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**Capacity of Biochar Filters for Onsite Wastewater Treatment: Removal of Phosphorus, Nitrogen and Pathogens in Impregnated Biochar and Two-stage Biochar Filters under Variable Loading Conditions – Technical Report**

**Biokol i små avloppsanläggningar: Rening av övergödande ämnen och patogener i impregnerad biokol och tvåstegbiokolanläggningar vid olika belastning – Teknisk rapport**

**Sahar S. Dalahmeh**

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## SAMMANFATTNING

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Svenska MiljöEmissionsData (SMED) rapporterade totala bruttobelastningen till Östersjön från små avloppsanläggningar 2017 till 303 ton fosfor och 3 133 ton kväve (Olshammar, 2018). Enligt Havs- och vattenmyndighetens allmänna råd (HVMFS 2016:17) om små avloppsanläggningar för hushållsspillvatten, bör reningen av totalfosfor (Tot-P) nå minst 70% för områden med normal miljöskyddsnivå och 90% för områden identifierade som hög nivå. Vidare bör reduktionen av organiskt material, BOD<sub>7</sub>, vara minst 90% för alla anläggningar (Havs- och vattenmyndighetens, 2016). Dessutom bör reningen av totalkväve (Tot-N) vara minst 50% i områden som är känsliga för kväve. Lösningar för att förbättra fosfor- och kvävereningen i små avloppsanläggningar samt för att nå Havs- och vattenmyndighetens allmänna råd finns på marknaden men många har visat sig ha brister i rening och robusthet. I första hand har systemlösningar och för små avloppsanläggningar utvecklats utifrån ett miljöskyddsperspektiv. Hygeniseringskapaciteten hos små avloppsanläggningar och hälsorisker kopplade till kvalitén på deras utgående vatten är lika viktiga som övergödande ämnen, särskilt i omvandlingsområden där dricksvattenförsörjningen sker med egna brunnar och/eller där utloppet från anläggningen går ut i en liten recipient med låg vattenomsättning. Hälsorisker är också viktiga att beakta där recipienten används för rekreation, till exempel för bad och fiske (Herman et al, 2017). Med hjälp av medel från Havs- och vattenmyndigheten genomförde vi på Institutionen för energi och teknik, SLU, fr.o.m. maj 2015 och t.o.m. juli 2019 en serie projekt som syftade till att utveckla biokol som filtermaterial för att förbättra reningen av olika föroreningar i avloppsvatten t.ex. kemisk syreförbrukning (COD), totalkväve (Tot-N), totalfosfor (Tot-P), patogener (E.coli och salmonella), läkemedelsrester och PFAS. Denna rapport är den tredje i serien *Biokol i små avloppsanläggningar*. Rapporten kompletterar våra två tidigare underlagsrapporter (SLU 2016:090 <https://pub.epsilon.slu.se/14611/> och SLU 2017:095 <https://www.havochvatten.se/hav/vagledning--lagar/vagledningar/sma-avlopp/stod-for-projekt-sma-avlopp/projekt-sma-avlopp-2016/projekt/2018-06-12-biokol-i-sma-avloppsanlaggningar.html>). I denna rapport sammanställs och publiceras våra senaste forskningsresultat om:

- 1- Effekter av fosfor belastning ( $194\text{--}1723 \text{ mg PO}_4\text{-P m}^{-2}\text{.dygn}^{-1}$ ) på reningskapaciteten i järn- och kalkimpregnerat biokolfiler jämfört med polonitinblandat biokol och obehandlat biokol.
- 2- Effekter av hydraulisk belastning på rening av fosfor samt på reduktion av E.coli och salmonella bakterier i järn- och kalkimpregnerat biokol jämfört med polonitinblandat biokol och obehandlat biokol.
- 3- Långsiktig rening av fosfor i impregnerat biokol under en driftsperiod på ca 2,75 år och livslängd på biokolfiler.
- 4- Långsiktig kväverening in tvåstegs biokolanläggningar med kombinerat vertikal- och horisontellt flöde och effekter av hydraulisk belastning på rening av kväve- och bakterier i sådana filter, och
- 5- Rening av läkemedelsrester och PFAS i biokol med olika behandlingar (med och utan

biofilm) jämfört med sand.

