed at the outlets of the control setup and plant setups ($p < 0.05$). The control setup provided 20% COD removal efficiency at both HLRs, whereas an average of 34% (5 cm/day) and 27% (16 cm/day) total COD removal efficiencies were observed for the plant setups (Table 5). No significant difference was observed in the total COD removal efficiencies between the plant setups and control setup ($p > 0.05$). Considering that COD is removed during the secondary treatment process, this study was designed to assess nutrient removal efficiencies at HRTs lower than the HRTs deployed for removal of organic carbon in CWs (4 days) (Table 2). Hence, low HRTs contributed to low COD removal efficiency in both the control and plant setups.

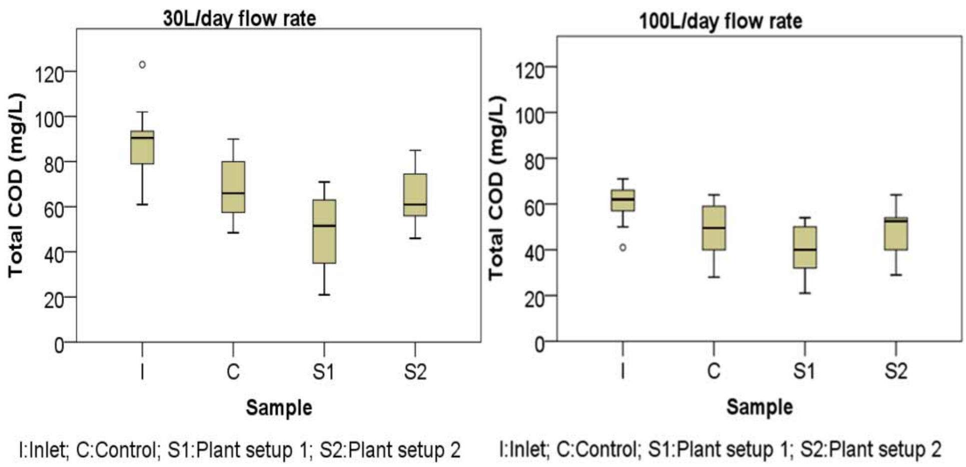


Figure 6 | Total COD levels at the inlet and outlet of control and plant setups.

We observed *Bacillus* spp. and *Pseudomonas* spp. in control setup (40%), plant setup 1 (62%) and plant setup 2 (66%). Relatively higher bacterial density contributed to higher but not statistically significant COD removal efficiency in the plant setups. Both *Bacillus* spp. and *Pseudomonas* spp. are known to remove COD brewery effluent efficiently. The study reported 82% COD removal by *Bacillus* spp. and up to 79% COD removal by *Pseudomonas* spp. (Oljira *et al.* 2018). Another study reported an overall COD reduction from 108 mg/l to 4 mg/L and 206 mg/L to 16 mg/L at 5 days HRT by *Pseudomonas* spp. and *Bacillus* spp. respectively (Adebayo *et al.* 2013). Mixed cultures of *Pseudomonas aeruginosa* and *Bacillus* spp. have also been used to remove COD in lipid-rich and pharmaceutical wastewater (Oljira *et al.* 2018).

Impact of hydraulic loading rate on the contaminant load reduction

We estimated contaminant load reduction efficiency using Equations (4) and (5). Figure 7 compares the contaminant load reduction at 5 cm/day and 16 cm/day of control and plant setups.

In the case of the control setup, either higher or similar contaminant removal efficiencies were observed at different HLRs. No significant difference was observed in Ammonium-N, Ortho-P and COD load reduction at both 5 cm/day and 16 cm/day, indicating that physical processes such as volatilisation, sorption and chemical adsorption to soil particles dominated contaminant removal and the same was not affected by HLR (Riley *et al.* 2005; Hedström 2006). Significantly higher; that is, 90% Nitrate-N load reduction was observed at 16 cm/day compared to 82% observed at 5 cm/day.

