White hard clam *(Meretrix lyrata)* shells media to improve phosphorus removal in lab-scale horizontal sub-surface flow constructed wetlands: performance, removal pathways, and lifespan

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Abstract

This work examined the phosphorus (P) removal from the synthetic pretreated swine wastewater using lab-scale horizontal sub-surface flow constructed wetlands (HSSF-CWs). White hard clam *(Meretrix lyrata*) shells (WHC) and *Paspalum atratum* were utilized as substrate and plant, respectively. The focus was placed on treatment performance, removal mechanisms and lifespan of the HSSF-CWs. Results indicated that WHC-based HSSF-CW with *P. atratum* exhibited a high P removal (89.9%). The mean P efluent concentration and P removal rate were 1.34±0.95 mg/L and 0.32±0.03 g/m^2/d, respectively. The mass balance study showed that media sorption was the dominant P removal pathway (77.5%), followed by microbial assimilation (14.5%), plant uptake (5.4%), and other processes (2.6%). It was estimated the WHC-based bed could work effectively for approximately 2.84 years. This

WHC-based HSSF-CWs technology will therefore pave the way for recycling Ca-rich waste materials as media in HSSF-CWs to enhance P-rich wastewater purification.

Keywords: Anaerobically digested swine wastewater; Ca-rich materials; Lifespan; Phosphorus removal efficiency; Removal pathways.

1. Introduction

In Vietnam, swine wastewater, characterized by high levels of organic matters and nutrients, is widely treated with anaerobic digestion lagoons (Nguyen, 2019). Since the quality of the effluent barely meets the requirements of QCVN40:2011/BTNMT, additional treatment is essential. Constructed wetlands (CWs) are known to have many advantages, such as being affordable, energy saving, simple, eco-friendly, effective (e.g. organic matters and nitrogen (N)), and suitable for small rural communities (Almuktar et al., 2018; Wu et al., 2015). However, one of the challenges concerning the widespread application of CWs is the poor and unstable phosphorus (P) removal capacity, this being 32.0-78.4% (Vohla et al., 2011; Žibienė et al., 2015). Therefore, enhancing the P treatment performance of CWs and developing a cost-effective and green method for decontamination of P in the anaerobically digested swine wastewater is critical to prevent receiving water bodies from eutrophication.

It is well recognized that in CWs, P can be removed via main pathways, such as media sorption, plant uptake and microbe assimilation (Wu et al., 2015). According to Luo et al. (2017) and Nguyen et al. (2014), polyphosphate accumulating organisms (PAOs) can retain P from wastewater. However, PAOs' P retention is limited to the temporary removal because microbial P is released back into wastewater when microbes die and decompose. The plant uptake and regular harvest can result in permanent P removal. However, the plant uptake normally contributes a low to medium percentage to total P removal of CWs, the percentages being: 4.72% for *Cyperus alternifolius* (Chan et al., 2008); 18.66% for *Canna australis* (Quan et al., 2016); and 22.5-59.6% for *Myriophyllum aquaticum* (Luo et al., 2017). Evidently,

substrate sorption plays a more important role than biological uptake (microbe assimilation and plant uptake) in removing P by the media-based CWs.

It is reported that P treatment performance of CWs can be improved by employing adsorptive/reactive media (Almuktar et al., 2018; Ballantine and Tanner, 2010). According to Žibienė et al. (2015), P removal in HSSF-CWs rose by 21% as a result of using dolomite, $CaMg(CO_3)_2$ instead of sand as the substrate. So far, great efforts have been made for developing P adsorbents. However, good P adsorbents are not always potential substrates because of their drawbacks when applied in the CWs, such as high cost (e.g. LWA, Filtralite P^{TM}), undesirable side-effects (e.g. red mud, steel slag) (Park et al., 2016). Therefore, selecting the proper media plays a crucial role in enhancing the CWs' P treatment performance. There was a strong correlation between Ca content and P retention of the media (Yang et al., 2018; Žibienė et al., 2015). Since bivalve shells contain large amounts of CaO (51-54.7%), they have potential for P sequestration (Nguyen et al., 2019). Many studies, examining bivalve shells as P adsorbents, demonstrate they can effectively retain P, such as cockle shell (Kim et al., 2018) and scallop shell (Yeom et al., 2009).

White hard clam (*Meretrix lyrata*) is a bivalve shell and it is widely cultivated in the coastal provinces of Vietnam. It is estimated that in the year 2020, the area and output of clam farming in Vietnam are 32,960 ha and 430,700 tons, respectively (Nguyen et al., 2019). Although clam farming creates jobs, improves incomes for local people, and promotes the seafood exports of Vietnam, it also generates a huge amount of clam shells (the ratio of clam to clam shell in weight is around 1:0.7) (Nguyen, 2019). Worldwide, bivalve shells can be used for many purposes, such as soil amendment, low-cost adsorbents, calcium supplements, construction materials, catalysts, artificial bones, bactericidal agents, and dehalogenation agents (Yao et al., 2014). However, in Vietnam, most of the clam shells are dumped in unsanitary landfills. Since clam shells are hard to decompose naturally and inadequate disposal of clam shells can cause serious environmental problems (e.g. bad odor and pathogen

growth), hundreds of million VND are paid annually by a seafood processing company for burying used clam shells (Nguyen, 2019). Thus, seeking for an alternative recycling of waste clam shells as beneficial products is urgently required in Vietnam.

Until now, very few studies have been published on recycling bivalve shells as filter media in the CWs for decontamination of P from high strength wastewater (Park et al., 2008). A lot of information is still missing about P removal pathways and lifespan of the CWs using bivalve shells media. To fill this research gap, we developed a HSSF-CWs system using white hard clam shells (WHC) media and the *Paspalum atratum* plant for remediating the simulated anaerobically digested swine wastewater. The preliminary results of this study showed that WHC exhibited properties essential for a potential filter media in CWs used for P elimination. In addition, *P. atratum* can tolerate and effectively remove P from aqueous solutions. Hence, the objectives of the present study are: (i) evaluating the effectiveness of WHC-based HSSF-CWs in eliminating P from the simulated anaerobically digested swine wastewater, (ii) elucidating the P removal pathways in the WHC-based HSSF-CWs, and (iii) estimating the lifespan of the WHC-based bed in the HSSF-CW.

2. Materials and Methods

2.1. Materials

2.1.1. Filter media

White hard clam *(M. lyrata)* shells (hereafter referred to as WHC), an adsorptive filter media, served as the main substrate in the HSSF-CWs. WHC was collected from Thai Binh Shellfish Co., Ltd., which is located in Nam Thinh commune, Tien Hai district, Thai Binh province (Coordinates: 20°23'34.1"N 106°34'28.3"E). This is one of the largest frozen clam processing factories in the north of Vietnam, with a processing capacity of over 20 tons/d for export to the European Union, Japan, Taiwan, and South Korea. Consequently, a large amount of clam shells (~14 tons/d) is generated by the company each day.

After being collected, WHC was washed with tap water to remove the clam meat leftover. Next, it was dried in the open air, followed by being transferred to a mechanical stone crushing plant in Chuong My district, Hanoi for grinding and screening to get the desired particle size (1.4-2 mm). The preliminary results of this study indicated that the optimal performance of WHC in terms of the P retention capacity and hydraulic conductivity could be achieved with this particle size range. Finally, the ground WHC was delivered to the CWs experimental site in Yen Hoa ward, Cau Giay district, Hanoi city. The physicochemical properties of WHC were as follows: P adsorption capacity (at C_{in} = 500 mg/L) of 10.34 mg/g; hydraulic conductivity of 0.7 cm/s, porosity of 43%, bulk density of 1.34 g/cm^3, total P content of 0.005 mg/g, metal oxides constituents of CaO 53.92%, MgO 0.07%, Fe_2O_3 0.017%, and Al_2O_3 < 0.01%. WHC, as an adsorptive/reactive main substrate (due to high CaO content), was used in HSSF-CWs to examine if it would augment the P abatement process.