I projektets första försök undersöktes reduktion av fosfatfosfor ( $\text{PO}_4\text{-P}$ ) och *E.coli* samt patogenen salmonella i biokol impregnerat med: 1) kalk, 2) järn eller 3) biokol blandat med polonit. Biokolet gjordes av tallbark som för behandlingarna 1 och 2 först impregnerades med kalciumoxid eller järnklorid och sedan pyrolyserades i 3-4 timmar vid  $350\text{-}400^\circ\text{C}$ . Kalciumoxid och järnklorid är två kemikalier som används för fosforbindningen. Ett tredje biokol blandades med det fosforbindande materialet polonit, som innehåller mycket kalk. Det impregnerade biokolet och polonitkolet jämfördes med obehandlat biokol, också av tallbark. Biokolen packades i kolonner och belastades med  $50\text{-}112\text{ L m}^{-2}\text{ dygn}^{-1}$  riktigt avloppsvatten från Kungsängsverket i Uppsala och fosforbelastning var  $194\text{-}1723\text{ mg PO}_4\text{-P m}^{-2}\text{,dygn}^{-1}$  ( $2\text{-}23\text{ mg PO}_4\text{-P L}^{-1}$ ). Detta försök pågick under 2,75 år.

I ett annat försök studerades biokolets kapacitet att rena avloppsvatten från ammonium ( $\text{NH}_4\text{-N}$ ), nitrat ( $\text{NO}_3\text{-N}$ ) och totalkväve (Tot-N) samt *ecoli* och patogen salmonella i ett tvåstegs biokolfilter; ett steg med vertikalt flöde följt av ett vattenfyllt steg med horisontellt flöde. Detta skapade ett filter med en syresatt första del följt av en syrefattig del, vilket är gynnsamt för de bakterier som renar bort kväve. Tvåstegs biokolfiltret belastades med  $23\text{-}50\text{ L m}^{-2}\text{ dygn}^{-1}$  riktigt avloppsvatten under 2,37 år.

I den tredje studien undersöktes rening av läkemedelsrester och PFAS i biokolbädd i laboratoriet. Denna studie pågick i sex månader. I studien undersöktes olika behandlingar av biokolfiltret: biokol med och utan biofilm (kluster av bakterier, som tillsammans bildar en slemmig yta där bakterierna lever och bryter ner de organiska ämnena) och jämförde deras beteende med rening av läkemedel och PFAS i sandfilter vid en belastning på  $50\text{ L m}^{-2}\text{ dygn}^{-1}$ . Vi jämförde biokolets reningskapacitet med sand eftersom sandfilter, sandbädd/markbädd, är den vanligaste tekniken för små och enskilda avlopp i Sverige samt i andra länder.

Den första studien visade att det järnkloridimpregnerade biokolet gav hög reduktion (95 %) och stabil fosforrening över 2,75 års driftperiod vid alla hydrauliska belastningar ( $50\text{-}112\text{ L m}^{-2}\text{ dygn}^{-1}$ ) och alla fosforbelastningar ( $194\text{-}1723\text{ mg PO}_4\text{-P m}^{-2}\text{,dygn}^{-1}$ ). Ökning och minskning av hydraulisk belastning mellan 50 och  $112\text{ L m}^{-2}\text{ dygn}^{-1}$  påverkade inte  $\text{PO}_4$ -reduktionen ( $>95\%$ ). Genomsnittlig koncentration av  $\text{PO}_4\text{-P}$  i renat avloppsvatten från järn impregnerat biokolfilter var stabil vid  $<0.05\text{ mg/L}$  under alla hydrauliska och fosfor belastningar förutom  $1723\text{ mg PO}_4\text{-P m}^{-2}\text{,dygn}^{-1}$ , då koncentrationen började öka efter 14 veckor. Maximal adsorptionskapacitet för detta filter var  $5,84\text{ mg per g biokol}$  och den beräknade livslängden var 33 månader vid en belastning av  $324\text{ mg PO}_4\text{-P m}^{-2}\text{,dygn}^{-1}$  eller  $4.4\text{ mg L}^{-1}$ . Fosforrening i biokolfilter impregnerat med kalk var hög ( $>87\%$  reduktion) vid låg fosforbelastning ( $194\text{ mg PO}_4\text{-P m}^{-2}\text{,dygn}^{-1}$ ) och den minskade vid högre belastning ( $256\text{-}1723\text{ mg PO}_4\text{-P m}^{-2}\text{,dygn}^{-1}$ ) och uppnådde i genomsnitt 46-50%. Det maximala adsorptionskapaciteten hos kalkimpregnerat biokol var  $3.85\text{ mg/g}_{\text{biokol}}$  och den beräknade livslängd var 7 månader på belastning av  $324\text{ mg PO}_4\text{-P m}^{-2}\text{,dygn}^{-1}$  ( $4.4\text{ mg L}^{-1}$ ). Ökning av hydraulisk belastning från 74 till  $112\text{ L m}^{-2}\text{ dygn}^{-1}$  förbättrade  $\text{PO}_4\text{-P}$  reduktion i kalkimpregnerat biokol men en minskning i reduktionen (87% till 71%) inträffades när den hydrauliska belastningen minskades igen till  $74\text{ L m}^{-2}\text{ dygn}^{-1}$ . Det polonitblandade biokolet

