Environmental Technology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tent20

Efficient removal of organic matter and nitrogen from municipal wastewater in multi-module biochar filters for onsite wastewater treatment

Makoto Shigei, Roger B. Herbert, Frank Persson, Ekaterina Sokolova & Sahar S. Dalahmeh

To cite this article: Makoto Shigei, Roger B. Herbert, Frank Persson, Ekaterina Sokolova & Sahar S. Dalahmeh (18 Sep 2024): Efficient removal of organic matter and nitrogen from municipal wastewater in multi-module biochar filters for onsite wastewater treatment, Environmental Technology, DOI: 10.1080/09593330.2024.2402096

To link to this article: https://doi.org/10.1080/09593330.2024.2402096

ENVIRONMENTAL TECHNOLOGY
https://doi.org/10.1080/09593330.2024.2402096

Taylor & Francis
Taylor & Francis Group

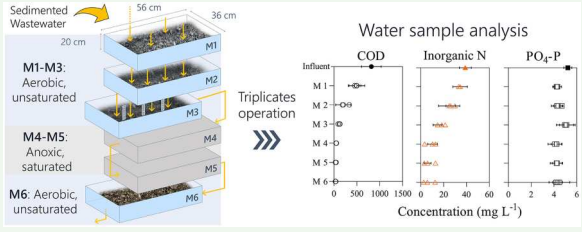
Efficient removal of organic matter and nitrogen from municipal wastewater in multi-module biochar filters for onsite wastewater treatment

Makoto Shigei [a], Roger B. Herbert [a], Frank Persson [b], Ekaterina Sokolova [a] and Sahar S. Dalahmeh[a]

[a]Department of Earth Sciences, Uppsala University, Uppsala, Sweden; [b]Division of Water Environment Technology, Chalmers University of Technology, Gothenburg, Sweden

ABSTRACT

Biochar is a promising material for wastewater treatment. This study assessed multi-module biochar filters (MmBFs) as onsite wastewater treatment systems (OWTSs), comprising movable modules filled with biochar to remove chemical oxygen demand (COD), nitrogen, phosphorus, and *Escherichia coli* (*E. coli*) in wastewater. The MmBF treats wastewater sequentially through six modules: three aerobic modules (M1-M3) for organic matter oxidation and nitrification, two anoxic modules (M4-M5) for denitrification, and an additional module (M6) for the removal of faecal bacteria using biochar and bark. The experiments ran for 381 days using three identical MmBF pilots with two distinct sampling periods, conducted under conditions relevant to OWTSs using municipal wastewater as influent. Water samples were taken from the influent, final effluent, and effluent of each module to evaluate the removal efficiency of organic matter, nitrogen, phosphorus, and *E. coli*. During the second sampling period, the results showed a 95 ± 2.1% removal of COD, along with a substantial removal of total inorganic nitrogen (71 ± 6.6%). However, phosphate removal was limited (3.4 ± 30.4%). *E. coli* removal decreased from 2.63 ± 0.93 $\log_{10}$ removal in the first sampling period to 1.8 ± 0.73 $\log_{10}$ removal in the second sampling period. In summary, the MmBFs showed promising potential in treating organic matter, nitrogen, and *E. coli*, making it an alternative option for OWTS. However, further exploration is needed to assess long-term performance, micropollutant removal, and biological activities. Design enhancements, especially for phosphorus removal are necessary.



ARTICLE HISTORY
Received 29 February 2024
Accepted 29 August 2024

KEYWORDS
Biochar; multi-module biochar filter; onsite wastewater treatment; organic matter; nitrogen

1. Introduction

Wastewater discharge from onsite wastewater treatment systems (OWTSs) is a substantial contributor to the pollution of many inland aquatic systems worldwide due to insufficient treatment and poor performance resulting from aging, malfunctioning, or inappropriate soil conditions [1, 2]. A study by Kinnunen et al. examined the effluent quality from 395 OWTSs in Sweden and Finland, revealing that 25% of Swedish systems had phosphorus levels and 10% had nitrogen levels that exceeded regulated limits [3]. In 2017, the anthropogenic net load of nitrogen (200 tons) and phosphorus (2,010 tons) from Swedish small-scale treatment systems to the surrounding sea basin accounted for 4% and 13% of the total anthropogenic load, respectively. In comparison, large-scale wastewater treatment plants contributed 28% and 15% [4]. This indicates that, even though fewer people use OWTSs, their nutrient leaching still poses a significant threat to the ecological health of the surrounding sea. In addition, it is estimated that around 40,000 people have unsafe drinking water wells near OWTSs due to high levels of *Escherichia coli* (*E. coli*) [5]. OWTSs have

CONTACT Makoto Shigei makoto.shigei@geo.uu.se Department of Earth Sciences, Uppsala University, Villavägen 16, SE-752 36 Uppsala, Sweden
Supplemental data for this article can be accessed online at https://doi.org/10.1080/09593330.2024.2402096

also been identified as significant sources of hazardous micro-pollutants such as pharmaceuticals and polyfluorinated alkyl substances [6].

The basic mechanism of traditional OWTSs involves a septic tank connected to a secondary treatment system. The secondary treatment system could be buried sand filters or soil infiltration systems that utilise the natural biological activity in the soil to treat wastewater [7]. Septic tank effluent is delivered by trench pipes to the sand filters or infiltration field. In these fields, biofilm formation, biodegradation, adsorption and precipitation aid in removing nitrogen and phosphorus [8]. However, the effectiveness of these systems is highly variable, often hindered by soil clogging issues [9]. Soil clogging in OWTS is primarily due to the accumulation of solids, biomat formation, and inadequate percolation, which restrict effluent dispersal and leads to system failure [9]. This challenge in OWTSs creates a demand for alternative filtration media that can maintain effective treatment capacity without the drawbacks of soil-based systems.

Biochar, a carbon-rich product derived from the thermal conversion of biomass, has gained significant attention as a potential alternative filtration medium for OWTS due to its high porosity, large specific surface area, and inherent ability to provide favourable environment for biofilm development essential for the biodegradation of organic pollutants. Furthermore, biochar has the potential to mitigate climate change impacts and promote circular economies by sequestrating carbon [10–12]. Moreover, the various chemical functional groups of biochar surfaces (e.g. hydroxyl, carboxyl, sulphonic acid group and amino functional groups) promote the adhesion of microorganisms on the surface of biochar [13, 14]. Surface adsorption onto biochar was reported as a predominant process for removing non-polar ions such as ammonium, phosphate, heavy metals, and pharmaceuticals [15]. Various factors including feedstock of biochar, pH, contact time, and the initial concentration of the pollutants influence the adsorption mechanisms [15].

Enaime et al. discussed the application of biochar for wastewater treatment, where it has several usages, such as support media during anaerobic digestion, filtration, and catalytic ozonation process [16]. Kaetzl et al. reported that, as a filtration support media, biochar had higher removal efficiency than sand with regard to chemical oxygen demand (COD) and *E. coli* in anaerobic biofilters [17]. Liang et al. tested a set of constructed wetlands under different ratios of biochar addition (0%, 10%, 20% and 30%) and found higher ammonium removal with increased biochar addition due to changes in the microbial community [18]. In addition, biochar could be used as a fertiliser after its use as a wastewater filtration medium [19, 20]. However, there is still limited research on the optimum design of biochar systems and their testing in pilot-scale experiments. Assayed et al. provided a new design of sand filter (Drawer Compacted Sand Filter; DCSF) which had several layers of sand placed in a movable drawer [21]. It showed high performance with 90% removal of organic matter, and the efficient design helped save space and provided easier maintenance for OWTSs [21]. The Multi-Soil-Layering (MSL) system, which has been under study since the nineteenth century, represents an advancement in soil infiltration technology designed to enhance permeability and treatment efficacy [22]. These systems employ layered configurations of soil mixture units and permeable layers of gravel or zeolite to create structured pathways that facilitate both aerobic and anoxic conditions. Previous studies focused on either horizontal flow MSL or vertical flow MSL. A recent study using a hybrid MSL system demonstrated significant removal of organic matter (79% COD removal), total nitrogen (27%), and phosphorus (76%), meeting regulations for reuse in non-potable water applications [23]. The positive outcomes of MSL systems and DCSF in improving infiltration and treatment have set a foundational premise for integrating layering structures into biochar-based pilot systems.