Yellow sand (q_{max} = 1.75 mg/g), as a conventional main substrate, was utilized for comparison purposes with WHC. Black sand (q_{max} = 0.97 mg/g), another conventional filter media, was used to cover the top layer of all HSSF-CWs units as the plant support matrix.

2.1.2. Plant characteristics

The plant examined with the HSSF-CWs system in the present work was *P. atratum* Swallen (common name: *Ubon paspalum*). This plant is native to Brazil and Bolivia. At present, it is widely distributed as sown forage in tropical regions, such as South America (e.g. Argentina, Bolivia, Brazil, Columbia), Southeast Asia (e.g. Indonesia, Philippines, Thailand, Vietnam), and Oceania (e.g. Australia). It is also abundantly available in the United States. The main attributes that make *P. atratum* a potential plant for use in HSSF-CWs are: waterlogging tolerance, easy cultivation, fast growth, high biomass yield (260-280 tons FW/ha/y), well-developed fibrous root system (up to 70 cm), and high P accumulation (0.30%). Besides, as a perennial grass (up to 4-6 years), *P. atratum* can enhance the stabilization of the HSSF-CWs. Moreover, *P. atratum* is a cultivar grass with high economic

value. Unlike other wild wetland plants with no or less beneficial use after harvest, *P. atratum* can be employed as animal feed (e.g. cow, goat, sheep, rabbit, and fish). In Vietnam, *P. atratum* is popularly cultivated in the Southwest region and in the cow farms of large economic enterprises, such as TH True Milk, Vinamilk, and Hoang Anh Gia Lai. Our bench experiments show that *P. atratum* has high potential for phytoremediation of P in aqueous solutions. To the best of our knowledge, this is the first time *P. atratum* has been subjected to P treatment processes in CWs. Hence, this study is expected to contribute to the list of potential wetland plants for solving the P-rich wastewater.

The *P. atratum* in the present study was collected in Dong Phong commune, Tien Hai district, Thai Binh province (Coordinates: 20°25'00.2"N 106°32'49.8"E). The plant was grown in the garden soil using seed (Category 1) purchased from Vietnam National University of Agriculture, Gia Lam, Hanoi. The 2-week old plant was taken out of the garden soil, transported to the study location in Hanoi. The plant leaves were cut off to mitigate water evaporation. After being washed with tap water to remove the residual soils, the plant stems of around 25 cm were kept in buckets of tap water in the shadow for 1-week adaptation before being transplanted in the HSSF-CWs. It took around 2 weeks for *P. atratum* to acclimatize and grow well in the HSSF-CWs.

2.1.3. Simulated wastewater

This study investigated the treatment performance of HSSF-CWs with synthetic wastewater, simulating the anaerobically digested swine wastewater from a commercial pig farm in Luong Xa village, Nam Dien commune, Chuong My district, Hanoi (Coordinates: 20°53'49.6"N 105°42'57.9"E). The pig farm covered an area of 1,700 m^2 with 1,100 pig heads. It is estimated that approximately 55 m^3/d of wastewater was generated and discharged into the surrounding environment from an anaerobic digester. The initial results of this study showed that the real anaerobically digested swine wastewater was characterized as follows: pH 8.1±0.3, BOD_5 299±48 mg/L, COD 1560±308 mg/L, total N (TN) 730±82 mg/L, total P

(TP) 151±26 mg/L, NH_4–N 371±38 mg/L, PO_4–P 52.9±7 mg/L, and turbidity 748±57 NTU. In order to simplify but imitate the nutrients found in real wastewater, the synthetic wastewater containing only NH_4-N and PO_4-P was prepared by dissolving the proper amounts of NH_4Cl and KH_2PO_4 in tap water. The initial investigations of our group show that the levels of PO_4-P and NH_4-N in tap water are negligible, thus having no effects on the composition of the synthetic wastewater. The concentrations of NH_4-N and PO_4-P in the synthetic wastewater were kept at 12.5 mg P/L and 125 mg NH_4-N/L, which were equivalent to those in the 4-fold diluted real anaerobically digested swine wastewater to protect *P. atratum* from potential toxic effects of ammonium (Hunt et al., 2002).

2.2. Constructed wetlands experiment set-up

This study developed the horizontal sub-surface flow constructed wetlands (HSSF-CWs) for eliminating P from simulated wastewater because it is well documented that HSSF-CWs are superior to vertical sub-surface flow constructed wetlands (VSSF-CWs) and free water surface constructed wetlands (FWS-CWs) for decontamination of P-rich wastewater (Almuktar et al., 2018). The investigation of the lab-scale HSSF-CWs system was conducted continuously for 117 days (in the period from 10 May 2019 to 3 September 2019). The lab-scale HSSF-CWs system was placed in a planting house, which was located at Yen Hoa ward, Cau Giay district, Hanoi city (Coordinates: 21°01'20.4"N 105°47'40.9"E). The area has a tropical climate with an average annual temperature of 23.5 °C, average annual precipitation of 1800 mm, and mean annual humidity of 79%. The planting house was covered with transparent polycarbonate roof panels to obtain sun light and protect the treatment system from effects of the rain water. There were four CW units in total in the HSSF-CWs system. A CW unit was constructed of stainless steel in the shape of a rectangle, with the dimensions of 68.5 cm in length, 33 cm in width, and 45 cm in height.

The efficient volume of each CW unit was 0.102 m^3. For the water to drain, the bottoms of all four CW units were covered by washed gravel (diameter 1-2 cm, porosity 52%) (Borin et al.,

2013). The top layers of all the four CW units were filled with black sand for plant cultivation. In the middle layer serving as the main substrate, the first two CW units were filled with WHC (diameter 1.4-2 mm, porosity 43%), whereas the last two CW units were packed with yellow sand (diameter 1-2 mm, porosity 34%).

The HSSF–CW1 and HSSF–CW3 were planted with the waterlogging tolerant grass *(P. atratum).* There were 2 rows, each row had 3 clusters and each cluster comprised 4 plants in each CW unit. The distance between 2 rows was 11 cm, whereas the distance between 2 clusters was around 17 cm. The plant density was about 106 plants per m^2. The HSSF–CW2 and HSSF–CW4 were not vegetated and used as the control experiments. The plant grew well throughout the monitoring period.

The lab-scale HSSF-CWs system was operated in a continuous flow regime, with the hydraulic loading rate (HLR) of 0.032 m/d for HSSF-CW1 & HSSF-CW2 and 0.027 m/d for HSSF-CW3 & HSSF-CW4. The hydraulic retention time (HRT) was 5.4 d in each CW unit. These hydraulic parameters were determined based on our initial investigations. At the HSSF-CWs inlet, a distributor silicon pipe (⌀ = 7 mm), which ran along a PVC plastic pipeline (⌀ = 21 mm), was placed horizontally and perpendicular to the flow direction. A drainage PVC plastic pipeline (⌀ = 21 mm) was placed at the bottom of each unit to facilitate effluent collection. At the outlet of each HSSF-CW, a PVC plastic pipe (⌀ = 21 mm) was placed vertically and parallel to the walls of the CWs unit to keep the water level inside the CW unit around 2 cm below the top layer surface. The effluents from four CW units were conveyed to one of two storage tanks using a PVC plastic pipe (⌀ = 34 mm). When one tank was full, another tank was utilized alternatively. The water in the filled tank was measured for P level and precipitated with lime to meet the QCVN40:2011/BTNMT (Column B) before discharging into the common drainage system. The simulated anaerobically digested swine wastewater was evenly loaded from a feed water tank to all four CWs units using four peristaltic pumps (HV-77200-50, Masterflex Cole-Parmer, USA). The mean wastewater

flowrate (around 5 mL/min) to all CW units were measured every day and adjusted if necessary to maintain a stable flowrate for the whole monitoring period.