hade låg fosforreduktion, ca 56 % vid belastningar på 194-653 mg PO<sub>4</sub>-P m<sup>-2</sup>.dygn<sup>-1</sup> och 34% på 1723 mg PO<sub>4</sub>-P m<sup>-2</sup>.dygn<sup>-1</sup>. Det obehandlade biokolet var sämre än alla filter och renade bort ca 15-44 % av fosfor under de olika hydrauliska och fosforbelastningarna. Bland filtren visade järnimpregnerat biokol (96%) det högsta avlägsnandet av PO<sub>4</sub>-P och var den minst känsliga för ökningen / minskningen av hydraulisk belastning, följt av kalkimpregnerat biokol (77-95%; figur 5). Obehandlat biokol var det minst effektiva när det gäller borttagning av PO<sub>4</sub>-P (-9-46%) och minst robust mot förändringar i hydraulisk belastning.

Koncentrationen av E.coli i inflödet varierade inom 3,9 - 4,75 Log<sub>10</sub> CFU mL<sup>-1</sup>. I genomsnitt innehöll utflödet från biokol impregnerat med kalk och obehandlat biokol högst antal E.coli. Utflödet från biokol impregnerat med järn och biokol med inblandad polonit innehöll lägst antal E.coli. Den genomsnittliga reduktionen av E.coli i alla filter var mellan 2,9 och 3,7 Log<sub>10</sub>. Med avseende på effekter av hydraulisk belastning, minskades reduktionen av E.coli i biokol impregnerat med järn och biokol inblandat med polonit när den hydrauliska belastningen ökade från 74 till 112 L m<sup>-2</sup> dygn<sup>-1</sup>. Den genomsnittligt högsta reduktionen av Salmonella spp. mättes i biokol impregnerat med järn och biokol blandat med polonit (3,99 - 4,25 Log<sub>10</sub>). Biokol impregnerat med kalk och obehandlat biokol reducerade Salmonella spp. sämre än de andra filtren (2,8-3,3 Log<sub>10</sub>).

Sammanfattningsvis, filter av järnimpregnerat biokol hade högsta reduktionen och adsorptionskapacitet av fosfor. Troligtvis skulle dessa filter kunna uppnå hög retention över lång tid särskilt om inflödet till filtret innehåller <3 mgL<sup>-1</sup> PO<sub>4</sub>-P och rena vatten med låga koncentrationer av E. coli och salmonella åtminstone upp till en belastning på 74 L m<sup>-2</sup> dygn<sup>-1</sup>.

Resultaten från försöket med kväverening i tvåstegs biokolfiltret visade effektiv långsiktig kväverening på 71 ± 12 % (genomsnitt av resultaten av senaste 52 veckorna av studiens 126 veckors driftperiod). Högsta reduktion av Tot-N var 89% och minsta var 47%. Totalt visade 60 % av mätningarna under de senaste 52 veckorna en reduktion av Tot-N på ≥70%, varav 44% av mätningarna visade ≥80%. Steget med vertikalflöde var viktigt för nitrifikation (omvandling av ammonium till nitrit och nitrat) och 65 ± 15% av Tot-N i inflödet omvandlades till nitrat. Den horisontella delen av systemet var viktigt för denitrifikation (omvandling av nitrit och nitrat till kvävgas eller lustgas) och mycket Tot-N renades bort (49 ± 14 %) i den delen. Långsiktig ammoniumreduktion i hela systemet var effektiv (93 ± 2 %). Mekanismerna för ammoniumreduktionen var dels nitrifikation/denitrifikation och dels adsorption. Stegvis ökning av hydraulisk belastning från 23 till 31 och sedan till 39 L m<sup>-2</sup> dygn<sup>-1</sup> påverkade nitrifikationen i steget med vertikalflöde och denitrifikationen i steget med horisontellt flöde samt reduktionen av Tot-N i hela systemet. Nitrifikationen ökade från 46 till 62% när den hydrauliska belastningen ökade från 23 till 31 L m<sup>-2</sup> dygn<sup>-1</sup>. När hydrauliska belastning ökade från 31 till 39 L m<sup>-2</sup> dygn<sup>-1</sup> minskade denitrifikationen från 46 till 25%. Den stegvisa ökningen av hydraulisk belastning minskade också reduktionen Tot-N i hela systemet från 81% till 59%. Analysen visade en direkt relation mellan organisk belastning och Tot-N-reduktion i hela systemet, d.v.s. att Tot-N-reduktion ökade när organisk belastning ökade och vice versa.