We developed the Multi-module Biochar Filter (MmBF) system to be operated after the sedimentation tank for onsite wastewater treatment. The MmBF system is a cabinet composed of movable drawers (modules) filled with biochar (Figure 1) at either aerobic or anoxic modules with aerobic conditions that have unsaturated downflow. The anoxic modules have saturated horizontal flow with low access to oxygen, to promote denitrification. The MmBF requires smaller space than soil-infiltration systems due to the compact design. Having removable modules facilitates the replacement of the biochar media when needed. It allows flexibility for adjusting the level of treatment according to wastewater quality and end-use of the treated wastewater. The advantage of this setup is that the system can be designed to achieve a certain quality of effluent water that makes it suitable for specific use. The main goal of this study was to investigate the performance of the MmBFs in removing different types of pollutants from wastewater. The specific objectives were to (i) present and describe the treatment components of the MmBF system, (ii) understand the hydraulic behaviour of the MmBF system, and (iii) explore the treatment efficiency of the MmBF system concerning the removal of organic matter, nitrogen, phosphorous, and *E. coli* from the wastewater. We hypothesise that the composition and surface properties of the biochar would provide a

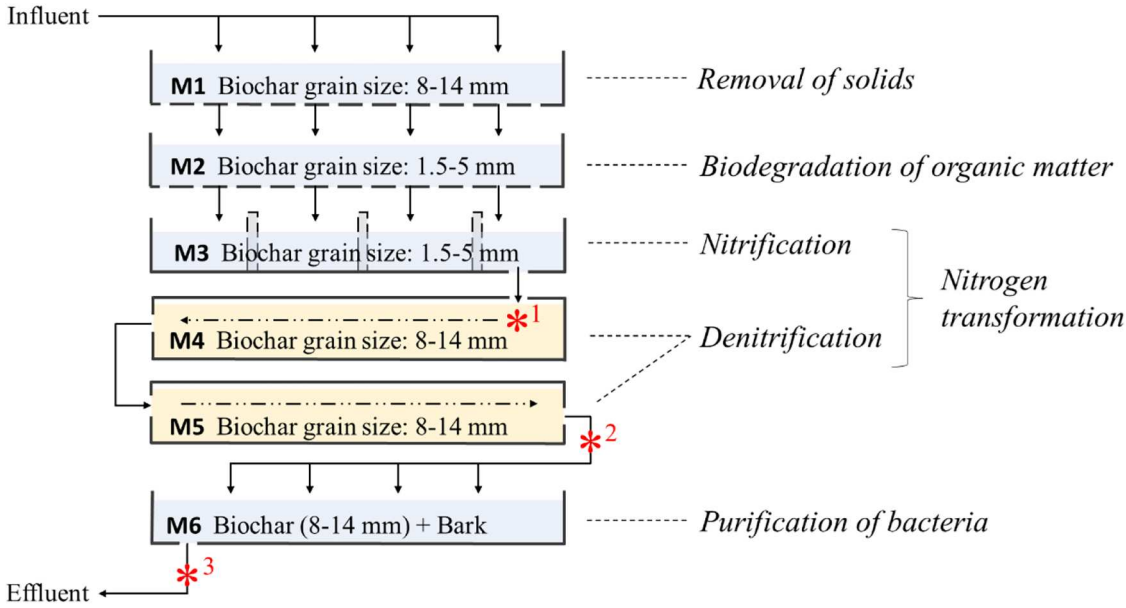


Figure 1. Schematic diagram of multi-module biochar filter (side view) with the contents and aim of each module (D: 20 cm W: 36 cm L: 56 cm). The points 1–3 denoted with *, are the points used for measuring electric conductivity (used for the hydraulic residence time calculation described in section 2.2). The dashed line in modules M1-M2 denotes the effluent openings/holes at the bottom of the modules. The vertical tubes with holes in them in M3 are used to force aeration of the module to improve nitrification.

high adsorption capacity and a favourable environment for microbial activity so that the MmBFs would remove organic matter (COD), nitrogen (Tot-N, NH_4-N, NO_3-N), phosphorous, and *E. coli* in the sequential treatment process with aerobic and anaerobic conditions.

2. Materials and methods

2.1. Description of Multi-module Biochar filter system (MmBF)

Three identical MmBFs composed of six modules were constructed, and each module was built from polyvinyl chloride plastic boxes (36 × 56 × 20 cm; surface area 0.20 m^2). Modules M1 to M5 were filled with biochar (see Table 1), while M6 was filled with a layer of pine bark topped with biochar. The bark enhances bacterial die-off due to tannins released from the bark [24]. The height of the media layer in each module was 15–17 cm. The biochar was commercially produced by Vindelkol AB (Umeå, Sweden) at 500°C for 8–14 hours, from hardwood. The bark was purchased from Plantagen Sverige AB (Uppsala, Sweden).

2.2. Determination of physical and hydraulic properties of biochar

The particle density (ρ_s), bulk density (ρ_b), and porosity (f) of solids were determined [25]. Calculations of the ρ_s, ρ_b, and f are described in section 2 of the supporting information (SI). The morphology of the biochar was analysed by a Zeiss Supra 35-VP (Carl Zeiss SMT, Oberkochen, Germany) field emission scanning electron microscope (SEM), equipped with a STEM detector for transmission microscopy and a Robinson BSD for backscatter detection. The EDAX Apex 4 (Amtekh, Mahwah USA) EDS-detector analysed the element composition for X-ray microanalysis. Element composition of the biochar surface was also analysed by Portable X-ray Fluorescence (pXRF) Spectrometry with a Bruker Tracer 5i XRF spectrometer (Bruker, Germany). The measured particle density (ρ_s) of the biochar was 0.45-0.78 g cm^{-3}. The mean (standard deviation) pore volume and porosity of modules 1–5 containing biochar were 25791 (1516) cm^3 and 80 (0.05) %, respectively. The elemental composition of the biochar, as determined by EDS was 86.1% C, 13.2% O, 0.2% K, and 0.4% Ca. SEM images are provided in Figure S1. The element composition measured >0.1% by portable XRF analysis was 0.5% Al_2O_3, 0.1% SiO_2, 0.2% K_2O, 0.9% Ca, 0.1% Mn, 0.1% Zn (Table S2).

The hydraulic residence time (HRT) of the system was determined through a tracer test conducted in MmBF 1 (Figure 2), based on the residence time distribution E(t) and cumulative residence time distribution F(t) of the tracer test data. Further information on the tracer test and the calculation of E(t) and F(t) can be found in S1 of SI. The electrical conductivity was measured at three specific sampling points (see the star symbol '*' in locations 1–3 in Figure 1). Point 1 was located at M3 and allowed for the determination of the HRT in the organic matter

Table 1. Description of biochar properties, operation condition, type of pollutants to remove, and treatment process in each module of the multi-module biochar filter system.

Module	Type of material	Particle size [mm]	Bulk density Mean ± SD [kg m^{-3}]	Porosity [cm^3 cm^{-3}]	Mode of flow	Organic loading rate [gCOD m^{-2} d^{-1}][a]	COD/ Inorganic N ratio[b]	Aeration condition	Expected treatment process and target pollutants
1 (Top)	Biochar	8–14	111 ± 1	0.75	Downflow, unsaturated	36	16.4	Aerobic	Sedimentation and biodegradation of solids and organic matter
2	Biochar	1.5–5	117 ± 1	0.85	Downflow, unsaturated	23	9.2	Aerobic	Oxidation of organic matter, Nitrification of ammonium
3	Biochar	1.5–5	116 ± 1	0.85	Downflow, unsaturated	11	6.1	Aerobic[c]	Nitrification of ammonium
4	Biochar	8–14	102 ± 0	0.78	Horizontal flow, saturated	5.2	6.4	Anoxic[d]	Denitrification of nitrate
5	Biochar	8–14	100 ± 0	0.78	Horizontal flow, saturated	2.4	5.3	Anoxic[d]	Denitrification of nitrate
6 (Bottom)	Biochar + Bark	8–14	546	0.78 (biochar)/ 0.32 (bark)[e]	Downflow, unsaturated	1.9	4.9	Aerobic	Inactivation of faecal bacteria

[a]Organic loading rate of period 2. See section 2.3 for further information on period 1.
[b]COD/Inorganic N was calculated from module specific data measured in period 2 (Table S5).
[c]The aeration in M3 was promoted by inserting three perforated pipes placed vertically through the filter media.
[d]To limit the oxygen access and achieve anoxic condition needed for denitrification, M4 and M5 modules were covered with lids to tightly close the modules.
[e]The porosity in the 6th module was calculated separately for biochar and bark.

oxidation and nitrification unit (M1-M3). Point 2, situated at the effluent of M5, enabled the determination of the HRT in both the nitrification and denitrification units (M1-M5). Point 3, located at the effluent of M6, facilitated the determination of the HRT of the entire system (M1-M6). For the calculation of the theoretical HRT from M4 to M5, the pore water volume was divided by the flow rate.