In the case of planted systems, except for Nitrate-N, contaminant removal efficiencies decreased at high HLR. Significantly higher Ammonium-N and Ortho-P load reductions were observed in plant

Table 5 | Concentrations and removal efficiencies of control and plant setups in different HLRs

Parameter	Inlet		Control		Control		Plant setup 1		Plant setup 1		Plant setup 2		Plant setup 2	
	HLR-5 cm/day Mean $\pm$ SD	HLR-16 cm/day Mean $\pm$ SD	HLR-5 cm/day Mean $\pm$ SD	HLR-16 cm/day Mean $\pm$ SD	HLR-5 cm/day % Removal	HLR-16 cm/day % Removal	HLR-5 cm/day Mean $\pm$ SD	HLR-16 cm/day Mean $\pm$ SD	HLR-5 cm/day % Removal	HLR-16 cm/day % Removal	HLR-5 cm/day Mean $\pm$ SD	HLR-16 cm/day Mean $\pm$ SD	HLR-5 cm/day % Removal	HLR-16 cm/day % Removal
pH ($n = 27$)	7.4 $\pm$ 0.2	7.31 $\pm$ 0.8	7.5 $\pm$ 0.2	7.4 $\pm$ 0.5	–	–	7.0 $\pm$ 0.2	7.4 $\pm$ 0.4	–	–	6.9 $\pm$ 0.1	6.9 $\pm$ 0.1	–	–
Conductivity (µS/cm) ($n = 27$)	1,517 $\pm$ 65	1,358 $\pm$ 35	1,500 $\pm$ 82	1,333 $\pm$ 34	–	–	1,549 $\pm$ 136	1,369 $\pm$ 56	–	–	1,552 $\pm$ 107	1,361 $\pm$ 53	–	–
Temperature (°C) ($n = 27$)	24.7 $\pm$ 1.8	28.8 $\pm$ 1.9	23.6 $\pm$ 1.5	28.0 $\pm$ 1.9	–	–	24.5 $\pm$ 1.9	28.2 $\pm$ 2.2	–	–	23.9 $\pm$ 1.8	28.2 $\pm$ 2.0	–	–
Dissolved Oxygen (mg/L) ($n = 4$)	0.2 $\pm$ 0.2	0.7 $\pm$ 0.9	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	–	–	0.01 $\pm$ 0.1	0.05 $\pm$ 0.05	–	–	0.09 $\pm$ 0.14	0.06 $\pm$ 0.06	–	–
Nitrate-N (mg/L) ($n = 26$)	40.4 $\pm$ 9.7	27.5 $\pm$ 6.2	6.6 $\pm$ 2.8	2.7 $\pm$ 3.0	82	90	5.7 $\pm$ 2.5	3.3 $\pm$ 3.1	85	87	7.2 $\pm$ 1.6	3.9 $\pm$ 1.8	81	85
Ammonium-N (mg/L) ($n = 21$)	10.4 $\pm$ 3.1	20.3 $\pm$ 6.3	7.2 $\pm$ 2.5	13.1 $\pm$ 2.1	28	31	1.2 $\pm$ 1.3	9.6 $\pm$ 2.6	87	49	0.8 $\pm$ 0.7	8.3 $\pm$ 2.9	92	55
Total Nitrogen (mg/L) ($n = 8$)	55.1 $\pm$ 11.9	43.9$\pm$ 7.7	25.1 $\pm$ 6.6	17.2 $\pm$ 4.0	52	60	15.0 $\pm$ 6.9	14.9 $\pm$ 6.1	71	65	15.1 $\pm$ 6.4	15.6 $\pm$ 6.6	71	63
Ortho-P (mg/L) ($n = 29$)	7.6 $\pm$ 0.4	8.3 $\pm$ 0.3	5.3 $\pm$ 0.5	5.81 $\pm$ 0.8	30	30	5.0 $\pm$ 0.5	7.1 $\pm$ 0.5	35	15	5.0 $\pm$ 0.6	7.0 $\pm$ 0.6	35	16
Total COD (mg/L) ($n = 14$)	88.6 $\pm$ 15.9	60.1 $\pm$ 8.3	69.0 $\pm$ 14.1	48.3 $\pm$ 10.6	20	20	48.8 $\pm$ 17.4	39.7 $\pm$ 10.2	43	34	64.5 $\pm$ 12.9	48.4 $\pm$ 11.0	25	20
E. coli (MPN/100 mL) ($n = 6$)	$7.0 \times 10^2 \pm 7.0 \times 10^2$	$8.1 \times 10^3 \pm 6.4 \times 10^3$	$3.8 \times 10^2 \pm 2.9 \times 10^2$	$2.9 \times 10^3 \pm 3.5 \times 10^3$	–	–	$1.8 \times 10^1 \pm 1.2 \times 10^1$	$8.9 \times 10^2 \pm 6.4 \times 10^2$	–	–	$0.8 \times 10^1 \pm 0.6 \times 10^1$	$8.4 \times 10^2 \pm 4.2 \times 10^2$	–	–
E. coli log reduction ($n = 6$)	–	–	0.22 $\pm$ 0.53	0.47 $\pm$ 0.35	–	–	1.62 $\pm$ 0.67	0.89 $\pm$ 0.38	–	–	1.90 $\pm$ 0.55	0.77 $\pm$ 0.65	–	–