I genomsnitt var reduktionen av E.coli i steget med vertikalflöde 1,53 Log<sub>10</sub> enheter medan den totala reduktionen i hela systemet var 2,95 Log<sub>10</sub>. Variationer i hydrauliska belastningar

påverkade inte reduktionen av E.coli i systemet. Reduktionen i steget med vertikalflöde var 1,1 - 2,1 Log<sub>10</sub> och i steget med horisontellflöde 2,5- 3,4 Log<sub>10</sub> vid de undersökta hydrauliska belastningarna.

Sammanfattningsvis visade tvåstegsbiokolfiltret tillräcklig kapacitet vad gäller kväveborttagning (> 70%). Trots variationen i den hydrauliska belastningen reducerade systemet i 80 % av mätningarna mer än 50% av Tot-N vilket visar på robusthet i systemet.

Studien om rening av läkemedelsrester och PFAS visade att biokolet både med och utan biofilm minskade i genomsnitt 95-99 % av läkemedelsresterna, bland annat karbamazepin (läkemedel mot epilepsi), diklofenak (inflammationshämmare), metoprolol (läkemedel mot högt blodtryck), ranitidin (läkemedel mot saltsyra i magsäcken) och koffein (uppgiggande). Det var betydligt mer än anläggningar med sandfilter som minskade 0-69 % av samma läkemedelsrester. Studien visade att biokol band upp karbamazepin och metoprolol på ytan samt att biofilmen renade ytan på biokolet från avloppsvattnets lättnedbrytbara organiska material. Detta gjorde det möjligt att behålla långvarig rening av läkemedelsrester i biokolfilter med biofilm. Dessutom, biokolet renade ranitidin och koffein effektivt genom antingen adsorption eller biologisk nedbrytning. Vad gäller högfluorerade ämnen (PFAS), visade biokolet hög reningsgrad (89 och 99 procent) för såväl substanserna PFHpA, PFOA, PFNA, PFHxA, PFDA, PFUnDA, PFDODA som FOSA och PFHxS. Biokolet kunde minska halterna av dessa substanser från 1000-3000 ng/L till <10-70 ng/L. Rening av PFAS i biokolet skedde mest via adsorption vilket innebär att de PFAS-ämnena som var lösta i avloppsvattnet fastnade på ytan av biokolet. Ovanstående PFAS-substanser innehåller många fluorerade kolgrupper (kallas också långfluorerad kolkedja, högfluorerade ämnen) som lätt fastnar på organiska ytor och därför fastnar de i biokolfiltret. Biokolet som designades för adsorption adsorberade olika mängd av olika substanser beroende på kolkedjelängd och funktionella grupper (t.ex. karboxylat och sulfonat). Till exempel, på ett gram biokol fastnade 168 nanogram av PFOA (8 kolkedjor och funktionell grupp karboxylat) och 119 nanogram av PFOS (8 kolkedjor och funktionell grupp sulfonat). De PFAS-substanser som har 4-6 fluorerade kolkedjor (som PFBA, PFPeA, PFHxA) är mer lösta i vatten så biokolet kunde inte separera dem helt från avloppsvattnet. Därför nådde biokolet en reningsgrad på 28-45 procent för PFBA, PFPeA, PFHxA. Biokolfilter där biofilm fanns nådde dock endast låg reduktion (15-50 procent) för de flesta PFAS-ämnen som studerades då högfluorerade ämnen är persistenta och bakterier svårtligen kan bryta ner dem.

Sammanfattningsvis har studierna visat att biokol är ett lovande material för avlägsnande av läkemedelsrester och PFAS med perfluorkolkelängd > C6 i småskalig avloppsrening. När det gäller rening av mikroföroreningar är det bästa sättet att använda biokolfilter som efterbehandling för att ta bort PFAS och läkemedelsrester efter att det mesta organiska materialet har tagits bort. Övergången till biokolanvändning som filtermaterial med alla dess nödvändiga drivkrafter som påverkar teknikutveckling, marknadspotential och institutionella aspekter behöver utredas. Ekonomisk analys och livscykelanalys av miljö- och resurspåverkan (t.ex. minskning av växthusgaser, koldioxiduttag, produktion av förnybar energi) av biokolbaserade små avloppsreningsystem behöver även genomföras.

Kunskapen som tagits fram under detta forskningsprojekt har använts och integrerats för att utveckla kompakta biokolsbaserade system för småskalig avloppsrening i form av biokolfilter uppbyggda av moduler, kassetter. Detta system består av flera kompakta kassetter fyllda med biokol som staplade på varandra kan användas för lokal avloppsvattenrening. Varje kassett i systemet kan designas för rening av vissa föroreningar. Tre system av har installerats i juli 2019 i ett hushåll i Gamla Uppsala och reningsresultatet följs för närvarande.