2.3. Wastewater flows and distribution system

Three identical MmBFs were deployed at the Uppsala wastewater treatment plant (Kungsängsverket) for the purpose of accessing real wastewater. The MmBFs received wastewater through intermittent dosing, with a total of five doses of 2 L each, administered every day between 8 AM and 12 PM. Each dose was delivered once per hour, with a duration of 15 minutes, using a set of peristaltic pumps (ALP09N, Albin Pump, France). Prior to the MmBFs, the raw wastewater underwent primary screening at the wastewater treatment plant, followed by the subsequent steps outlined below. (i) The wastewater was continuously pumped from the subterranean wastewater flow of the plant to a dosing tank (Figure 2). This dosing tank served the purpose of facilitating convenient access to the incoming raw wastewater. (ii) From the dosing tank, a peristaltic pump (Watson Marlow Peristaltic pump 520S, Fisher Scientific, Sweden) transferred 40 L of wastewater to the sedimentation tank once daily. The wastewater remained in the sedimentation tank for a duration of 16.5 hours. (iii) Following the sedimentation process, the wastewater was conveyed to each MmBF through a 2 L reservoir. The wastewater from the reservoir was pumped to the top module of each replicated system and then flowed downwards to the lower modules by gravity. The daily flow rate amounted to 10 L per day, resulting in a hydraulic loading rate (HLR) of 50 L m^{-2} d^{-1}. It is worth noting that this HLR aligns with the conventional sand filters for OWTSs [26]. The organic loading rate (OLR) applied to the top module was 12 gCOD m^2 d^{-1} in period 1 and 36 gCOD m^2 d^{-1} in period 2. Information on periods 1 and 2 is presented in the next section.

2.4. Filter operation, sample collection and analyses

The multi-module system was operated continuously starting from 25th January 2021. The pollutant removal in the multi-module systems was investigated during two separate sampling periods. The first period was from 1st February (day 1) until 7th April 2021 (day 66) as the initial trial period [27]. The second period was from 27th October 2021 (day 269) until 16th February 2022 (day 381) for assessment of long-term performance. The influent water sample from the sedimentation tank (Figure 2) and the effluent water sample from outlet M6 were collected once every week. Additional water samples from each module of M1-M6 were collected once every four weeks. The samples were analysed for

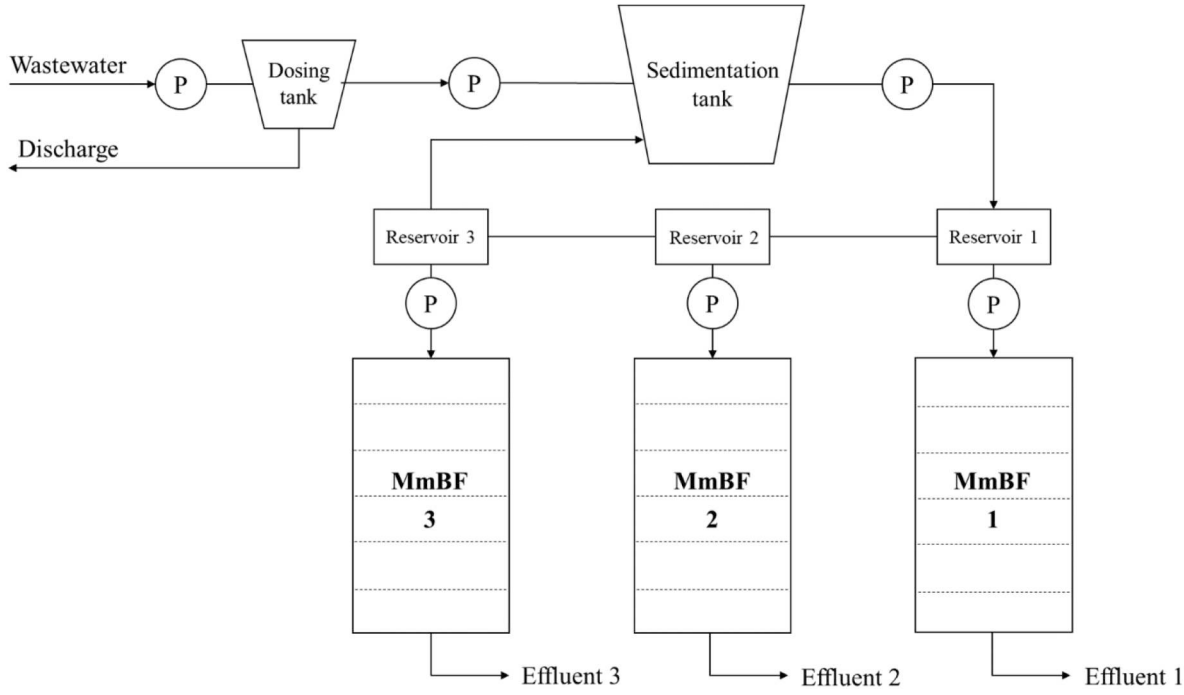


Figure 2. Schematic diagram of the experimental setup of the multi-module biochar filters (MmBFs), including the wastewater distribution system. Pump positions are indicated by letter P.

the following parameters: pH, total suspended solids (TSS), total volatile solids (TVS), chemical oxygen demand (COD), ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), total nitrogen (Tot-N), phosphate (PO_4-P), total phosphorus (Tot-P), and *E. coli*. Tot-N was not measured during sampling period 2.

2.5. Methods of chemical and microbiological analyses

The pH was measured using a pH meter (AE150 pH Benchtop Meter, Fisher Scientific). TSS and TVS of the water samples were determined according to the methods 2540 D and 2540 E of the Standard Method for the Examination of Water and Wastewater [28]. The concentrations of COD, NH_4-N, NO_3-N, NO_2-N, Tot-N, PO_4-P, and Tot-P were determined for the influent and effluents by using the Spectroquant® test kits for COD (detection range 100–1500 mg L^{-1} and 10–150 mg L^{-1}, respectively), NH_4-N (detection range = 2.0-150 mg-N L^{-1}), NO_3-N (detection range = 0.1-25.0 mg-N L^{-1}), NO_2-N (detection range = 0.007-3.28 mg-N L^{-1}), Tot-N (detection range = 0.1-25.0 mg-N L^{-1} as measured with NO_3-N kit after the digestion), PO_4-P (detection range = 0.01-5.0 mg-P L^{-1}), and Tot-P (detection range = 0.01-5.0 mg-P L^{-1} as measured with NO_3-N kit after the digestion), respectively (Merck KGaA, Darmstadt, Germany), followed by colorimetric analysis using a Nova 60 photometer (Merck KGaA, Darmstadt, Germany). The kit information is provided in Table S1. The analytical quality was tested using blank samples and control standard solutions of known substance concentrations for every measurement series. Data values below the method detection limit are presented as a value at the detection limit. To analyse *E. coli*, the water sample was diluted 10–10^4 times in phosphate-buffered saline solution with the detergent Tween®20 (SVA, Sweden). A 100 L sample of each dilution was added to Petri dishes of Chromocult agar (Miclev, Sweden). The *E. coli* plates were incubated at 37°C for 24 h. Colonies were enumerated after incubation up to 200 colony-forming units (CFU) mL^{-1}. Counts above 200 were considered too numerous to count due to the difficulty of identifying individual colony-forming units.