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

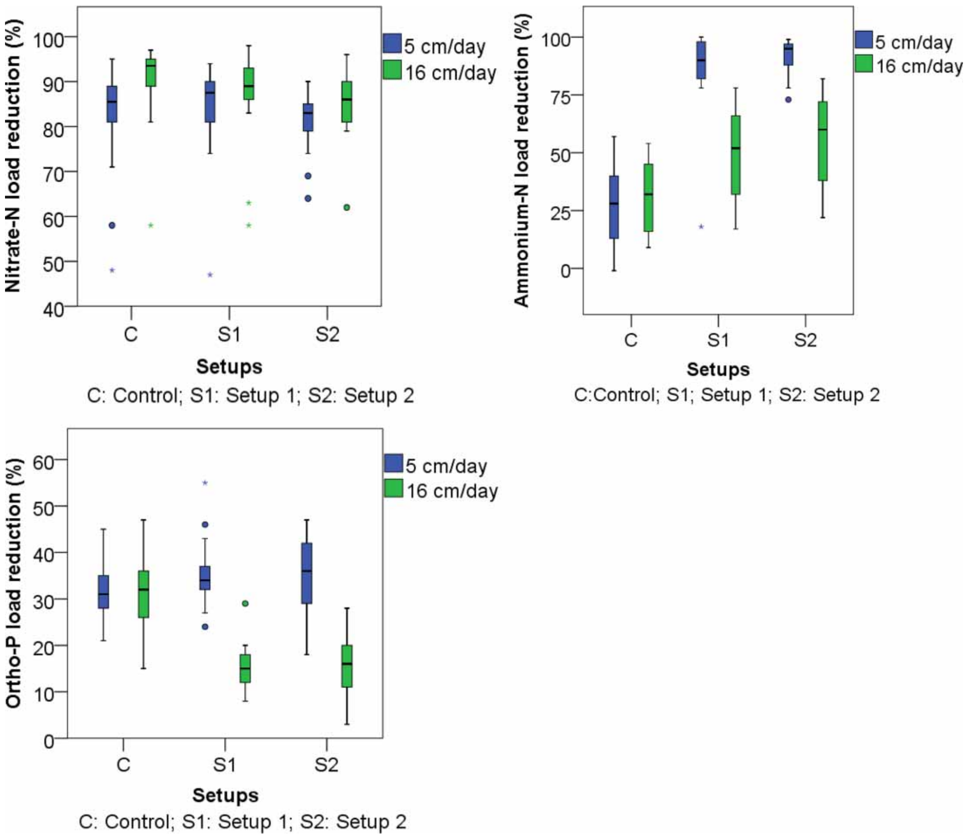


Figure 7 | Contaminant load reduction in control and plant setups at different HLRs.