## GENERAL BACKGROUND AND OBJECTIVES

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Swedish Environmental Emissions Data (SMED) show a total gross load from small and onsite wastewater facilities of 303 tons of phosphorus (P) and 3133 tons of nitrogen (N) (Olshammar, 2018). According to the general recommendation by the Swedish Agency for Marine and Water Management for small and onsite wastewater treatment systems (HVMFS 2016: 17), removal of total phosphorus (Tot-P) should be at least 70% in areas with normal environmental protection level and 90% in areas identified as having sensitive waters. The reduction in organic material (biological oxygen demand, BOD<sub>7</sub>) should be at least 90% for all onsite treatment plants and the removal of total nitrogen (Tot-N) should be at least 50% in areas sensitive to nitrogen (Havs- och vattenmyndighetens, 2016). The microbiological quality of effluent from onsite systems is important, especially in facilities constructed in communities where drinking water is supplied by individual wells and/or where the outlet from the facility goes into a small recipient water body with low water turnover. The microbiological quality is also important when the recipient is used for recreation, for example for bathing and fishing (Herrmann et al., 2017). Solutions for improved removal of phosphorus and nitrogen in small-scale onsite wastewater treatment systems are available on the market, but many have proven to have shortcomings in terms of purification and robustness. With funding from the Swedish Agency for Marine and Water Management, from May 2015 to April 2019 the Department of Energy and Technology at the Swedish University of Agricultural Sciences (SLU) carried out a series of projects aimed at developing biochar as a filter material to improve removal of various pollutants from wastewater, e.g. chemical oxygen demand (COD), Tot-N, Tot-P, pathogens (*Escherichia coli* and *Salmonella*), pharmaceutical residues and per- and polyfluorinated alkyl substances (PFASs). This report is the third in the series *Capacity of Biochar Filters for Onsite Wastewater Treatment*, complementing the two previous reports (SLU 2016: 090 <https://pub.epsilon.slu.se/14611/> and SLU 2017: 095 [https://www.havochvatten.se/hav/vagledning--lagar/vagledningar/sma\\_avlopp / standing-for-design-small-drain / projects-small-sewage-2016 / projects / 2018-06-12-biochar-the-small-avloppsanlaggningar.html](https://www.havochvatten.se/hav/vagledning--lagar/vagledningar/sma_avlopp / standing-for-design-small-drain / projects-small-sewage-2016 / projects / 2018-06-12-biochar-the-small-avloppsanlaggningar.html)).

The overall aim of this report is to disseminate knowledge needed for development and optimisation of biochar filters as a new technology for purification of wastewater in small onsite systems, thus preventing the eutrophication of lakes, streams and coastal waters. Specific objectives of this report are to:

1. Demonstrate and assess the effects of changing hydraulic loading conditions on removal of phosphorus and coliform bacteria and pathogens (*Escherichia coli* and *Salmonella*) in iron- and calcium-impregnated biochar (up to 144 weeks) at constant and varying hydraulic load (56-120 L m<sup>-2</sup> day<sup>-1</sup>).
2. Demonstrate and assess the effects of phosphorus loading rate (194-1723 mg PO<sub>4</sub>-P m<sup>-2</sup> day<sup>-1</sup>) on treatment capacity in iron- and calcium-impregnated biochar.
3. Describe the effects of changing hydraulic loading rate on removal of nitrogen and pathogens in two-stage biochar filters with combined vertical and horizontal flow.

4. Describe and assess the performance of biochar filters with and without biofilm in removal of pharmaceutical residues and PFASs, and compare it with that of sand.

The remainder of this report is divided into three sections dealing with these objectives. Section 1 summarises the research findings on phosphorus and pathogen removal in impregnated biochar filters; Section 2 summarises the research findings on removal of nitrogen and pathogens in combined vertical-horizontal flow biochar filters; and Section 3 summarises the research findings on pharmaceuticals and PFAS removal.

# 1. IMPREGNATED BIOCHAR FILTERS FOR ENHANCED REMOVAL OF PHOSPHORUS

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## 1.1 BACKGROUND

Anthropogenic disposal of phosphorus (Tot-P) via wastewater discharge and agricultural runoff are major contributors to eutrophication of many inland aquatic systems worldwide (Smith, 2003). Strict regulations on the quality of treated wastewater are applied to protect water bodies in Sweden and high-tech solutions are used for efficient removal of phosphorus from wastewater to meet the environmental regulations. However, wastewater quality from small-scale onsite wastewater systems stands is sub-standard as regards phosphorus removal (Heinonen-Tanski & Matikka, 2017) and onsite systems are considered a significant source of anthropogenic phosphorus, with a gross load of 303 tonnes (Olshammar, 2018).