Removal efficiency (%) was calculated from the influent concentration and effluent concentration using Equation 1 where C_{in} is the influent concentration and C_{out} is the effluent concentration. The removal rate was calculated using Equation 2 where HLR is 50 L m^{-2} $d-^1$. The removal rate per HRT cycle of functional unit (M1-M3, M4-5, M6; g m^{-2}) was calculated by multiplying each functional unit's HRT to the whole removal rate.

$$\textit{Removal efficiency}\ (\%) = \left(1 - \frac{C_{out}}{C_{in}}\right) \times 100 \qquad (1)$$

$$\textit{Removal rate}\ (g\ m^{-2}\ day^{-1}) = (C_{in} - C_{out}) \times HLR \qquad (2)$$

2.6. Statistical analyses

An analysis of one-way ANOVA followed by Tukey's multiple comparisons test at a 95% confidence level was employed to assess variations in effluent concentrations among the three MmBFs for each analysed pollutant. In addition, a comparison of mean concentrations during sampling periods 1 and 2 was carried out using unpaired T-tests (two-tailed, 95% confidence level) for each pollutant's concentration in both the influent and effluent of each MmBF. To ensure the validity of these tests, several assumptions such as normal data distribution and equal variance were visually inspected through quantile-quantile plots, homoscedasticity plots, and residual plots. All statistical analyses were conducted using GraphPad Prism version 9.1.0 for Windows (GraphPad Software, San Diego, California, USA).

3. Results and discussion

3.1 Hydraulic residence time

The average HRT calculated by using the residence time distribution E(t) was: 168 h for M1-M3, 189 h for M1-M5, and 324 h for M1-M6 (Figure 3). The resulting average HRT for each functional unit was 168 h for the organic matter oxidation and nitrification unit (M1-M3), 21 h for the denitrification unit (M4-M5), and 135 h for the bacteria purification unit in M6. The theoretical residence time in total for M4 and M5 (saturated modules) was 152 h which is much longer than the measured HRT of 21 h. This suggests that water may have followed a preferential flow path near the surface of the biochar, and not through the bulk of the media.

3.2 General water quality and removal of organic matter

The pH in the MmBF system was stable (7-8) during the whole experiment period and there was no observation of significant pH change between the influent and effluent (Table S3). The mean electrical conductivity was 1.12 ms cm^{-1} in the influent and 0.88 ms cm^{-1} in the effluent. The slight removal in electrical conductivity in the MmBF is likely due to biochar's adsorption and ion exchange capabilities, retention of particulate matter, and microbial activity, all of which reduce the concentration of dissolved ions.

The reported BOD concentration in septic tank effluent in Sweden varies significantly, ranging from 1.1 to 950 mg L^{-1} depending on the facility [29, 30], and their mean concentration ± standard deviation (86.3 ± 82.3 mg L^{-1}) is generally lower than the influent COD concentration entering the MmBFs [30]. The mean concentration of COD ± standard deviation (SD) in the influent during period 1 was 239 ± 71 mg L^{-1}, which was significantly lower than that of period 2 (712 ± 240 mg L^{-1}; $p < 0.0001$; Table S3), though the influent COD concentrations in period 2 fluctuated

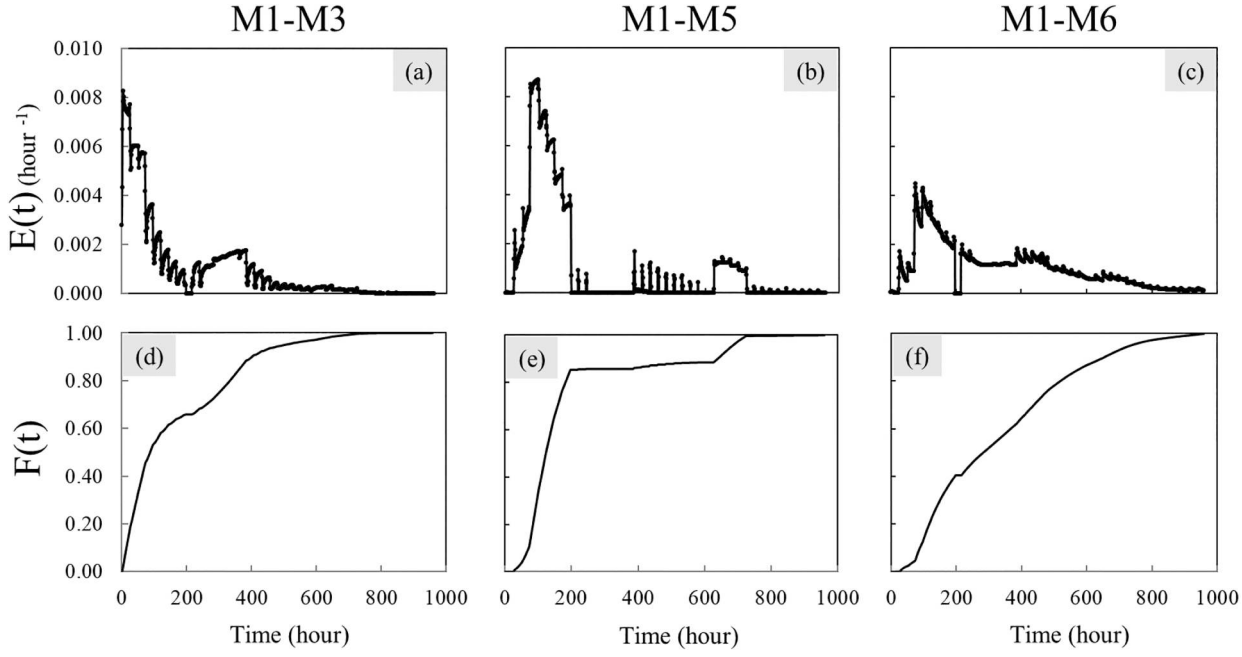


Figure 3. Residence time distribution E(t) and cumulative residence time distribution curve F(t) of the multi-module systems as measured in filter 1. The operation was interrupted during 264–360 hours due to a technical problem. The zero values in figure b and c indicate lack of data.