setups at low HLR (5 cm/day) ($p < 0.05$). This could be attributed to the longer average HRT at low HLRs. Studies suggests that high HRTs encourage nutrient uptake and contribute to higher removal efficiencies in planted systems. Several studies have reported increased Ammonium-N, Phosphorus and organic matter removal efficiency at low HLRs (Çakir *et al.* 2015; Mesquita *et al.* 2018). Dan *et al.* (2011) reported 1.1% to 8.2% of Nitrogen mass removal and 0.6% to 2.2% of Phosphorus mass removal by plant uptake, with highest removal observed at 8 cm/day HLR. An average of 50% decline in the Ortho-P and Ammonium-N load reduction at 16 cm/day was observed in the plant setups. Also, as compared to conventional septic tank systems (anaerobic process), significant decrease in Ammonium-N levels was observed in both planted and unplanted systems. Studies have reported significant increase in Ammonium-N levels in the effluent from septic tanks (Nasr & Mikhaeil 2013).

Both unplanted and planted systems showed increased or similar Nitrate-N removal efficiency at high HLR (16 cm/day) respectively. Exposure to high organic loads (16 cm/day) promoted the growth of microbes, leading to the formation of thick biofilms and anoxic sites, which favours denitrification activity (Dalahmeh *et al.* 2014), thereby contributing to increased denitrification rates and Nitrate-N removal (Shen *et al.* 2015). In addition to this, the organic carbon present in the soil contributed to a higher C/N ratio that might have promoted the growth of denitrifying bacteria. Hence, both the increased loading rate and organic carbon in soil might have contributed to the high Nitrate-N load reduction at high HLR in the control setup.

CONCLUSION

This study the demonstrated the suitability of the unplanted/control setup for enhancing the growth of anaerobic and facultative bacteria. In addition, the ability of anaerobic and facultative bacterial

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

colonies to grow on NA (pour plate method) indicates its suitability for identifying and quantifying microflora in soils and other media.

Both control and plant setups exhibited similar Nitrate-N removal efficiency at both HLRs. Control setup provided suitable conditions for the growth of denitrifying bacteria. Eighty percent of the bacterial species identified in the control setup contributed to the denitrification process. In the case of planted setups, additional nutrient uptake by plants might have contributed to Nitrate-N removal at high HLR. Both the soil organic carbon and thick anoxic biofilms within the systems significantly contributed to Nitrate-N removal.

Both control and plant setups showed an absence of nitrifying bacteria. Low oxygen and high organic carbon inhibited the growth of nitrifying bacteria in the control and plant setups. Physical processes dominated removal of Ammonium-N, Ortho-P and COD in the control setup. Similar removal efficiency was observed at both HLRs in the control setup, indicating the presence of sorption sites rather than HLRs as a limiting factor for contaminant removal. At low HLR, biological processes (uptake by plants) dominated the removal of Ammonium-N in the plant setup. Ammonium-N removal efficiency decreased with increase in HLR.

Presence of diverse phosphate solubilizing bacterial species (66%–68%) in the plant setup contributed to high Ortho-P removal at low HLRs. The uptake of Ortho-P by plants is limited by the HRT; as a result, lower Ortho-P removal efficiencies (50% less) were observed at lower HRTs (high HLR).

Higher *Bacillus* spp. and *Pseudomonas* spp. levels (diversity and density) (62%–66%) contributed to higher COD removal in the plant setups as compared to the control setup. Whilst HLR did not have any effect on the COD removal efficiency in the control setup (dominated by physical process), COD removal efficiency decreased at high HLR in the plant setups.

This study demonstrated that unplanted constructed wetlands are suitable to treat effluents from secondary wastewater treatment systems that are based on the aerobic process such as the activated sludge process. The secondary treated effluent from such systems is low in COD and has high Nitrate-N and Ortho-P levels that can be easily treated by unplanted CW provided an additional carbon source is made available in the filter media. Whereas, planted wetlands are suitable to treat effluents from treatment systems based on anaerobic processes such as septic tanks. The effluent from such systems are low COD and have high Ammonium-N and Ortho-P levels that can be easily treated with planted CW systems.