2.3. Sample collection and analysis

Wastewater samples were collected 47 times, whereas plant and media samples were taken at the start and end of the 117-day monitoring period. There were 5 sampling points of wastewater including 1 common inlet and 4 outlets of HSSF-CWs. For filter media, 3 samples at the start and 8 samples at the end of the experiment were collected. For plant, 3 samples at the beginning and 6 samples at the end of the experiment were collected. For each sampling point, 3 samples were taken to determine the average value. In total, there were 705 samples of wastewater, 33 samples of media, and 27 samples of plant.

After being collected, the wastewater samples were preserved according to the standard methods for examination of wastewater (Rice and Bridgewater, 2012). All samples were then immediately transported to the laboratory of the Master's Program in Environmental Engineering, VNU Vietnam Japan University. In the laboratory, the plant samples were first washed with tap water, dried in the air, cut into different parts (e.g. leaf, stem, and root) and measured for fresh weights. Next, the cut plant samples were dried in the precision mechanical convection oven (PR305220M, Thermo Scientific, USA) at 70 °C to a constant weight. After being cooled down to room temperature (25 °C), the dried plant samples were measured for dry weights using a precision balance (MS3002TS/00, Mettler Toledo, China). Finally, the dried plant samples were ground into fine powder and kept in a tight glass bottle. The media samples were first washed with saturated sodium chloride (NaCl) solution to eliminate the residual P on the media surface (Korkusuz et al., 2007). In the next step, they were dried in a precision mechanical convection oven (PR305220M, Thermo Scientific, USA) to a constant weight. After being cooled down to room temperature (25 °C), the media samples were retained in tight glass bottles for determining P.

The P concentration in wastewater was analyzed according to method 365.3 (U.S. EPA, 1983) using UV-VIS Diode Array Spectrophotometer (S2100, Unico, USA). The wastewater pH was determined with Seven Compact™ pH/Ion (S220-Kit, Mettler Toledo, Switzerland). The plant samples were first digested in accordance with the TCVN 8551:2010 method using Kjeldahl Digestion Unit (DK6, Velp, EU). Then, TP content in the dried *P. atratum* was determined following the method 365.3 (U.S. EPA, 1983) with UV-VIS Diode Array Spectrophotometer (S2100, Unico, USA). The inorganic P binding forms in the spent media taken from the HSSF-CWs were examined using the sequential extraction method, deemed to be suitable for the Ca-rich media (Korkusuz et al., 2007). The P in microbial biomass was analyzed according to the extraction method (Rozari et al. (2016). All analyses were triplicated.

2.4. Calculation

The P removal efficiency of the HSSF-CW was calculated according to the following Eq. 1 (Korkusuz et al., 2007):

$$RE = \frac{(C_{in} - C_{out})}{C_{in}} * 100 \quad (1)$$

where *RE* is the P removal efficiency (%), while C_{in} and C_{out} represent the inlet and outlet P concentrations, respectively (mg/L).

The P sorption capacity of a filter media in the HSSF-CW was determined as follows:

$$q = \frac{(C_i - C_e)}{m} * V \quad (2)$$

where q is defined as the P sorption capacity of the substrate (mg/g); C_i and C_e represent the P concentration in the wastewater before and after adsorption (mg/L), respectively; V indicates the volume of wastewater (L); and m is the mass of the substrate (g).

The P mass balance in the HSSF-CWs was evaluated using the following equations (Luo et al., 2017; Tang et al., 2016):

$$\Delta m = L_{in} - L_{out} \quad (3)$$

$$L_{in} = C_{in} * HLR = C_{in} * \frac{Q}{A} = \frac{C_{in} * V_{in}}{A * T} \quad (4)$$

$$L_{out} = C_{out} * HLR = C_{out} * \frac{Q}{A} = \frac{C_{out} * V_{out}}{A * T} \quad (5)$$

$$\Delta m = \frac{(C_{in} * V_{in}) - (C_{out} * V_{out})}{A * T} \quad (6)$$

where Δm is the total P removal rate (g/m^2/d) of the HSSF-CWs; L_{in} and L_{out} are the P loading rates for the inlet and the outlet, respectively (g/m^2/d); C_{in} and C_{out} represent the inlet and outlet P concentration, respectively (mg/L ~ g/m^3); V_{in} and V_{out} are the inlet and outlet watewater volume (m^3); Q is the flow rate (m^3/d); A is the HSSF-CW unit area (m^2); and T indicates the period of time between two continuous sampling times (d).

The total P removal rate (Δm) of the HSSF-CW can also be expressed by the following equation:

$$\Delta m = R_{Substrate\ sorption} + R_{Plant\ uptake} + R_{Microbe\ assimilation} + R_{Others} \quad (7)$$

$$R_{Substrate\ sorption} = \frac{C_{substrate} * M_{substrate}}{A * T} \quad (8)$$

$$R_{Plant\ uptake} = \frac{C_{plant} * M_{plant}}{A * T} \quad (9)$$

$$R_{Microbial\ assimilation} = \frac{M_{P\ microbe}}{A * T} \quad (10)$$

$$R_{Others} = \Delta m - R_{Substrate\ sorption} - R_{Plant\ uptake} - R_{Microbe\ assimilation} \quad (11)$$

where $C_{substrate}$ and C_{plant} represent the P contents in the substrate after use in HSSF-CWs and in the plant after harvest from HSSF-CWs (g/kg), respectively; $M_{substrate}$ and M_{plant} are the mass of the substrate and plant (kg), respectively; $M_{P\ microbe}$ is the mass of P accumulated in the microbial biomass (g).

Since there were 2 kinds of substrates in each CWs unit, the P removal by substrate sorption in a CW unit was the sum of the P removals by two substrates.

The lifespan of the media bed in the HSSF-CWs was calculated using Eq. 12 (Chan et al., 2008) as follows.

$$L_l = \frac{\sum(q_{substrate} * M_{substrate})}{Q * C_{in}} \quad (12)$$

where L_l is the lifespan of the media bed (y); $q_{substrate}$ represents the P sorption capacity of the substrate (mg/g ~ g/kg); $M_{substrate}$ is the mass of the substrate (kg); Q is the inlet flowrate (m^3/d); C_{in} is the inlet P concentration (mg/L ~ g/m^3). In this work, the lifespans of the media beds in HSSF-CWs were estimated using both q_{max} (determined with C_{in} range of 0-500 mg/L) and q_{real} (determined at the real C_{in} of 12.5 mg/L) for the comparison purpose. Since there were 2 kinds of substrates in each CWs unit, the P mass captured by the bed media was the sum of the P mass retained by two substrates.

2.5. Statistical analysis

SPSS 20.0 package was used for statistical analyses of data. All data were tested for goodness of fit to a normal distribution. Data were log transformed where necessary to achieve homogeneity of variance. Significant differences among different HSSF-CWs were evaluated using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ indicating statistical significance. Pearson correlation coefficients (r) were used to express the associations between CaO content and P sorption capacity.

3. Results and Discussion

3.1. Phosphorus treatment performance of the HSSF-CWs

Data on inlet and outlet P concentrations were analyzed to determine the P removal efficiency and P removal rate of CW units. The treatment performance of the HSSF-CWs was evaluated based on the P removal percentage, P removal rate, and by making a comparison between the outlet P concentrations with the permissible level according to QCVN40:2011/BTNMT (Column B).

The mean P removal efficiencies in HSSF-CW1, HSSF-CW2, HSSF-CW3, and HSSF-CW4 over 117-day monitoring period were 89.9%, 78.8%, 57.2%, and 44.4%, respectively. It

can be observed that the P removal efficiencies in the WHC-based HSSF-CW1 and HSSF-CW2 (78.8-89.9%) were substantially greater than those in the yellow sand-based HSSF-CW3 and HSSF-CW4 (44.4-57.2%) ($p < 0.001$). This outcome proves the significance of using adsorptive/reactive materials as substrates to improve CWs' removals of P. In addition, the P removal efficiencies in the HSSF-CW1 and HSSF-CW2 using WHC media (78.8-89.9%) were equal to and higher than those in other CWs treating the anaerobically digested swine wastewater, such as: 78.2-89.8% in the SF-CWs filled with the paddy soil (Luo et al., 2017); 61% in the HSSF-CWs packed with gravel, coarse sand, and zeolite (Borin et al., 2013); and < 50% in the HSSF-CW using loamy sand and compacted clay (Hunt et al., 2002). In contrast, the yellow-based HSSF-CW3 and HSSF-CW4 exhibited similar or poorer P removal efficiencies (44.4-57.2%) comparing with the above studies. Evidently, WHC as adsorptive/reactive media was more effective in removing P than yellow sand and other conventional substrates.