A septic tank system with subsequent soil or sand infiltration bed is the most common treatment system for onsite wastewater treatment locally and globally (Olshammar, 2018; US EPA, 2002). In such infiltration systems, the capacity to bind phosphorus depends on pH and on the availability of surfaces rich in aluminium (Al), iron (Fe) and calcium (Ca) (Arias *et al.*, 2001; Hylander *et al.*, 2006). The phosphate ions ( $\text{PO}_4^{3+}$ ) adsorbed to the surface of the bed material can also react with iron (Fe), aluminium (Al) or calcium minerals to form strong precipitates or surface complexes. The pH in the material affects the reaction. At low pH,  $\text{PO}_4^{3+}$  ions react more easily with iron and aluminium, forming *e.g.*  $\text{FePO}_4 \cdot \text{H}_2\text{O}$ . At higher pH,  $\text{PO}_4^{3+}$  ions more easily form complexes with calcium ions, such as  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{Ca}_4\text{H}(\text{PO}_4) \cdot 3\text{H}_2\text{O}$  (US EPA, 2002). In addition, individual small sewage treatment systems near drinking water wells present a potential health hazard by spreading pathogens to the drinking water source. During the period 1995-2003, approximately 70% of all water-borne disease outbreaks in Sweden took place around private drinking water wells in the vicinity of small wastewater treatment systems (Lindberg *et al.*, 2005). The most common cause of disease outbreaks relating to private drinking water wells is small sewage plants in the environment (Stenström *et al.*, 1994).

In Sweden, natural sand is considered a finite resource and there is a desire to protect it from excavation and excessive exploitation. Thus, there is a need to identify another filter media for onsite wastewater treatment, as a complement or replacement for sand. One cost-effective treatment method for on-site wastewater treatment is use of biochar filters. Biochar is a material of organic origin charred at high temperatures (300-800 °C) in the absence of oxygen (Downie *et al.*, 2009) and is characterised by large specific surface and high porosity. Forest residues and other organic waste products (*e.g.* agricultural waste and sewage sludge) can be utilised as a substrate for biochar production, thereby contributing to sustainable management and re-utilisation of waste products (He *et al.*, 2016). The main aim of the studies summarised in this section of the report was to evaluate the potential of biochar impregnated with iron, calcium and Polonite® as a filter material for phosphorus adsorption in small-scale onsite wastewater systems. Specific objectives were to (i) assess the long-term adsorption capacity of phosphate in biochar impregnated separately with calcium oxide, iron chloride or Polonite® and (ii)

evaluate the adsorption of phosphate in impregnated biochar column filters in response to different hydraulic loading rates and phosphate loading rates.

In the following subsections, we present and discuss retention of phosphate in unimpregnated biochar and in biochar filters impregnated with calcium, iron and Polonite® during 143 weeks of operation. We describe phosphate retention capacity under variable hydraulic loading rates (HLR) and phosphorus loading rates (PLR) and estimate the theoretical service life of the different impregnated biochar filters based on their capacity for retaining phosphate. We also describe the efficiency of impregnated biochar filters in removing COD during 143 weeks of operation. Finally, we summarise removal of *E. coli* and *Salmonella* spp. in the filters and describe the effects of HLR on removal of these microorganisms.