ACKNOWLEDGEMENTS

This research was supported by a research grant from the Royal Norwegian Embassy (RNE) and the Ashoka Trust for Research in Ecology and the Environment (ATREE). The authors would like to thank Pavan Muttepawar (Trainee) and Sumita Bhattacharyya (Doctorate student) at ATREE for helping us with the analysis of water samples and maintenance and upkeep of the experimental setup.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Adebayo, G. B., Jimoh, A. A., Odebunmi, E. O. & Oke, M. A. 2013 Kinetics study of bioremediation of industrial effluents by *Pseudomonas* spp and *Bacillus* spp using chemical oxygen demand (COD). *Research Journal of Agriculture and Environmental Management* **2**(9), 261–269.

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

Almuktar, S. A. A. N., Abed, S. N. & Scholz, M. 2018 Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. *Environmental Science and Pollution Research* **25**(24), 23595–23623.

Aneja, K. R. 2007 *Experiments In Microbiology, Plant Pathology And Biotechnology*. New Age International Publishers, Daryaganj, New Delhi, India.

APHA 2005 *Standard Methods for Examination of Water and Wastewater*, 21st edn. American Public Health Association, Washington, DC.

Betts, G. 2006 23 - Other spoilage bacteria. In: *Food Spoilage Microorganisms* (C. de W. Blackburn, ed.). Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp. 668–693. Available from: http://www.sciencedirect.com/science/article/pii/B9781855739666500237 (Accessed May 4, 2020).

Çakir, R., Gidirislioglu, A. & Çebi, U. 2015 A study on the effects of different hydraulic loading rates (HLR) on pollutant removal efficiency of subsurface horizontal-flow constructed wetlands used for treatment of domestic wastewaters. *Journal of Environmental Management* **164**, 121–128.

Cao, Y., Fu, D., Liu, T., Guo, G. & Hu, Z. 2018 Phosphorus solubilizing and releasing bacteria screening from the rhizosphere in a natural wetland. *Water* **10**(2), 195.

Capodaglio, A. 2017 Integrated, decentralized wastewater management for resource recovery in rural and peri-urban areas. *Resources* **6**(2), 22.

Coleman, J., Hench, K., Garbutt, K., Sexstone, A., Bissonnette, G. & Skousen, J. 2001 Treatment of domestic wastewater by three plant species in constructed wetlands. *Water, Air, and Soil Pollution* **128**(3/4), 283–295.

Collison, R. S. & Grismer, M. E. 2013 Nitrogen and COD removal from domestic and synthetic wastewater in subsurface-flow constructed wetlands. *Water Environment Research* **85**(9), 855–862.

Dalahmeh, S. S., Pell, M., Hylander, L. D., Lalander, C., Vinnerås, B. & Jönsson, H. 2014 Effects of changing hydraulic and organic loading rates on pollutant reduction in bark, charcoal and sand filters treating greywater. *Journal of Environmental Management* **132**, 338–345.

Dan, T. H., Quang, L. N., Chiem, N. H. & Brix, H. 2011 Treatment of high-strength wastewater in tropical constructed wetlands planted with *Sesbania sesban*: horizontal subsurface flow versus vertical downflow. *Ecological Engineering* **37**(5), 711–720.

Dong, X. & Reddy, G. B. 2010 Soil bacterial communities in constructed wetlands treated with swine wastewater using PCR-DGGE technique. *Bioresource Technology* **101**(4), 1175–1182.

Drzewiecka, D. 2016 Significance and roles of *proteus* spp. bacteria in natural environments. *Microbial Ecology* **72**(4), 741–758.