The P removal efficiencies of the planted HSSF-CWs were always higher than those of the unplanted HSSF-CWs ($p < 0.001$), representing 3.1% and 11.0% for the WHC-based HSSF-CW and yellow sand-based HSSF-CW, respectively. The lower increment in the P removal efficiency of the WHC-based HSSF-CW can be due to stronger growth of microbes in this unit. Consequently, the P capture capacity of WHC was declined more substantially as a result of thicker biofilm on WHC compared to yellow sand. The obtained results indicate the contribution of the *P. atratum* to the total P removal of the HSSF-CWs. The finding is in harmony with the result obtained by Liu et al. (2012), reporting that the vegetated wetland showed 12% greater P removal than the unplanted wetland.

Table 1 represents the P loading rates of the HSSF-CW1, HSSF-CW2, HSSF-CW3, and HSSF-CW4, which were 0.35, 0.39, 0.38, and 0.42 $g/m^2/d$, respectively. The mean P removal rates in the HSSF-CW1, HSSF-CW2, HSSF-CW3, and HSSF-CW4 were 0.32, 0.31, 0.22, and 0.19 $g/m^2/d$, respectively. The P removal rates of HSSF-CW1 and HSSF-CW2 using

WHC media were higher than that of FWS-CWs using paddy soil media (0.044-0.247 g/m²/d) (Luo et al., 2017). Conversely, HSSF-CW3 and HSSF-CW4 using yellow sand media showed equivalent to or lower P removal rates than the above study. This suggests that WHC-based HSSF-CWs exhibit better P removal rates than yellow sand-based HSSF-CWs. This finding matches well with the result on the P removal efficiency mentioned above. The P removal rates of the planted HSSF-CWs were only slightly higher than those of the unplanted HSSF-CWs, regardless of the media categories (WHC or yellow sand). This indicates the minor role of *P. atratum* in the total P removal of the HSSF-CWs.

Table 1

Evaluating the treatment performance based solely on the removal efficiency may lead to data misinterpretation. This is evidenced by Luo et al. (2017), revealing that the outlet P concentration was still high (17.0 mg/L) in spite of a relatively high P removal efficiency (70.1%) in the SF-CWs. This highlights the necessity of examining the outlet P concentration in addition to P removal efficiency. Fig. 1 illustrates changes in the outlet P concentrations of HSSF-CWs over the study period. It is clear that after 117 days of operation, the outlet P concentrations of the WHC-based HSSF-CWs were far below the permissible level (6 mg/L) according to QCVN40:2011/BTNMT (Column B). Contrarily, the outlet P concentrations in the yellow sand-based HSSF-CWs reached the permissible level very quickly, which was just more or less than 1 month after operation. This can be attributed to higher P sorption capacity of WHC (10.34 mg/g) than yellow sand (1.753 mg/g). The outlet P concentrations of the *P. atratum* planted HSSF-CWs were always lower than that in the unplanted HSSF-CWs ($p < 0.001$), suggesting the contribution of *P. atratum* to the total P removal of the HSSF-CWs. These results prove that WHC-based HSSF-CWs can effectively decontaminate P from the simulated anaerobically digested swine wastewater.

Figure 1

Regarding these CWs' practical application, it was found that the P removal efficiency of WHC in the real anaerobically digested swine wastewater only fell by 10.9% compared to that in the simulated wastewater. Besides, the P removal efficiency by WHC in the real wastewater was reasonably high (75.6%). This indicates the negative effects in the real wastewater were at acceptable levels. Moreover, as this study focused on enhancing the P removal efficiency of CWs, the treatment system was designed with a single HSSF-CW unit. In practice, as the anaerobically digested swine wastewater contains several contaminants (e.g. organic matter, TSS, N, etc.), the actual system is normally a treatment train, which includes many treatment stages and several treatment units. Of which, the primary treatment stage (e.g. screening, filtration, coagulation, etc.) can effectively remove TSS while the secondary treatment stage (e.g. activated sludge, uflow anaerobic sludge blanket, membrane bioreactor. etc.) can significantly reduce the contents of organic matters. Therefore, CWs as the tertiary/polishing treatment stage can be used for enhancing P removal without significant effects of the TSS or organic matters.

3.2. Phosphorus removal pathways in the HSSF-CWs

3.2.1. Substrate sorption

Substrates in the sub-surface flow CWs are also known as filling materials or media support matrix. Substrates play a crucial role in removing P by the CWs because of versatile functions, such as adsorbent for P sequestration, carrier for biofilm development, and medium for plant growth (Yang et al., 2018). In the present study, P was abundantly retained by substrates (WHC and yellow sand) of the HSSF-CWs after operating for 117 days. The P sequestration ability of substrates were evaluated via the P removal rate, which was determined using Eq. 8 with the input data in Table 2.

Table 2

As summarized in Table 3, the P removal rates via substrate sorption in the HSSF-CWs varied in the 61.2-95.5 ($g/m^2/y$) range, depending on the category of substrates (WHC, yellow

sand) and the presence of the plant (*P. atratum)*. It can be observed that the P removal rates of substrate sorption were higher than those of *P. atratum* uptake (6.3 - 10.5 g/m^2/y) or microbe assimilation (2.8-16.7 g/m^2/y) from 4 to 36 times ($p < 0.001$). This proves that substrates serve as an important component in the media – plant – microbe interaction system for P decontamination.

Table 3

The P removal rates of the WHC-based HSSF-CWs (112-115 g/m^2/y) were substantially greater than those of the yellow sand-based HSSF-CWs (68.6-80.1 g/m^2/y) ($p < 0.01$). This can be attributed to the greater P sorption capacity of WHC (10.34 mg/g) in comparison with yellow sand (1.753 mg/g), as a consequence of higher CaO content in WHC (53.92%) than yellow sand (0.73%). The CaO content and P sorption capacity in the filter media (WHC, yellow sand, and black sand) was strongly correlated ($r = 0.997$, $p < 0.001$). The result was even higher than that ($r^2 = 0.51$) obtained by Vohla et al. (2011) for numerous types of filter media from the literature review. Similarly, Žibienė et al. (2015) stated that dolomite, $CaMg(CO_3)_2$, as the filter media in the VF-CW was 21% more efficient than sand when removing P.

It is interesting to note that the P removal rates of the substrates (WHC and yellow sand) in the planted HSSF-CWs were lower than those in the unplanted HSSF-CWs. This can be explained by the competing effects of biological uptake *(P. atratum* and microbes*)* on the P sequestration of the substrates (WHC and yellow sand) in the planted HSSF-CWs (Korkusuz et al., 2007). It can be seen from Table 3 that the P removal rates of substrate sorption were reversely proportional to those of microbe assimilation regardless of the categories of media (WHC or yellow sand). This is possibly because the P adsorption sites can be blocked by the biofilm coating on the media particles (Chan et al., 2008).