## **1.2 MATERIALS AND METHODS**

### **1.2.1 Preparations for impregnation of biochar**

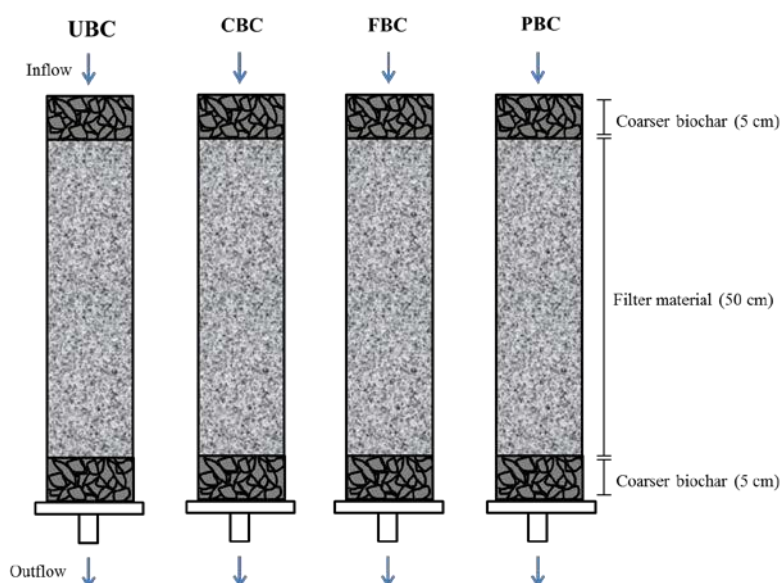
Four types of biochar were tested for phosphate-phosphorus ( $\text{PO}_4\text{-P}$ ) removal: (i) unimpregnated biochar (UBC); (ii) biochar impregnated with ferric chloride ( $\text{FeCl}_3$ ) (FBC); biochar impregnated with calcium oxide ( $\text{CaO}$ ) (CBC) and biochar mixed with Polonite® (PBC). Impregnation of the biochar was performed according to the following method: Pine bark with particle size 1-5 mm was saturated with a solution of  $\text{FeCl}_3$  (VWR, Stockholm) or  $\text{CaO}$  (VWR, Stockholm) before pyrolysis. The  $\text{FeCl}_3$  and  $\text{CaO}$  was of purity 97% and 95%, respectively, and the  $\text{FeCl}_3$  or  $\text{CaO}$  to bark ratio was 0.3 w/w. After being immersed in the solution for 24 hours at room temperature, the bark was dried at 100 °C for another 24 hours. Finally, the impregnated bark was pyrolysed in a muffle stove at 400 °C for 4 hours according to recommendations by Agraifioti *et al.* (2014). Unimpregnated biochar was produced by pyrolysis of the pine bark without impregnation. After pyrolysis, half the unimpregnated biochar was mixed with Polonite® gravel, at a Polonite®/biochar ratio of 0.3.

### **1.2.2 Column filters of impregnated biochar**

Four 60 cm high acrylic glass columns with 4.5 cm inner diameter were separately filled with UBC, CBC, FBC or PBC biochar to form a filtration layer of 55 cm ( $n=1$  for each type of biochar). Below and on the top of the filtration biochar layer, 5 cm coarse untreated biochar (8-15 mm particle size) was added to prevent clogging of the top of the filter and facilitate drainage at the bottom (Figure 1).

### **1.2.3 Operating conditions of the filters**

The filters were tested over an operating period of 143 weeks, starting from October 2016 until end of July 2019. During the operating period, removal of pollutants by the filters was investigated under different loading conditions, mimicking start-up, steady state, operation pauses and variable loading rates (HLR and PLR). The experimental set-up during the different operating periods is presented in the following subsections.



**Figure 1.** Column filters filled with unimpregnated biochar (UBC), biochar impregnated with calcium oxide (CBC), biochar impregnated with ferric chloride (FBC) and biochar mixed with Polonite® (PBC). Diagram taken from previous reports in this series (Dalahmeh, 2018) (Stenström, 2017).

### 1.2.3.1 Start-up, pause and restart period

Removal of  $\text{PO}_4\text{-P}$  and COD in the biochar filters was investigated during the start-up period of 26 weeks. During this period, the filters were loaded at a HLR of  $56 \text{ L m}^{-2} \text{ day}^{-1}$ , an organic loading rate (OLR) of  $24 \pm 10 \text{ g COD m}^{-2} \text{ day}^{-1}$  and a PLR of  $167 \pm 74 \text{ mg PO}_4\text{-P m}^{-2} \text{ day}^{-1}$ . After that, the filters were paused for 20 weeks and then reloaded with wastewater under similar loading rates as in the start-up phase for a period of 7 weeks. The pause in operation was intended to mimic discontinuity of wastewater flows due to vacation by household members or technical problems leading to stops in filter operation.

### 1.2.3.2 Changing hydraulic loading rate

The response of filters to changing hydraulic loading conditions was investigated with respect to removal of  $\text{PO}_4\text{-P}$ , COD, *E. coli* and *Salmonella* spp. During this trial, the HLR was first increased from 56 to  $74 \text{ L m}^{-2} \text{ day}^{-1}$  and then to  $112 \text{ L m}^{-2} \text{ day}^{-1}$ . Thereafter, the HLR was decreased again to  $74 \text{ L m}^{-2} \text{ day}^{-1}$  (18 weeks). The filters received wastewater at the planned HLR without modifying the quality of the wastewater for all wastewater parameters except for *Salmonella* spp. Detectable colonies of *Salmonella* spp. were not found in the raw wastewater and therefore cultivated *Salmonella* spp. was added to the wastewater used to feed the filters, to reach a final concentration of  $10^6\text{-}10^8 \text{ colony-forming units (CFU) mL}^{-1}$  in the wastewater. This mimicked episodic contamination of wastewater with *Salmonella* spp., e.g. due to salmonella infection within a household using an onsite wastewater system. The concentrations of  $\text{PO}_4\text{-P}$  and the OLR and PLR applied during the HLR trial are summarised in Table 1.