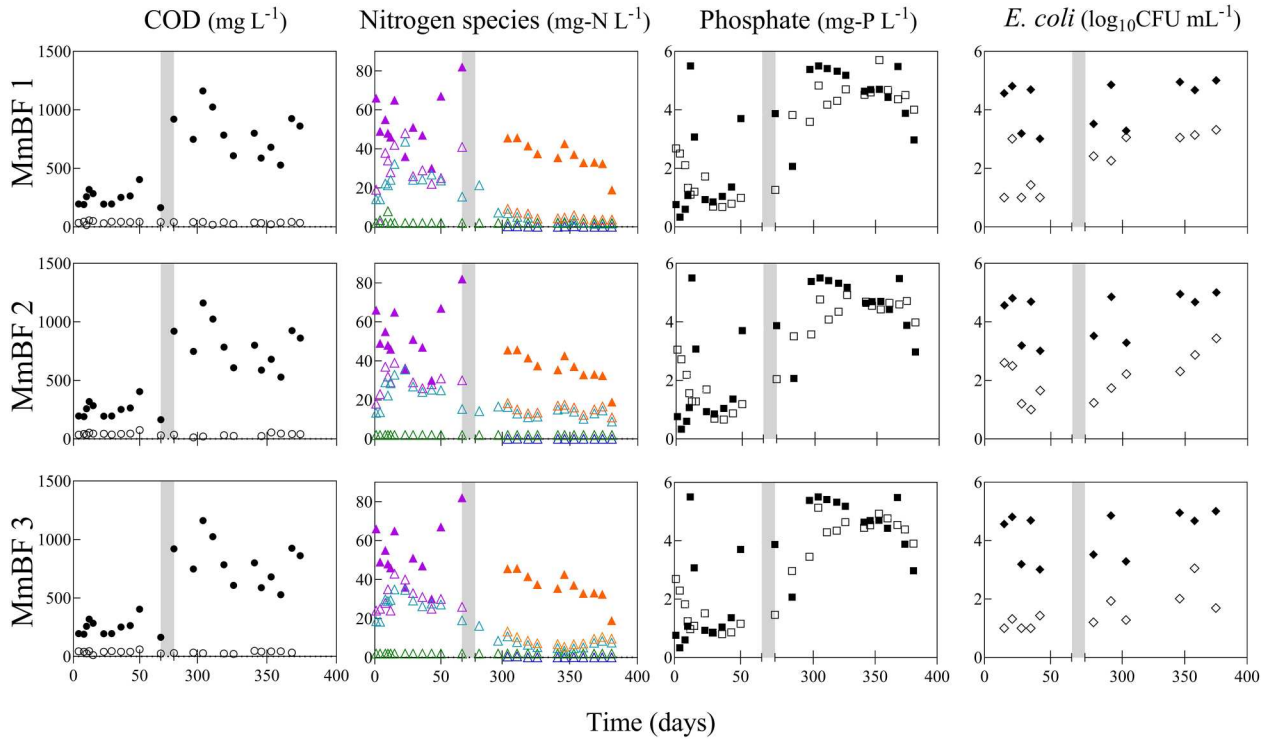


Figure 4. Concentration of COD (●, ○), nitrogen species (▲, △), phosphate (■, □), and *E. coli* (◆, ◇) in influent (solid symbols) and effluent (empty symbols) of the multi module biochar filter (MmBF). The nitrogen species include total nitrogen (▲, △), total inorganic N (▲, △), NH_4-N (△), NO_2-N (△) and NO_3-N (△), with their respective colors represented in the figure as purple, orange, green, blue, and light blue. MmBF 1, 2, and 3 are the identical replicates of the multi-module systems, all operated at a hydraulic loading rate of 50 L m^{-2} d−^{1}. The grey bar shows the gap between sampling periods 1 and 2. Tot-N was analysed only during the sampling period 1, and NO_2-N was analysed only during the sampling period 2, so the concentration of total inorganic N is shown for sampling period 2 by summing up the concentration of NH_4-N, NO_3-N, and NO_2-N. The effluent concentration of NH_4-N is reported as detection limit (2 mg-N L^{-1}) when it was under the detection limit. Detailed information is reported in the Table S3 in SI.

between 528 and 1162 mg L^{-1} (Figure 4). The low influent COD concentrations during period 1 could be attributed to the use of a test kit with a low detection range of 10–150 mg L^{-1}. The COD concentration was measured with a 3 mL sample in the test cell after two-fold dilution. Since the distribution of TSS in the sample may not have been homogeneous, COD concentrations may reflect the sample surface where samples for dilution were obtained, but not the bulk solution.

The mean COD concentration in the effluents was 35.7 ± 11.3 mg L^{-1} for the MmBFs 1–3 throughout periods 1 and 2, with no statistical difference in the concentrations among the triplicate MmBFs nor the sampling periods ($p > 0.05$). The resulting mean COD removal in period 1 was 83 ± 4% (removal rate of 9.9 ± 3.3 g m^{-2} d^{-1}). In contrast, for period 2, the mean COD removal was 95 ± 2% for MmBFs 1-3, corresponding to a removal rate of 32.4 ± 11.4 g m^{-2} d^{-1} (Table S4). According to the results of period 2, the top three modules M1-M3 achieved most of the COD removal as intended, accounting for 84% of the total COD removal with a removal rate of 241.1 g m^{-2} per cycle for M1-M3. In contrast, module M6 contributed minimally to the overall COD removal, with only 0.1 g m^{-2} per cycle of M6 (Figure 5). It should be pointed out that the OLR on M1, M2 and M3 (36, 23, and 11 gCOD m^{-2} d^{-1}, respectively) are considered sufficient to sustain active biofilm in these modules. The TSS concentration measured in period 2 was 213 ± 82 mg L^{-1} in the influent and 25 ± 5.1 mg L^{-1} in the effluents (Table S3), resulting in a mean removal of 88 ± 4% (Table S4).

The removal of COD is expected to be achieved mainly through the mineralisation of organic matter in the biofilm. A small fraction of the organic matter could have been removed in the upper module by adsorption during the initial period of operation, as suggested by Khurshid et al. [31] The SEM picture of used biochar in MmBFs showed the developed biofilm on the biochar (Figure S1). The C/N/P ratio (COD/Inorganic N/ Phosphate) in the influent of MmBFs was 712:36:4 = 178:9:1 in period 2 which is compatible to the study of biological nutrient removal in sequencing batch reactor (C/N/P = 56:7:1) especially when it is lower C/N/P ratio after the aerobic modules [32]. The

COD removal reported in the current study was comparable to that reported in the study of Kaetzl et al. [17], who tested municipal wastewater treatment with anaerobic biofilters using biochar from *Miscanthus* grass, resulting in 74 ± 18% COD removal. Their filter bed depth was 60 cm of 5 cm diameter column and the HLR rate was 1250 L m^{-2} d^{-1}, which is about 25 times higher than the present study (50 L m^{-2} d^{-1}). Kaetzl et al. [17] discussed their OLR being too high to achieve sufficient operation time (366 ± 126 gCOD m^{-2} d^{-1}) and the need for a lower HLR and a larger area of the filter. The lower OLR in the present study likely explains its more efficient COD removal. Perez-Mercado et al. [33] also tested the column-type filter with pine-spruce biochar with a comparably low load (OLR of 20 ± 5 g BOD_5 m^{-2} d^{-1} and HLR of 34 L m^{-2} d^{-1}) and achieved over 90% COD removal.

3.3 Transformation of nitrogen species

The total nitrogen concentration in the influent in period 1 was 52 ± 15 mg-N L^{-1}. Most of it was inorganic in the form of NH_4-N (51 ± 18 mg-N L^{-1}; Table S3). The influent concentration of NH_4-N showed a significant difference between the sampling periods 1 and 2 (51 ± 18 mg-N L^{-1} and 36 ± 12 mg-N L^{-1}, respectively; $p = 0.0096$). In-house measurements at the treatment plant showed higher Tot-N concentrations in period 2, indicating potential variations in influent organic nitrogen content (Table S6). The reported mean Tot-N concentration in Swedish septic tank effluent is 45 ± 30 mg-N L^{-1} [29] and is similar to the influent concentration measured in our study.

Ammonium was consistently removed at nearly 100% during sampling periods 1 and 2, with no difference in the performance of the three replicates ($p > 0.05$; Figure 4). Most of the ammonium was nitrified into nitrite and nitrate in the upper aerated modules (see Figure 5), but some biological assimilation into biomass likely occurred. While previous studies have reported the high adsorption capacity of biochar for ammonium [15, 34], adsorption was likely minor in the MmBF as most ammonium was converted to nitrate (Figure 5). As for the performance of the different modules in nitrogen transformation, the module-specific sampling showed that 54% of the NH_4-N was removed in M1 to M2 in MmBF 1 and 88% was removed in MmBF 2 and 3, with corresponding increases in NO_2-N and NO_3-N concentrations (Figure 5, Table S5).

Module M2 in MmBF 1 had a higher COD concentration (Table S5), leading to a higher C/Inorganic N ratio (11) than in the other two MmBFs that had a C/Inorganic N ratio of 8. The higher ratio in M2 of MmBF 1 likely resulted in a lower nitrification rate due to competition for oxygen between nitrifying– and aerobic heterotrophic bacteria [35]. However, the long HRT (168 h) of all the unsaturated upper modules (M1-3) was apparently sufficient for efficient ammonium oxidation in all three MmBFs (Figure 5).

More effective denitrification was achieved in period 2 (71 ± 7% removal of inorganic N) compared to period 1 (41 ± 18% removal of Tot-N; Table S4), leading to a lower mean effluent concentration of NO_3-N in period 2 (8.1 ± 3.7 mg-N L^{-1}) than that of period 1 (24.1 ± 7.0 mg-N L^{-1}; Table S3; $p < 0.0001$). The improved denitrification could be due to the higher COD concentration in period 2 (OLR = 36 gCOD m^{-2} d^{-1}), providing more