The P content captured in the WHC after use in the WHC-based HSSF-CW was 74 mg/kg. This was equivalent to that in the spent blast furnace granulated slag (70 mg/kg) as the

substrate in the vertical flow reed beds (Korkusuz et al., 2007). This finding suggests that WHC can be a potential substrate for P elimination in the CWs. The percentages of inorganic P binding forms were declined in the following order: Ca-P (44%) > loosely bound P, LBP (35%) > Fe-P (10%) > Mg-P (8%) > O-P (2%) > Al-P (1%). It demonstrates that Ca-P and LBP were the major inorganic P binding forms. This was supported by Korkusuz et al. (2007), who revealed that CaO and LBP were pre-dominant forms of mineral P in the blast furnace granulated slag (BFGS) taken from the vertical flow reed bed. This similarity can be attributed to the high CaO content in both WHC (53.92%) and BFGS (33.53%) as the filter media. According to Krogstad et al. (2005), Ca-P was more available to plants than Fe-P and Al-P. As well, our previous study showed that WHC did not contain considerable amounts of toxic elements. These results pave the way for the spent WHC media to be recycled as Ca-rich and P-rich soil amendments.

3.2.2. Plant uptake

The plant is considered to be an important design component in the CWs. The main functions of wetland plants in the remediation of P-rich wastewater include: i) uptake and accumulation of P in their cells, (ii) provide a living medium for microorganisms (e.g. carrier, carbon source, oxygen), and (iii) prevent water clogging (Almuktar et al., 2018). The data on the role of *P. atratum* in the total P removal of the HSSF-CWs are given in Table 3. It can be seen that the P removal rates of *P. atratum* in the HSSF-CWs were relatively low (6.27-10.47 $g/m^2/y$) compared to those of substrates (WHC, yellow sand) ($p < 0.001$). Additionally, the contribution of *P. atratum* to the total P removal of the HSSF-CWs was lower in the WHC-based HSSF-CW. This is possibly explained by the greater role of WHC compared to yellow sand as the media. In the same trend, the P removal rate via plant uptake in the WHC-based HSSF-CW (6.27 $g/m^2/y$) was lower than that in the yellow sand-based HSSF-CW (10.47 $g/m^2/y$) ($p < 0.001$). This suggested that P was less effectively accumulated by *P. atratum* in

the WHC-based HSSF-CW. The fact that WHC can lower the plant uptake of P should be interest for future work on this subject.

The P removal rate of *P. atratum* (6.27-10.47 g/m^2/y) was higher than those of: *Ceratophyllum demersum* (1 g/m^2/y); *Pistia stratiotes* and *Potamogeton pectinatus* (4 g/m^2/y), *Cyperus papyrus* (1 g/m^2/y); and Canna and reed mixed plantation (1.14 g/m^2/y). It was, however, lower than those of *P. australis* (12 g/m^2/y), bulrush (3.7-16.3 g/m^2/y), cattail (2.0-20.6 g/m^2/y), *Typha latifolia* (18 g/m^2/y), and *M. aquaticum* (15.1-40.9 g/m^2/y) (Hunt et al., 2002; Luo et al., 2017; Quan et al., 2016). It is evident that *P. atratum* exhibited an intermediate P removal rate comparing with other wetland plants.

According to Table 4, the P content in the whole plant of *P. atratum* fluctuated from 3.16 to 4.02 mg/g, which was equivalent to the 0.32-0.40% range. This is consistent with the discovery by Vo et al. (2017) that the percentage of P in the emergent wetland plants fluctuated from 0.13% to 1.07%. The P content in *P. atratum* had the same of magnitude as or higher than those of other emergent macrophyte plants in CWs, such as *Brophytes* (0.1%), *Helophytes* (0.15%) (Demars and Edwards, 2008), *P. australis* (0.2%), *Typha domingensis* (0.23%), *Schoenoplectus validus* (0.26%), and *Eleocharis acuta* (0.34%) (Greenway et al., 2003). It should be noted that *P. atratum* exhibited more P content than the most commonly used wetland plants, for example *P. australis* and *T. domingensis*. The results suggest that *P. atratum* can be a promising plant for removing P in the HSSF-CWs although it is not a P hyperaccumulator, which concentrates at least 2% P in the foliar (Delorme et al., 2000).

Table 4

The P content in the whole plant is an important indicator for the potential of a macrophyte to uptake and accumulate P as biomass. As the overall P abatement of a plant also depends on its total biomass, both P content and total biomass of the plant need to be examined (Almuktar et al., 2018). Using data on fresh weight and dry weight of the *P. atratum* obtained from HSSF-CW1 and HSSF-CW3 over a 117-day period (Table 4), the

biomass yield of the *P. atratum* was estimated to be 129-185 ton FW/ha/y or 1.91-2.51 kg DW/m^2/y. This is equivalent to that of *M. aquaticum* (1.95-4.90 kg DW/m^2/y) in the pilot-scale FWS-CWs treating anaerobically digested swine wastewater (Luo et al., 2017).

The P allocation in different parts of *P. atratum* harvested from HSSF-CWs using WHC-based media and yellow sand-based media is displayed in Fig. 2. It is evident that the P mass accumulated in different parts of *P. atratum* was declined in the order: stem > leaf > root for both categories of substrates (WHC or yellow sand). This outcome is supported by Shane et al. (2004), who found that P was mainly concentrated in the aboveground parts of the *Hakea prostrata* at a high P concentration range in wastewater and vice versa. The majority amount of P was accumulated in the aboveground parts of *P. atratum,* accounting for 92.6-95.1%. This feature favors the permanent removal of P by CWs via plant uptake and regular harvest. A similar trend was indicated for *T. latifolia, Oryza sativa,* and *Ipomoea aquatica* while the opposite trend was observed for *Zizania latifolia, Lactuca sativa* (Chen et al., 2017).

Figure 2

3.2.3. Microbial assimilation

The P removal rates of microbes in the HSSF-CWs were calculated based on the P amount extracted from the microbial biomass (Rozari et al., 2016). Based on the input data in Table 2, the P removal rates via microbe assimilation in the HSSF-CW1, HSSF-CW2, HSSF-CW3, and HSSF-CW4 were estimated to be 16.68, 15.62, 5.12, and 2.78 (g/m^2/y), respectively. These were smaller than those of substrate sorption and equivalent to those of plant uptake. It is clear that the P removal rates of microbial assimilation were considerably higher in the WHC-based HSSF-CWs compared to those of yellow sand-based HSSF-CWs ($p < 0.001$). This can be explained by the fact that more shelters for microbes were available in WHC as a result of higher porosity in WHC (43%) compared to that in yellow sand (34%). Another reason may be relevant to the more abundance of Ca and Mg elements in WHC than in yellow sand as essential nutrients for microbial growth (Bertrand et al., 2015).

The P removal rates of microbes in the HSSF-CWs planted with *P. atratum* were always greater than those in unplanted HSSF-CWs, regardless of the category of substrates (WHC, yellow sand). It suggests that the presence of *P. atratum* can enable the microbial activities, thereby accelerating their P accumulation. This can be explained by the fact that plants provide a carrier for biofilm development and release useful organic compounds (often citric and oxalic acids) for the microbial growth (Faulwetter et al., 2009; Meng et al., 2014; Tang et al., 2016). In addition to the organic matters exudated via plant roots, organic matters can also be produced as microbial biomass by autrophic microbes via the following reaction:

$$NH_4^+ + 1.815\ O_2 + 0.1304\ CO_2 = 0.0261\ C_5H_7O_2N + 0.973\ NO_3^- + 0.921\ H_20 + 1.973\ H^+ \quad (13)$$

The dead microbes can be a source of organic matters for living microbes. These sources of organic matters can provide carbon sources for microbe's growth and activities. For that reason, microorganisms could still be present and active in the CWs operated with plant, WHC and synthetic wastewater without addition of external carbon source.

3.2.4. Other processes

It was reported that in CWs, P can also be removed from wastewater by other routes, such as chemical precipitation and evaporation as phosphine (PH_3). The phosphine, also known as hydrogen phosphide, is formed by the reaction of water with calcium phosphide (Ca_3P_2) (Chen et al., 2017). In this study, the P removal rates of other processes in the HSSF-CWs were calculated using Eq. 11 and the results are represented in Table 3. Accordingly, P was removed via other processes at a rate of 1.10-3.34 $g/m^2/y$, which was smallest among all the P removal routes ($p < 0.001$). It is suggested by this result that other processes play a minor role in the P removal of HSSF-CWs.