Gaballah, M. S., Abdelwahab, O., Barakat, K. M. & Aboagye, D. 2020 A novel horizontal subsurface flow constructed wetland planted with *Typha angustifolia* for treatment of polluted water. *Environmental Science and Pollution Research* **27**(22), 28449–28462.

Gajewska, M., Skrzypiec, K., Jóźwiakowski, K., Mucha, Z., Wójcik, W., Karczmarczyk, A. & Bugajski, P. 2020 Kinetics of pollutants removal in vertical and horizontal flow constructed wetlands in temperate climate. *Science of The Total Environment* **718**, 137371.

García, J., Rousseau, D. P. L., Morató, J., Lesage, E., Matamoros, V. & Bayona, J. M. 2010 Contaminant removal processes in subsurface-flow constructed wetlands: a review. *Critical Reviews in Environmental Science and Technology* **40**(7), 561–661.

Gerba, C. P. 2015 Indicator Microorganisms. In: *Environmental Microbiology*. Elsevier, New York, NY, pp. 551–564. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780123946263000235 (Accessed May 4, 2020).

Ghosh, D. & Gopal, B. 2010 Effect of hydraulic retention time on the treatment of secondary effluent in a subsurface flow constructed wetland. *Ecological Engineering* **36**(8), 1044–1051.

Gorgoglione, A. & Torretta, V. 2018 Sustainable management and successful application of constructed wetlands: a critical review. *Sustainability* **10**(11), 3910.

Gottschall, N., Boutin, C., Crolla, A., Kinsley, C. & Champagne, P. 2007 The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater, Ontario, Canada. *Ecological Engineering* **29**(2), 154–163.

He, T., Guan, W., Luan, Z. & Xie, S. 2016 Spatiotemporal variation of bacterial and archaeal communities in a pilot-scale constructed wetland for surface water treatment. *Applied Microbiology and Biotechnology* **100**(3), 1479–1488.

Hedström, A. 2006 *Reactive Filter Materials for Ammonium and Phosphorus Sorption in Small Scale Wastewater Treatment*. Luleå University of Technology, Luleå, Sweden.

Higa, T. & Kinjo, S. 1989 *Effect of Lactic Acid Fermentation Bacteria on Plant Growth and Soil Humus Formation*. University of The Ryukus, Okinawa, Japan, p. 6.

Hu, Y.-S., Li, C.-X., Sun, F.-L., Wu, K. & Jia, X.-C. 2007 Improved culturability of soil bacteria using proper combination with various culturing medium. *Wei Sheng Wu Xue Bao (Acta Microbiologica Sinica)* **47**(5), 882–887.

Iasur-Kruh, L., Hadar, Y., Milstein, D., Gasith, A. & Minz, D. 2010 Microbial population and activity in wetland microcosms constructed for improving treated municipal wastewater. *Microbial Ecology* **59**(4), 700–709.

Jamwal, P., Mittal, A. K. & Mouchel, J.-M. 2008 Effects of urbanisation on the quality of the urban runoff for Delhi watershed. *Urban Water Journal* **5**(3), 247–257.

Jamwal, P., Zuhail, T., Urs, P. R., Srinivasan, V. & Lele, S. 2015 Contribution of sewage treatment to pollution abatement of urban streams. *Current Science* **108**(4), 9.

Jamwal, P., Phillips, D. & Karlsrud, K. 2019 Assessing local materials for the treatment of wastewater in open drains. *Water Science and Technology* **79**(5), 895–904.

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

Jamwal, P., Biswas, D. & Phillips, D. 2020 Provisional green infrastructure: trans-disciplinary approaches to address contamination in urban streams. *Water Science and Technology* **82**(11), 2209–2219.

Kahlon, R. S. 2016 Biodegradation and Bioremediation of Organic Chemical Pollutants by *Pseudomonas*. In: *Pseudomonas: Molecular and Applied Biology* (R. S. Kahlon, ed.). Springer International Publishing, Cham, Switzerland, pp. 343–417. Available from: http://link.springer.com/10.1007/978-3-319-31198-2_9 (Accessed May 4, 2020).