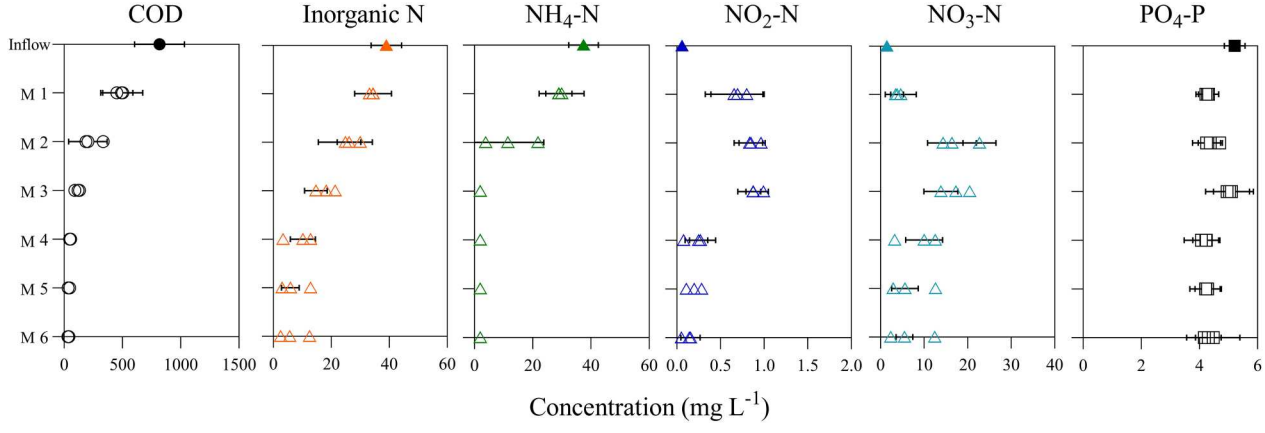


Figure 5. Concentrations of COD (●, ○), Inorganic N (▲, △), NH_4-N (▲, △), NO_3-N (▲, △), NO_2-N (▲, △), and PO_4-P (■, □) in the influent (solid symbol) and in the water collected in the outlet of each module M1-M6 (empty symbols) The three data points and error bars for each module show the mean concentrations and standard deviations of each module of three filters through the sampling occasions (n = 3 for Inorganic N, NH_4-N, NO_3-N, and NO_2-N, n = 4 for COD and PO_4-P). The error bar is not shown if it is shorter than the symbol size.

carbon for denitrification than what was available in period 1 (OLR = 12 gCOD m^{-2} d^{-1}). The mean COD/Inorganic N ratio of the wastewater from M3 to M4 across three MmBFs in period 2 was 6 (Table 1) which was lower than the several studies regarding sequencing batch reactors [32, 36] that achieved higher nitrogen removal with C/N ratio of 8-10. The efficiency of denitrification in MmBF 2 in period 2 was lower than in the other two MmBFs (Table S4), as NO_3-N in the effluent of MmBF 2 (13.3 mg-N L^{-1}) was significantly higher than that of MmBF 1 (3.8 mg-N L^{-1}; $p < 0.0001$) and MmBF 3 (7.1 mg-N L^{-1}; Table S3; $p = 0.0009$). The lower denitrification efficiency in MmBF 2 (39 ± 6%, Table S4) could partly be caused by a lower influent concentration of COD to M4 (92 mg L^{-1}, Table S5). This resulted in a difference in the COD/Inorganic N ratio among three MmBFs in the water entering M4, with a ratio of 3.9 in MmBF 2 compared to 7.2 and 7.1 in MmBF 1 and MmBF 3, respectively (Table S5). All three MmBFs had similar low effluent COD concentrations (35.7 ± 11.3 mg L^{-1}), suggesting that the remaining COD was not bioavailable. Despite that M4 and M5 were designed to have anoxic environments to promote denitrification, the denitrification was mainly achieved in M4, and there was no substantial decline in inorganic N concentrations after M4 (Figure 5; Table S5). The heterotrophic bacteria in M4 may have consumed most of the readily available organic matter coming from M3 (OLR = 5 gCOD m^{-2} d^{-1}; 44–276 mgCOD L^{-1}), so denitrifiers in M5 were left short of readily available organic carbon (influent to M5 contained 40–55 mgCOD L^{-1}), limiting further denitrification in M5.

The HRT of the denitrification unit (M4 and M5) was 21 h, while the theoretical HRT through M4 to M5 was 152 h. The relatively short HRT in the modules M4 and M5 suggests that there was preferential flow in the saturated modules. Denitrification could be enhanced by preventing preferential flow and thus increasing contact time with denitrifying bacteria. One possible solution could be adding baffles in M4 and M5.

It should be noted that there was no external carbon source added to the denitrification module (M4-M5) in our study. Zhou et al. [37] reported that the adsorption–desorption of organics on biochar and dissolved organic matter released from biochar were the main contributing factors for denitrification. In our study, the organic matter originating from the wastewater or from the decaying bacteria was likely a limiting factor for denitrification. Hence, nitrogen removal could be increased by supplying external carbon. In future studies, it would be interesting to amend the biochar in M4 and M5 with nature-based organic media such as bark, wood chips, sedge or even compost to allow a release of organic carbon in M4 and M5. Hellman et al. [38] investigated how different types of substrates, including bottle sedge, barley straw, and pine woodchips, influenced microbial activity and community composition in bioreactors for nitrate removal in water at low temperatures (10°C). Their result showed that the highest nitrate removal rates were obtained in the reactors amended with sedge and straw [38]. With an organic amendment, a more efficient use of the entire filter, including M5, may be possible. The performance of the three MmBFs was reproducible when it came to organic matter and ammonium removal, as evidenced by the minor variations in effluent concentrations (Table S3), and all had a steady pH of 7-8. However, denitrification was indeed less reproducible (Table S4). A steady supply of an organic substrate would possibly contribute to more stable denitrification with less variation between the MmBFs.

3.4 Removal of phosphorus and E. coli

The incoming wastewater had a phosphate concentration of 1.25 ± 1.63 mg-P L^{-1} during sampling period 1 and 4.04 ± 1.03 mg-P L^{-1} during sampling period 2 which was significantly higher than period 1 ($p < 0.0001$, Table 1). During period 1 sampling, the influent had lower phosphate concentrations compared to the effluent, particularly during the first 10 days (Figure 4). Higher effluent than influent concentrations were also observed during period 2 from several samplings (Figure 5). The observed release of phosphate from the filter could be attributed to that the filters were previously tested for a short period at a local household, and the restart of operation in period 1 may have washed out desorbed phosphate and sloughed biofilm. The low adsorption capacity of phosphorus in MmBFs can be explained by the element composition of the biochar used, which consisted of 86.1% carbon, 13.2% oxygen, 0.24% potassium, and 0.42% calcium, with only trace fractions of other metals. The low metal content may have contributed to the low adsorption capacity since metals in biochar materials promote chemical reactions that facilitate the formation and adsorption of metal phosphate compounds onto the biochar surface [39, 40].

The MmBF design did not have a specific module targeted for phosphorus removal, but it would be advisable to include one module for phosphorus removal by metal-promoted adsorption. Alternatively, the biochar used in this study was commercially made of hardwood such as oak or ash but it could be substituted with corn cob biochar, which has been reported by Kizito et al. [41]

to exhibit high adsorption capacities for phosphate, measured at 3.3 ± 0.6 mg-P per g of biochar. Vidal et al. studied nine different OWTSs in Sweden regarding phosphorus removal [42]. They reported Tot-P concentration in the septic tank effluent in the range of 6-29 mg-P L^{-1}, indicating that improving phosphorous removal is indeed an important treatment goal [42]. Future studies could focus on improving the performance of the MmBF by exploring different filter materials for phosphorus removal such as modified biochar or the biochar made of different feedstock.

The mean effluent concentration of *E. coli* was 1.30 ± 0.49 log_{10}CFU mL^{-1} during period 1 and 2.22 ± 0.61 log_{10}CFU mL^{-1} during period 2 (Figure 4), with the difference between the two periods not being significant ($p = 0.0978$). The removal efficiency between the influent and the effluent was 2.3 ± 0.9 log_{10} units during period 1 and 1.8 ± 0.7 log_{10} units during period 2 (Table S4). Inactivation of bacteria was planned to occur in the last module (M6), which utilises a combination of biochar and bark. The bark serves as a significant source of antibacterial phenolic compounds known as tannins, which were expected to deactivate bacteria upon reaching the final module as suggested by Das et al. [43] and Kaczmarek [24]. In addition to its chemical properties, biochar also possesses physical filtration capabilities due to the presence of micropores in the biochar (Figure S1). This enables biochar to effectively retain bacteria through physical removal. Hence, during the transport through the MmBFs, *E. coli* may well have been removed in the modules prior to M6. It is important to acknowledge that the MmBFs exhibited a long HRT across all modules (M1-M6), lasting 324 hours. The longer HRT could extend the contact time with the biochar and facilitate physical filtration and adsorption [44]. Previous studies utilising biochar in stormwater biofilters have reported higher removal of *E. coli* by biochar amended filters (2.32 log_{10} unit removal) than by sand filters (0.29 log_{10} unit removal) [45]. A prolonged operation of the system may reduce the effectiveness of tannin, as indicated by a slight increase in the effluent concentration of *E. coli* during period 2 (Figure 4). However, with longer operation periods, a more developed biofilm may have formed, making it challenging to determine if tannin alone contributed to the removal of *E. coli*. It should be noted that module-specific sampling for *E. coli* was not conducted in this study. Therefore, it is difficult to determine which module was primarily responsible for *E. coli* removal in the MmBFs.