3.2.5. Mass balance study

The P removal from the simulated swine wastewater can be classified into losses via substrate sorption, plant uptake, microbe assimilation, and other processes (Fig. 3).

Figure 3

As is illustrated by Fig. 3, substrate sorption was a principal route for P removal, representing 41.3-69.7% of the inflow in all the HSSF-CWs, regardless of the substrates (WHC or yellow sand) and presence of the plant (*P. atratum)*. Microbe assimilation was another important P elimination pathway and it accounted for 1.8-13.0% of the inflow. The plant uptake represented 4.9-7.5% of the flow, indicating that it was only a minor P removal route. The contribution of other processes to the total P removal was marginal, accounting for only 0.8-2.3% of the inflow. A similar result was reported by Chan et al. (2008) for coal slag bed planted with *C. alternifolius* treating domestic wastewater. Accordingly, the contribution of different components to the total P removal of the CWs declined in the following order: adsorption (69.11%) > microbial processes (26.18%) > plant (4.72%). This is supported by Quan et al. (2016) and Wu et al. (2015), reporting that the plant uptake covered a minor part of the total P abatement, achieving 14.74% and 10.76-34.17%, respectively. These results agree well with the finding by Luo et al. (2017) that the plant played a minimal role in the heavily loaded treatment system.

The contribution of microbe assimilation to total P removal was augmented, whereas the role of substrate sorption was diminished in the planted HSSF-CWs. Therefore, while *P. atratum* can enhance the P removal of microbes, it may hinder the P retention of the substrate. The lower P removal rates of substrate sorption were found in the HSSF-CWs with greater P removal rates of microbes, indicating the negative effects of biofilm on the P attachment to the media surface.

3.3. Estimating lifespans of the media beds in HSSF-CWs

The lifespan of CWs indicates the long-term sustainability of CWs (Korkusuz et al., 2007). For this reason, this research content has received much attention of scientists worldwide. In this study, HSSF-CW1 had the same media bed as HSSF-CW2. Similar thing occurred with HSSF-CW3 and HSSF-CW4. Therefore, though the HSSF-CWs system in this

study consisted of four HSSF-CW units, there were only two categories of the media bed, namely the WHC-based bed and the yellow sand-based bed.

The maximum P adsorption capacity of WHC, black sand and yellow sand (C_{in} range of 0-500 mg/L) was 10.34, 0.97 and 1.75 g/kg, respectively. The respective mass of WHC and black sand and yellow sand was 79.77, 19.10, and 77.17 kg. The expected quantity of P can be adsorbed by the WHC-based bed (including black sand and WHC) and the yellow sand-based bed (including black sand and yellow sand) was 0.843 and 0.154 kg, respectively. Based on daily operation, the estimated P loadings to the WHC-based bed and yellow sand-based bed were 0.029 and 0.032 kg/y, respectively. Consequently, the lifespans of the WHC-based bed and yellow sand-based bed were 29.24 and 4.84 year, respectively.

According to Korkusuz et al. (2007), the P sorption isotherm was not appropriate for predicting the longevity of the blast furnace granulated slag (BFGS) bed in the VSSF-CWs. The reason was that the P content retained in the spent BFGS media (70 mg/kg) was substantially lower than the maximum P adsorption capacity (9150 mg/kg) and the equilibrium P sorption capacity (150 mg/kg) obtained with the real inlet P concentration. In other words, the use of q_{max} can result in an over-estimation of the lifetime of CWs. The reduction in the actual P content of the media compared to q_{max} can be attributed to the competing effects of biological uptake (e.g. plant and microbes), organic matter, and suspended solids (Druze et al., 2002). Another reason was the fact that the inlet P concentrations in the real CWs are often smaller than those in the isotherm tests for determining q_{max} (Ádám et al., 2006). For these reasons, Chan et al. (2008) suggested the real P adsorption capacity (q_{real}) to be 50% of the q_{max} value for the CWs using coal slag as substrate for treating domestic wastewater. Accordingly, the lifespans of the WHC-based bed and yellow sand-based bed were shrunk to 14.62 and 2.42 years, respectively.

In this work, both q_{max} (10,340 mg/kg) and q_{real} (1,026 mg/kg), which were respectively determined with the C_{in} range of 0-500 mg P/L and the real C_{in} of 12.5 mg P/L, were utilized

to estimate the lifespan of the media bed in the HSSF-CWs for comparison purposes. It was proposed that the q_{real} (1,026 mg/kg) could be employed to calculate the CWs' lifespan because it was closer to the P content in the spent WHC media (74 mg/kg) (Table 2) than the q_{max} (10,340 mg/kg). Using the same method, the lifespans of the WHC-based bed and yellow sand-based bed were determined to be 2.84 and 0.07 year, respectively. These results agree well with the data on the outlet P concentrations from WHC-based HSSF-CWs and yellow sand-based HSSF-CWs. Accordingly, the P levels in the effluents of yellow sand-based HSSF-CWs exceeded the permissible level (6 mg/L) according to QCVN40:2011/BTNMT (Column B) at more or less than 1 month of operation. In contrast, the outlet P concentrations of WHC-based HSSF-CWs were still far below the permissible level. It confirms that using q_{real}, which was achieved with real Ci, instead of q_{max} in estimating longevity of HSSF-CWs can provide a more accurate prediction.

Regarding the operational sustainability, desorption or dissolution of the adsorbed P from the spent WHC during CWs operation with real wastewater should be marginal. The reason is that desorption of P from the spent WHC as $Ca_3(PO_4)_2$ can occur via dissolution in the strongly acidic medium or replacing PO_4^{3-} with other anions. However, the anaerobically digested swine wastewater is normally neutral or lightly alkaline (Nguyen, 2019). Also, the significant effects of DO, oxygen reduction potential, organic matter, and hydraulic loading rate on the P desorption from the spent substrate cannot be found. Therefore, it is expected that the media is used until the effluent P concentrations of CWs exceed the discharge standard. As P was mainly removed by sorption on the media, the high levels of P in the effluents can indicate the media is being exhausted. For the recovery of filter media, the supply of P-rich wastewater will be replaced by providing nutrition solutions lacking of P for plant growth. In addition, bioaugmentation using phosphate solubilizing bacteria (e.g. *Pantoea agglomerans*, *Microbacterium_laevaniformans*, *Pseudomonas putida*, etc.) will be applied in CWs. As a result, the adsorbed P as $Ca_3(PO_4)_2$ will be converted into $CaHPO_4$,

which can be sequentially uptaken and accumulated by the wetland plants. By harvesting the plant biomass, the P can be permanently removed from the media.

4. Conclusions

WHC significantly augmented the P removal of HSSF-CWs compared to yellow sand. The mean P efluent concentration and P removal rate were 1.34±0.95 mg/L and 0.32±0.03 $g/m^2/d$, respectively. The key P removal mechanism was WHC sorption. Ca-P and LBP were major mineral P binding forms in spent WHC media. Higher P contents were allocated in aboveground parts of *P. atratum*. WHC-based bed exhibited a substantially longer lifespan than yellow sand-based bed. In short, WHC is a promising media in HSSF-CWs for intensifying P elimination from the P-rich wastewater. This enables the recycling of Ca-rich waste materials for the same purpose.