Kramer, S. 2016 *Nitrosomonas eutropha*: a study of the effects of nitrosomonas on pathogenic bacterium and the effects of current hygiene habits on the colonization of nitrosomonas within our normal flora. *JCCC Honors Journal* **7**(1), 3.

Lee, C., Fletcher, T. D. & Sun, G. 2009 Nitrogen removal in constructed wetland systems. *Engineering in Life Sciences* **9**(1), 11–22.

Llanos-Lizcano, A., Barraza, E., Narvaez, A., Varela, L. & Caselles-Osorio, A. 2019 Efficiency of pilot-scale horizontal subsurface flow constructed wetlands and microbial community composition operating under tropical conditions. *International Journal of Phytoremediation* **21**(1), 34–42.

Massoud, M. A., Tarhini, A. & Nasr, J. A. 2009 Decentralized approaches to wastewater treatment and management: applicability in developing countries. *Journal of Environmental Management* **90**(1), 652–659.

Mcmullen 2018 *How to Grow Cattails From Seeds | Home Guides | SF Gate*. Available from: https://homeguides.sfgate.com/grow-cattails-seeds-70962.html (Accessed April 17, 2020).

Mesquita, C., Albuquerque, A., Amaral, L. & Nogueira, R. 2018 Effectiveness and temporal variation of a full-scale horizontal constructed wetland in reducing nitrogen and phosphorus from domestic wastewater. *ChemEngineering* **2**(1), 3.

Nasr, F. A. & Mikhaeil, B. 2013 Treatment of domestic wastewater using conventional and baffled septic tanks. *Environmental Technology* **34**(16), 2337–2343.

Nautiyal, C. S., Rehman, A. & Chauhan, P. S. 2010 Environmental *Escherichia coli* occur as natural plant growth-promoting soil bacterium. *Archives of Microbiology* **192**(3), 185–193.

Oljira, T., Muleta, D. & Jida, M. 2018 Potential applications of some indigenous bacteria isolated from polluted areas in the treatment of brewery effluents. *Biotechnology Research International* **2018**, 1–13.

Pham, V. & Kim, J. 2016 Improvement for isolation of soil bacteria by using common culture media. *Journal of Pure and Applied Microbiology* **10**(1), 49–59.

Philip, L., Murty, B. S. & Sundaramoorthy, S. 2012 *Guidelines for Decentralized Wastewater Management*. MoUD Centre of Excellence in DWWM, Department of Civil Engineering, Indian Institute of Technology, Madras – Chennai.

Philippot, L., Piutti, S., Martin-Laurent, F., Hallet, S. & Germon, J. C. 2002 Molecular analysis of the nitrate-reducing community from unplanted and maize-planted soils. *Applied and Environmental Microbiology* **68**(12), 6121–6128.

Quan, Q., Shen, B., Zhang, Q. & Ashraf, M. A. 2016 Research on phosphorus removal in artificial wetlands by plants and their photosynthesis. *Brazilian Archives of Biology and Technology* **59**(spe). Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-89132016000200701&lng=en&tlng=en (Accessed April 17, 2020).

Rajan, R. J., Sudarsan, J. S., Nithiyanantham, S., Rajan, R. J., Sudarsan, J. S. & Nithiyanantham, S. 2018 Microbial population dynamics in constructed wetlands: review of recent advancements for wastewater treatment. *Environmental Engineering Research* **24**(2), 181–190.

Riley, K. A., Stein, O. R. & Hook, P. B. 2005 Ammonium removal in constructed wetland microcosms as influenced by season and organic carbon load. *Journal of Environmental Science and Health, Part A* **40**(6–7), 1109–1121.

Rodríguez, H. & Fraga, R. 1999 Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* **17**(4–5), 319–339.

Saeed, T., Haque, I. & Khan, T. 2019 Organic matter and nutrients removal in hybrid constructed wetlands: influence of saturation. *Chemical Engineering Journal* **371**, 154–165.