3.5 General discussion

The MmBF system is designed as a secondary treatment unit in an OWTS. Thus, in a real onsite application, the influent wastewater quality would be similar to septic tank effluent, even though the pilot MmBF in this study used municipal wastewater from a large-scale treatment plant. The MmBFs operated for 381 days without any signals of clogging or deterioration in COD and nitrogen removal. The removal efficiency of COD and nitrogen remained stable throughout period 2 with lower variability compared to period 1 (Table S4). The long-term performance of the MmBF system was very good when compared to other conventional OWTSs (see Table 2) such as drawer compacted sand filters (operational period 110 days, [21]), multi-soil layering system (583 days, [23]), and sand filters (7days, [8]). Furthermore, the MmBF system performed better than the OWTS guidelines of the Swedish Environmental Protection Agency (EPA) (Table 2) for COD and nitrogen.

According to the Swedish Agency for Marine and Water Management, the estimated daily wastewater volume from individual water consumers is 170 L [46]. The configuration of the tested MmBF occupied an area of approximately 4 m^2, encompassing the filter triplicates, pumps, and sedimentation tank. While design specifics should be tailored to individual cases and account for variables like resident count, HLR, and wastewater characteristics, the present MmBF configuration necessitates a filter surface area of 10–17 m^2 to

Table 2. Pollutant removal efficiency of MmBF and other OWTSs with the required efficiency by the Swedish EPA (%).

	HLR (L m^{-2} d^{-1})	COD	Nitrogen	Phosphorus	*E. coli*	Source
MmBF[A]	50	95	71	3	98.4	Present study
Swedish EPA requirement[B]	-	90	(50)	70 (90)	N/A	[46]
Drawer Compacted Sand Filter	72	94	N/A	N/A	99.9	[21]
Multi-Soil Layering[C]	250	79	27	76	99.9	[23]
Sand filter	270	82	N/A	65	N/A	[8]
Sandfilter + *P*-filter[SE]	N/A	99	N/A	96	99.9	[47]
Package plant + coagulant[SE]	N/A	96	N/A	78	96.8	[47]

[A]The MmBF results are from period 2, nitrogen data is shown as inorganic N concentration and phosphorus data is shown as phosphate removal.
[B]The values in parentheses are for areas with sensitive land use.
[C]The *E. coli* removal was converted from the reported 2.88 log_{10} unit to %.
[SE]Monitoring studies of Swedish full-scale OWTSs used year-round, covering different seasons. COD column shows BOD reduction.

effectively manage daily loads from households of three to five residents with the same HLR condition as in this study (50 L m^{-2} d^{-1}). There exists a compelling need to assess the system's performance under elevated HLR and ascertain the system's corresponding capacity at the conditions applied in other studies (Table 2), in order to provide insights into the scalability and adaptability of the system. The MmBF system showed promising results in removing *E. coli*. However, to guarantee long-term microbial quality and maintain high removal efficiencies, periodic maintenance, media replacement, and potential design optimisations are recommended since period 2 had a lower *E. coli* removal than period 1.

Additionally, while the MmBFs are presented in this study as systems for household wastewater including summer houses or hostels in rural areas, there is also a potential for utilising such systems in different settings. For example, the systems could be installed in the basement of multi-storey buildings and the treated water could be recycled for irrigation. The various applications of MmBF system would contribute to decentralisation, resource recovery and mitigation of climate change impact that are major goals for new development in the urban and peri-urban areas of Sweden and other places.

4. Conclusions

The pilot-scale multi-module biochar filter (MmBF) system which consists of six modules (M1-M6) for onsite wastewater treatment was subjected to triplicate testing using municipal wastewater. The MmBF systems exhibited notable efficacy in the removal of organic matter and *E. coli*, while nitrogen removal ranged from 40% to 80%. The results revealed that organic matter degradation primarily occurred in modules M1-M2, nitrogen removal through nitrification in M2-M3 and denitrification in M4-M5. However, phosphorus removal was insufficient and the use of modified biochar may be necessary in future studies. The modular design of the MmBF system, with specific objectives for each module, demonstrated that most of the treatment occurred between M1 and M4. For future work, it is recommended to investigate the microbial community in each module, consider the addition of an external carbon source to improve denitrification, perform module-specific analysis of *E. coli* concentrations, and explore different configurations of the MmBF system to enhance phosphorus removal.

Acknowledgements

This work was funded by the Swedish Research Council (VR) (project grant number 2018-05791) and the Swedish Research Council for Sustainable Development FORMAS (project grant number 2019-01257 and 2018-00261). The authors extend their gratitude to Uppsala Vatten och Avfall AB for facilitating the pilot MmBFs within the Kungsängsverket wastewater treatment plant. Special thanks are extended to Mr. Oscar Gotlind for his assistance during the experiment for the operation and troubleshooting.

Author Contributions by CRediT

M. Shigei: conceptualisation, methodology, formal analysis, investigation, visualisation and writing – original draft preparation; R. Herbert: supervision and writing – reviewing and editing; F. Persson: supervision and writing – reviewing and editing; E. Sokolova: writing – reviewing and editing; S. Dalahmeh: supervision, conceptualisation, methodology, writing – reviewing and editing and funding acquisition.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Swedish research council for sustainable development (Svenska Forskningsrådet Formas): [Grant Number 2018-00261,2019-01257]; Swedish Research Council (Vetenskapsrådet): [Grant Number 2018-05791].

Data availability statement

The data that support the findings of this study are available from the corresponding author, MS, upon reasonable request.

ORCID

Makoto Shigei http://orcid.org/0000-0001-7785-0044
Roger B. Herbert http://orcid.org/0000-0002-7561-757X
Frank Persson http://orcid.org/0000-0002-0269-9375
Ekaterina Sokolova http://orcid.org/0000-0002-5715-8007

References

[1] Roth S, Ejhed H, Olsson öberg M, et al. Maintenance regulation of small wastewater treatment facilities. Stockholm: IVL Swedish Environmental Research Institute; 2016.
[2] Swedish EPA. Wastewater Treatment in Sweden 2020 Swedish EPA publications 2020.
[3] Kinnunen J, Rossi PM, Herrmann I, et al. Factors affecting effluent quality in on-site wastewater treatment systems in the cold climates of Finland and Sweden. J Clean Prod. 2023;404:136756, doi:10.1016/j.jclepro.2023.136756
[4] SwAM. Näringsbelastningen på Östersjön och Västerhavet 2017 [The nutrient load on the Baltic Sea and the North Sea] . Swedish Agency for Marine and Water Management. 2017.(In Swedish)