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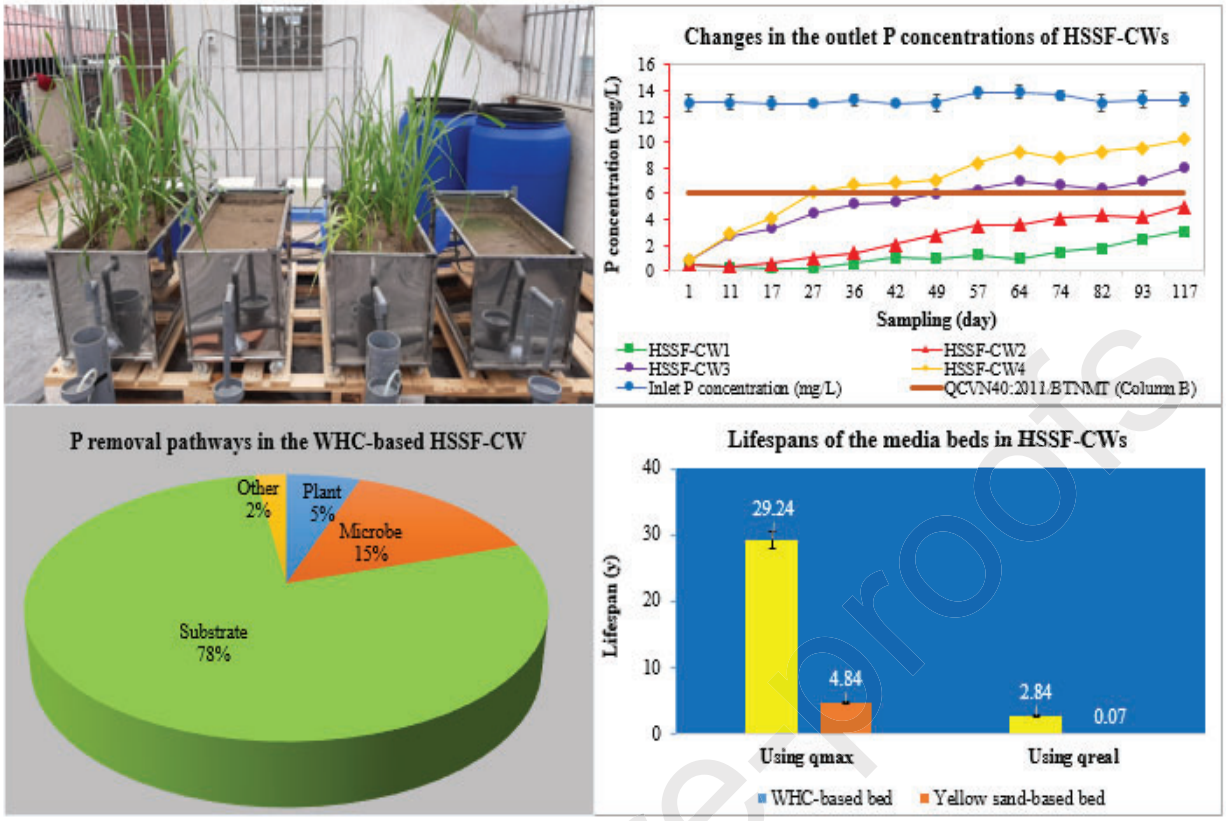
GRAPHICAL ABSTRACT



38.

HIGHLIGHTS

- P removal from simulated wastewater using lab-scale HSSF-CWs was examined.
- *Paspalum atratum* was utilized as the emergent wetland plant for very first time.
- WHC-based HSSF-CW planted with *Paspalum atratum* removed up to 89.9% of inflow P.
- WHC sorption was the dominant P removal pathway in HSSF-CWs.
- Utilizing WHC as a reactive filter media apparently extended lifespans of HSSF-CWs.

39.

FIGURE CAPTIONS

Figure 1

Changes in the outlet P concentrations of HSSF-CWs over the time.

Figure 2

Distribution of the accumulated P in different parts of *P. atratum* from HSSF-CWs using WHC-based media and yellow sand-based media.

Figure 3

Phosphorus removal pathways in the HSSF-CWs using WHC-based media or yellow sand-based media, with or without *P. atratum.*