Saeid, A., Prochownik, E. & Dobrowolska-Iwanek, J. 2018 Phosphorus solubilization by bacillus species. *Molecules* **23**(11), 2897.

Samsó, R. & García, J. 2013 Bacteria distribution and dynamics in constructed wetlands based on modelling results. *Science of The Total Environment* **461–462**, 430–440.

Sharma, C. & Sharma, S. 2018 *Centralized Versus Decentralized Wastewater Treatment and Reuse: A Feasibility Study for NITTTR Campus, Chandigarh*.

Shehzadi, M., Afzal, M., Khan, M. U., Islam, E., Mobin, A., Anwar, S. & Khan, Q. M. 2014 Enhanced degradation of textile effluent in constructed wetland system using Typha domingensis and textile effluent-degrading endophytic bacteria. *Water Research* **58**, 152–159.

Shen, Z., Zhou, Y., Liu, J., Xiao, Y., Cao, R. & Wu, F. 2015 Enhanced removal of nitrate using starch/PCL blends as solid carbon source in a constructed wetland. *Bioresource Technology* **175**, 239–244.

Singh, S., Haberl, R., Moog, O., Shrestha, R. R., Shrestha, P. & Shrestha, R. 2009 Performance of an anaerobic baffled reactor and hybrid constructed wetland treating high-strength wastewater in Nepal – a model for DEWATS. *Ecological Engineering* **35**(5), 654–660.

Suma, B. N. & Srinivasa, C. V. 2015 Location identification for water conservation and quality assurance. *Aquatic Procedia* **4**, 1134–1141.

Vaish, M., Price-Whelan, A., Reyes-Robles, T., Liu, J., Jereen, A., Christie, S., Alonzo, F., Benson, M. A., Torres, V. J. & Krulwich, T. A. 2018 Roles of *staphylococcus aureus* Mnh1 and Mnh2 antiporters in salt tolerance, alkali tolerance, and pathogenesis. *Journal of Bacteriology* **200**(5). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5809693/ (Accessed May 4, 2020).

Water Practice & Technology Vol 16 No 2
doi: 10.2166/wpt.2021.017

Wang, Q., Xie, H., Ngo, H. H., Guo, W., Zhang, J., Liu, C., Liang, S., Hu, Z., Yang, Z. & Zhao, C. 2016a Microbial abundance and community in subsurface flow constructed wetland microcosms: role of plant presence. *Environmental Science and Pollution Research* **23**(5), 4036–4045.

Wang, W., Ding, Y., Ullman, J. L., Ambrose, R. F., Wang, Y., Song, X. & Zhao, Z. 2016b Nitrogen removal performance in planted and unplanted horizontal subsurface flow constructed wetlands treating different influent COD/N ratios. *Environmental Science and Pollution Research* **23**(9), 9012–9018.

Weerakoon, G., Jinadasa, K., Herath, G. B. B., Mowjood, M. I. M. & Van Bruggen, J. J. A. 2013 Impact of the hydraulic loading rate on pollutants removal in tropical horizontal subsurface flow constructed wetlands. *Ecological Engineering* **61**, 154–160.

Wu, Y., He, T., Chen, C., Fang, X., Wei, D., Yang, J., Zhang, R. & Han, R. 2019 Impacting microbial communities and absorbing pollutants by *canna indica* and *cyperus alternifolius* in a full-scale constructed wetland system. *International Journal of Environmental Research and Public Health* **16**(5), 802.

Yang, L., Chang, H.-T. & Huang, M.-N. L. 2001 Nutrient removal in gravel- and soil-based wetland microcosms with and without vegetation. *Ecological Engineering* **18**(1), 91–105.

Zhu, H., Yan, B., Xu, Y., Guan, J. & Liu, S. 2014 Removal of nitrogen and COD in horizontal subsurface flow constructed wetlands under different influent C/N ratios. *Ecological Engineering* **63**, 58–63.

First received 27 July 2020; accepted in revised form 7 February 2021. Available online 22 February 2021