[5] Swedish EPA. Miljömålen : svensk konsumtion och global miljöpåverkande Facto2010 [Environmental goals: Swedish consumption and global environmental impact Fact2010]. Stockholm: Swedish EPA Publications; 2010.(In Swedish)
[6] Schaider LA, Rodgers KM, Rudel RA. Review of organic wastewater compound concentrations and removal in onsite wastewater treatment systems. Environ Sci Technol. 2017;51:7304–7317. doi:10.1021/acs.est.6b04778
[7] Beal CD, Gardner EA, Menzies NW. Process, performance, and pollution potential: A review of septic tank – soil absorption systems. Soil Res. 2005;43:781, doi:10.1071/SR05018
[8] Bali M, Gueddari M. Removal of phosphorus from secondary effluents using infiltration–percolation process. Appl Water Sci. 2019;9:54, doi:10.1007/s13201-019-0945-5
[9] McKinley JW, Siegrist RL. Soil clogging genesis in soil treatment units used for onsite wastewater reclamation: a review. Crit Rev Environ Sci Technol. 2011;41:2186–2209. doi:10.1080/10643389.2010.497445
[10] He M, Xu Z, Hou D, et al. Waste-derived biochar for water pollution control and sustainable development. Nat Rev Earth Environ. 2022;3:444–460. doi:10.1038/s43017-022-00306-8
[11] Quispe JIB, Campos LC, Mašek O, et al. Use of biochar-based column filtration systems for greywater treatment: A systematic literature review. J Water Process Eng. 2022;48:102908, doi:10.1016/j.jwpe.2022.102908
[12] Xiang W, Zhang X, Chen J, et al. Biochar technology in wastewater treatment: A critical review. Chemosphere. 2020;252:126539, doi:10.1016/j.chemosphere.2020.126539
[13] Bolan S, Hou D, Wang L, et al. The potential of biochar as a microbial carrier for agricultural and environmental applications. Sci Total Environ. 2023;886:163968, doi:10.1016/j.scitotenv.2023.163968
[14] Wang X, Guo Z, Hu Z, et al. Recent advances in biochar application for water and wastewater treatment: a review. PeerJ. 2020;8:e9164, doi:10.7717/peerj.9164
[15] Dai Y, Wang W, Lu L, et al. Utilization of biochar for the removal of nitrogen and phosphorus. J Clean Prod. 2020;257:120573, doi:10.1016/j.jclepro.2020.120573
[16] Enaime G, Baçaoui A, Yaacoubi A, et al. Biochar for wastewater treatment—conversion technologies and applications. Appl Sci. 2020;10:3492, doi:10.3390/app10103492
[17] Kaetzl K, Lübken M, Nettmann E, et al. Slow sand filtration of raw wastewater using biochar as an alternative filtration media. Sci Rep. 2020;10:1229, doi:10.1038/s41598-020-57981-0
[18] Liang Y, Wang Q, Huang L, et al. Insight into the mechanisms of biochar addition on pollutant removal enhancement and nitrous oxide emission reduction in subsurface flow constructed wetlands: microbial community structure, functional genes and enzyme activity. Bioresour Technol 2020;307:123249, doi:10.1016/j.biortech.2020.123249
[19] Li S, Chan CY, Sharbatmaleki M, et al. Engineered biochar production and its potential benefits in a closed-loop water-reuse agriculture system. Water (Basel). 2020;12:2847, doi:10.3390/w12102847
[20] Werner S, Kätzl K, Wichern M, et al. Agronomic benefits of biochar as a soil amendment after its use as waste water filtration medium. Environ Pollut. 2018;233:561–568. doi:10.1016/j.envpol.2017.10.048
[21] Assayed A, Chenoweth J, Pedley S. Drawer compacted sand filter: a new and innovative method for on-site grey water treatment. Environ Technol. 2014;35:2435–2446. doi:10.1080/09593330.2014.909886
[22] Chen X, Luo AC, Sato K, et al. An introduction of a multi-soil-layering system: a novel green technology for wastewater treatment in rural areas. Water Environ J. 2009;23:255–262. doi:10.1111/j.1747-6593.2008.00143.x
[23] Zidan K, Mandi L, Hejjaj A, et al. Efficiency of a new hybrid multi-soil-layering eco-friendly technology for removing pollutants from domestic wastewater under an arid climate. J Water Process Eng. 2023;51:103482, doi:10.1016/j.jwpe.2022.103482
[24] Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials—a minireview. Materials (Basel). 2020;13:3224, doi:10.3390/ma13143224
[25] Hillel D. Introduction to soil physics. Cambridge, MA: Academic press; 1982.
[26] US EPA. Onsite wastewater treatment systems manual - Revised February 2002. US: National Service Center for Environmental Publications (NSCEP); 2003.
[27] Shigei M. Multi-module biochar filter for on-site wastewater treatment [Internet]. Uppsala: SLU, Dept. of Energy and Technology; 2021 [cited 2024 Apr 17]. Available from: https://stud.epsilon.slu.se/17357/.
[28] Baird RB., Eaten A.D., Rice E.W.. Standard Methods For The Examination Of Water And Wastewater. 23Ed. US: American Water Works Association (AWWA, WEF and APHA); 2017.
[29] Vidal B.. Avloppsvattens inkommande och utgående koncentrationer från minireningsverk i norra Sverige och Finland (Version 1) [Influent and effluent concentrations of wastewater from small-scale treatment plants in northern Sweden and Finland.] [Dataset].2023. Luleå tekniska universitet. doi:10.5878/48t5-qm50
[30] Vidal B, Kinnunen J, Hedström A, et al. Treatment efficiency of package plants for on-site wastewater treatment in cold climates. J Environ Manage 2023;342:118214, doi:10.1016/j.jenvman.2023.118214
[31] Khurshid H, Mustafa MRU, Rashid U, et al. Adsorptive removal of COD from produced water using tea waste biochar. Environ Technol Inno. 2021;23:101563, doi:10.1016/j.eti.2021.101563
[32] Puig S, Ll C, Balaguer MD, et al. Biological nutrient removal by applying SBR technology in small wastewater treatment plants: carbon source and C/N/P ratio effects. Water Sci Technol. 2007;55:135–141.
[33] Perez-Mercado L, Lalander C, Berger C, et al. Potential of biochar filters for onsite wastewater treatment: effects of biochar type, physical properties and operating conditions. Water (Basel). 2018;10:1835, doi:10.3390/w10121835
[34] Liang P, Yu H, Huang J, et al. The review on adsorption and removing ammonia nitrogen with biochar on its

mechanism. MATEC Web of Conferences. 2016;67:07006, doi:10.1051/matecconf/20166707006
[35] Hanaki K, Wantawin C, Ohgaki S. Nitrification at low levels of dissolved oxygen with and without organic loading in a suspended-growth reactor. Water Res 1990;24:297–302. doi:10.1016/0043-1354(90)90004-P
[36] Keller J, Watts S, Battye-Smith W, et al. Full-scale demonstration of biological nutrient removal in a single tank SBR process. Water Sci Technol. 2001;43:355–362. doi:10.2166/wst.2001.0157
[37] Zhou X, Wang R, Liu H, et al. Nitrogen removal responses to biochar addition in intermittent-aerated subsurface flow constructed wetland microcosms: enhancing role and mechanism. Ecol Eng. 2019;128:57–65. doi:10.1016/j.ecoleng.2018.12.028
[38] Hellman M, Hubalek V, Juhanson J, et al. Substrate type determines microbial activity and community composition in bioreactors for nitrate removal by denitrification at low temperature. Sci Total Environ. 2021;755:143023, doi:10.1016/j.scitotenv.2020.143023
[39] Dalahmeh SS, Stenström Y, Jebrane M, et al. Efficiency of iron- and calcium-impregnated biochar in adsorbing phosphate from wastewater in onsite wastewater treatment systems. Frontiers in Environmental Science [Internet]. 2020 [cited 2022 Dec 6];8. Available from: https://www.frontiersin.org/articles/10.3389fenvs.2020.538539.
[40] Takaya CA, Fletcher LA, Singh S, et al. Phosphate and ammonium sorption capacity of biochar and hydrochar from different wastes. Chemosphere. 2016;145:518–527. doi:10.1016/j.chemosphere.2015.11.052
[41] Kizito S, Lv T, Wu S, et al. Treatment of anaerobic digested effluent in biochar-packed vertical flow constructed wetland columns: role of media and tidal operation. Sci Total Environ. 2017;592:197–205. doi:10.1016/j.scitotenv.2017.03.125
[42] Vidal B, Hedström A, Herrmann I. Phosphorus reduction in filters for on-site wastewater treatment. J Water Process Eng. 2018;22:210–217. doi:10.1016/j.jwpe.2018.02.005
[43] Das AK, MdN I, MdO F, et al. Review on tannins: extraction processes, applications and possibilities. S Afr J Bot. 2020;135:58–70. doi:10.1016/j.sajb.2020.08.008
[44] Kristian Stevik T, Aa K, Ausland G, et al. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Res 2004;38:1355–1367. doi:10.1016/j.watres.2003.12.024
[45] Mohanty SK, Cantrell KB, Nelson KL, et al. Efficacy of biochar to remove *Escherichia coli* from stormwater under steady and intermittent flow. Water Res 2014;61:288–296. doi:10.1016/j.watres.2014.05.026
[46] SwAM. Havs- och vattenmyndighetens allmänna råd (HVMFS 2016:17) om små avloppsanläggningar för hushållsspillvatten [Swedish Agency for Marine and Water Management's general advice on small sewer systems for domestic wastewater].Swedish Agency for Marine and Water Management; . 2016; . (In Swedish)
[47] Vidal B.. Small Sanitation Systems – Treatment Efficiency, Sustainability and Implementation (PhD dissertation, Luleå University of Technology).Retrieved from https://urn.kb.se/resolve?urn=urn:nbn:se:ltu:diva-92702 2022.