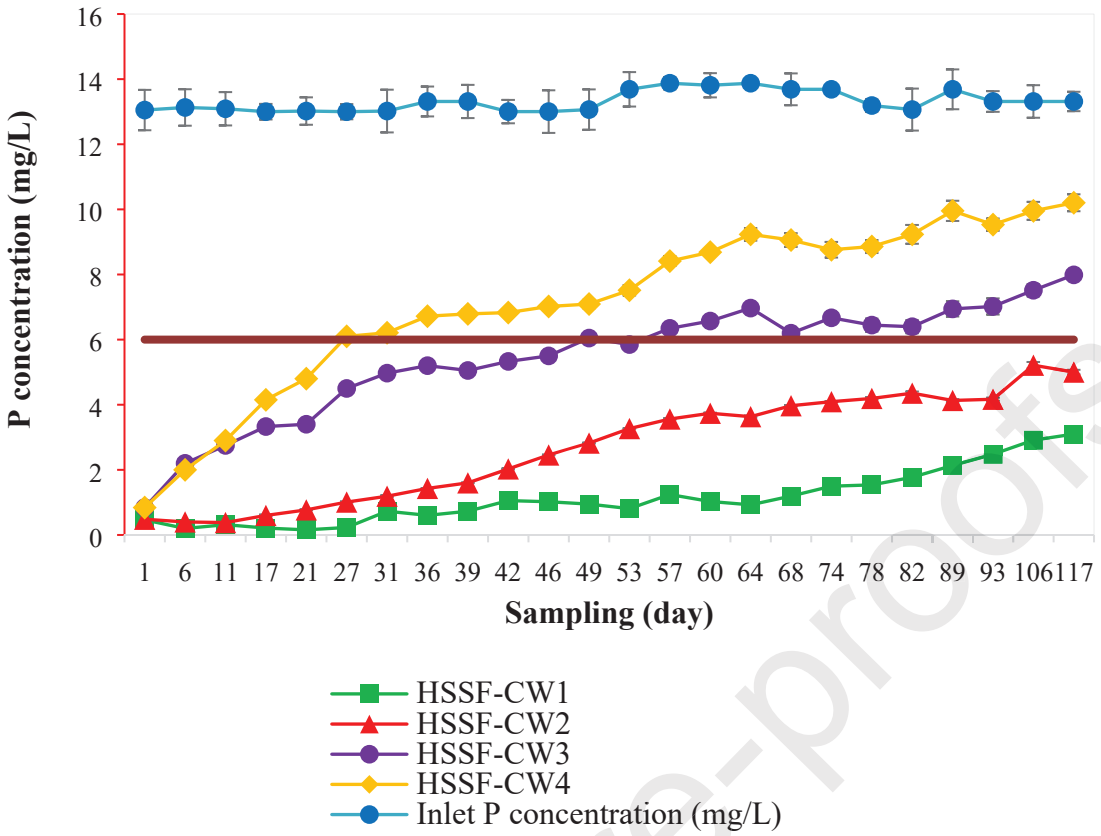


Figure 1

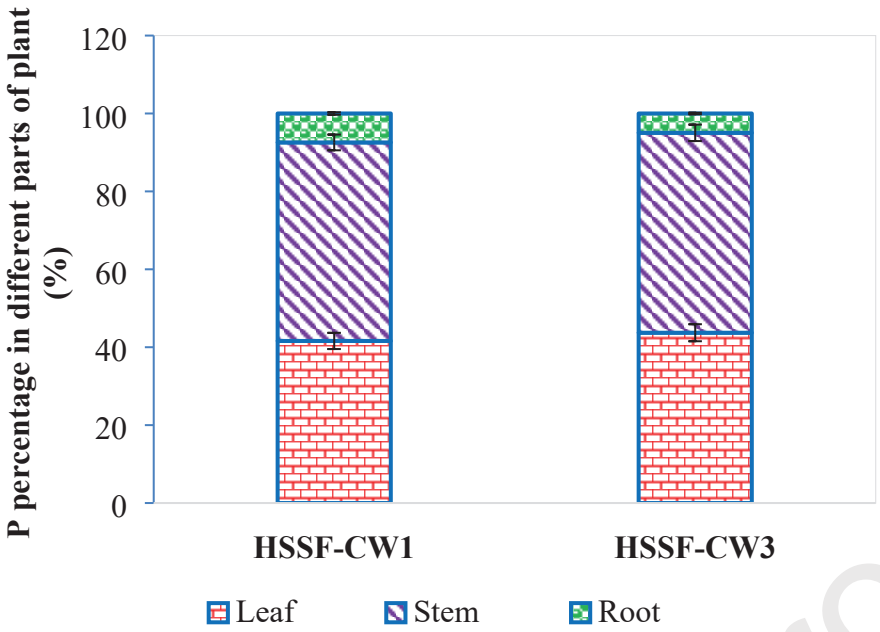


Figure 2

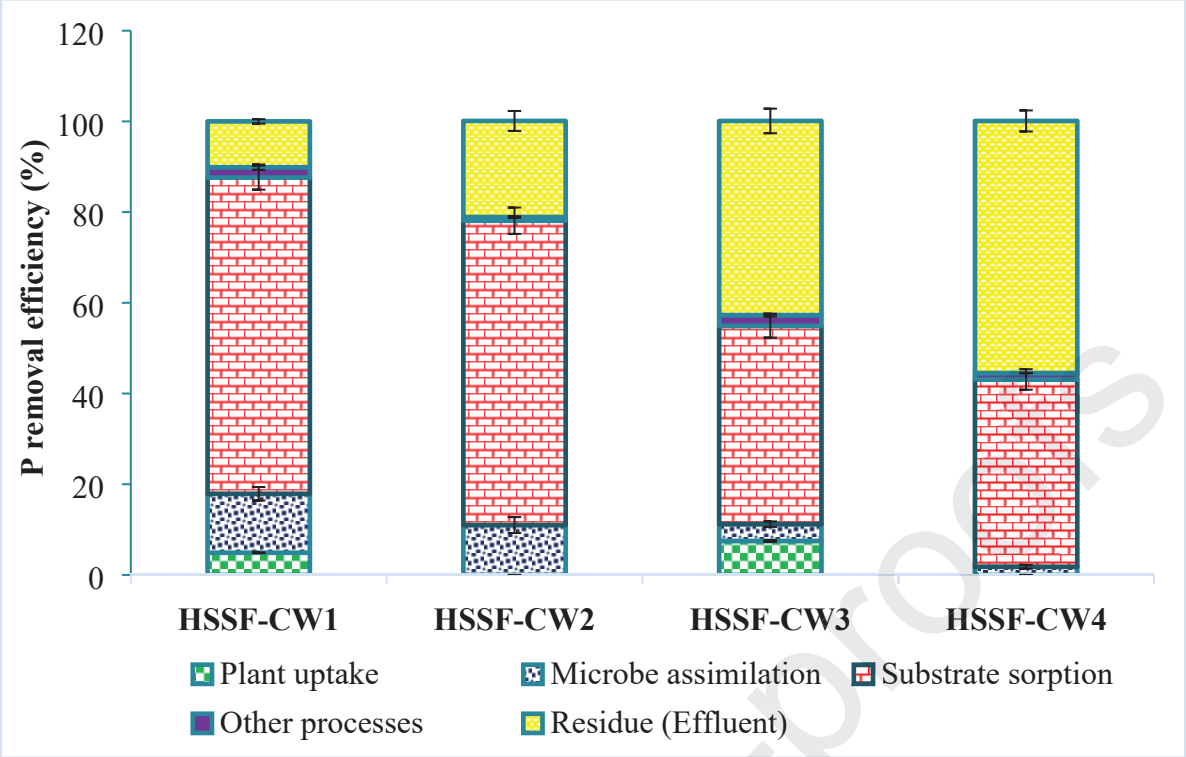


Figure 3

40.

TABLES

Table 1

The phosphorus treatment performance of the HSSF–CWs.

Parameter	Unit	HSSF–CW1	HSSF–CW2	HSSF–CW
Inlet P concentration	mg/L	13.3±0.4 [a]	13.3±0.4 [a]	13.3±0.4 [a]
Inlet P loading rate	g/m^2/d	0.35±0.02 [a]	0.39±0.05[b]	0.38±0.03[b]
Hydraulic loading rate	m/d	0.026±0.001 [a]	0.029±0.004 [b]	0.029±0.002
Outlet P concentration	mg/L	1.34±0.95 [a]	2.94±1.66[b]	5.66±1.79[c]
P removal rate	g/m^2/d	0.32±0.03 [a]	0.31±0.08 [a]	0.22±0.05[b]
P removal efficiency	%	89.9±7.1 [a]	78.8±12.3[b]	57.2±13.1[c]

Values followed by different letters in the same row are significantly different (One-way Anova, Tukey HSD, $p < 0.05$).

Table 2

Phosphorus removal by substrate sorption and microbe assimilation in the HSSF–CWs for 117-day monitoring period.

CWs unit	Substrate	Substrate sorption			Microbial	
		Mass of substrate (g)	P content in spent substrate (mg/g)	P mass captured by spent substrate (mg)	Mass of substrate (g)	Microbia in su (m
HSSF–CW1	Black sand	19,098	0.031±0.005	584.00±95.85	19,098	0.007
	WHC	79,768	0.074±0.004	5885.98±331.15	79,768	0.014
HSSF–CW2	Black sand	19,098	0.042±0.003	793.48±61.22	19,098	0.008
	WHC	79,768	0.077±0.004	6124.60±279.34	79,768	0.012
HSSF–CW3	Black sand	19,098	0.051±0.003	971.22±61.22	19,098	0.005
	Yellow sand	77,172	0.045±0.003	3462.82±203.59	77,172	0.004
HSSF–CW4	Black sand	19,098	0.128±0.006	2437.56±122.43	19,098	0.003
	Yellow sand	77,172	0.028±0.004	2180.30±311.00	77,172	0.002

Table 3

The P removal rates (g/m^2/y) of different routes in the HSSF–CWs.

CWs unit	Main substrate	*P. atratum*	Substrate sorption	Plant uptake	Microbe assimilation	Other processes	Total
HSSF–CW1	WHC	106 plants/m^2	89.3±3.4 [a]	6.27±0.09 [a]	16.7±2.0 [a]	2.97±0.76 [a]	115±1 [a]
HSSF–CW2	WHC	None	95.5±4.1 [a]	-	15.6±1.0 [a]	1.10±0.25 [b]	112±3 [a]
HSSF–CW3	Yellow sand	106 plants/m^2	61.2±3.6 [b]	10.5±0.3 [b]	5.12±0.45 [b]	3.34±0.43 [a]	80.1±3.8 [b]
HSSF–CW4	Yellow sand	None	63.7±3.5 [b]	-	2.78±0.15 [c]	2.10±0.08 [a]	68.6±3.6 [c]

Values followed by different letters in the same column are significantly different (One-way Anova, Tukey HSD, $p < 0.05$).

Table 4

The P removal by *P. atratum* plant uptake in the HSSF-CWs for 117-day monitoring period.

CWs unit	Part of plant	Fresh weight (g)	Dry weight (g)	P content in dry weight (mg/g)	P mass captured via plant uptake (mg)
HSSF–CW1	Leaf	398.22±34.25	69.88±4.42	2.72±0.16	189.60±0.88
	Stem	490.74±43.19	61.94±0.18	3.73±0.12	230.82±6.81
	Root	84.30±3.62	11.92±0.03	2.82±0.09	33.62±1.08
	Whole plant	973.26±78.83	143.74±4.24	3.16±0.13	454.03±6.59
HSSF–CW3	Leaf	620.08±53.95	98.06±4.67	3.38±0.09	331.68±23.60
	Stem	666.96±58.66	78.06±0.18	4.99±0.07	389.78±5.16
	Root	103.72±4.56	12.80±0.03	2.93±0.12	37.46±1.44
	Whole plant	1390.76±125.17	188.92±4.83	4.02±0.25	758.91±19.91

41.

Author Contribution Statement

T. A. H. Nguyen: investigation, writing - original draft, methodology, formal analysis, data curation, project administration

H. H. Ngo: investigation, supervision, conceptualization, review & editing

W. S. Guo: investigation, review & editing

T. H. H. Nguyen: methodology, formal analysis, resources, review