**Table 1.** Loading conditions in the study on changing hydraulic loading rate (HLR). OLR = organic loading rate, PLR = phosphate-phosphorus ( $\text{PO}_4\text{-P}$ ) loading rate. Values shown are mean  $\pm$  SD

ID	HLR (L m <sup>-2</sup> day <sup>-1</sup> )	Duration (week)	PO <sub>4</sub> -P (mg L <sup>-1</sup> )	OLR (g COD m <sup>-2</sup> day <sup>-1</sup> )	PLR (mg PO <sub>4</sub> - P m <sup>-2</sup> day <sup>-1</sup> )	Parameters investigated
HLR 56	56±0 <sup>1</sup>	30	2.99 ± 1.33	25±11	187±74	PO <sub>4</sub> -P, COD, pH, EC <sup>5</sup> , <i>Salmonella</i> spp. and <i>E. coli</i> <sup>5</sup>
HLR74-1	74±0 <sup>2</sup>	7	1.37 ± 0.93	51±39	101 ± 69	PO <sub>4</sub> -P, COD, pH, EC <i>Salmonella</i> spp. and <i>E. coli</i>
HLR74-2	74±0 <sup>3</sup>	23	2.60 ± 0.86	28±10	193±63	PO <sub>4</sub> -P, COD, pH and EC
HLR74	74±0 <sup>4</sup>	30	2.04 ± 1.06	38±29	151±80	
HLR112	112±0	10	2.43 ± 1.60	52±15	271±178	PO <sub>4</sub> -P, COD, pH, EC <i>Salmonella</i> spp. and <i>E. coli</i>

<sup>1</sup> This loading period included the start-up and restart of the filter after reaching stable state.

<sup>2</sup> Prior to this loading period, the filters received wastewater at 56 L m<sup>-2</sup> day<sup>-1</sup>.

<sup>3</sup> Prior to this loading period, the filters received wastewater at 112 L m<sup>-2</sup> day<sup>-1</sup>.

<sup>4</sup> Average loading conditions during the periods HLR74-1 and HLR74-2.

<sup>5</sup> Electrical conductivity.

### 1.2.3.3 Increasing phosphate loading rate

The removal of PO<sub>4</sub>-P and COD in the UBC, CBC, FBC and PBC columns in response to increased PLR was investigated during this trial. The filters were subjected to step-wise increasing PLR while the HLR was kept constant at 74 L m<sup>-2</sup> day<sup>-1</sup> (Table 2). During this trial, the filters received 194±65 mg PO<sub>4</sub>-P m<sup>-2</sup> day<sup>-1</sup> for 18 weeks, 324±27 mg PO<sub>4</sub>-P m<sup>-2</sup> day<sup>-1</sup> for 23 weeks, 653±61 mg PO<sub>4</sub>-P m<sup>-2</sup> day<sup>-1</sup> for 11 weeks and 1723±39 mg PO<sub>4</sub>-P m<sup>-2</sup> day<sup>-1</sup> for 14 weeks. The concentrations of all parameters in the raw wastewater remained unmodified during this trial, except PO<sub>4</sub>-P and Tot-P. The PO<sub>4</sub>-P concentration was adjusted to give the target PLR by adding 15, 30, 90 and 150 mL of PO<sub>4</sub> standard solution (1000 mg L<sup>-1</sup>) to 1 L raw wastewater. The PO<sub>4</sub>-P concentration in the raw wastewater was 2.6±0.86 mg PO<sub>4</sub>-P L<sup>-1</sup>. Inflow characteristics, HLR, OLR and PLR of the feed wastewater during this trial are summarised in Table 2.

**Table 2.** Operating and loading conditions during the trial on increasing phosphate-phosphorus (PO<sub>4</sub>-P) loading rate (PLR). OLR = organic loading rate, HLR = hydraulic loading rate. Values shown are mean± SD

ID	PLR (mg PO <sub>4</sub> -P m <sup>-2</sup> day <sup>-1</sup> )	Duration (week)	PO <sub>4</sub> -P (mg L <sup>-1</sup> )	OLR (g COD m <sup>-2</sup> day <sup>-1</sup> )	HLR (L m <sup>-2</sup> day <sup>-1</sup> )	Parameters investigated
PLR-194	194±64	18	2.60 ± 0.86	26±11	74±0	PO <sub>4</sub> -P, Tot-P, COD,
PLR-324	324±27	23	4.36 ± 0.37	28±8	74±0	pH and EC <sup>1</sup>
PLR-653	653±61	13	8.78 ± 0.82	40±10	74±0	PO <sub>4</sub> -P, Tot-P, COD
PLR-1723	1723±39	14	23.19 ± 0.53	53 ± 4	74±0	and pH

<sup>1</sup> Electrical conductivity.

### 1.2.4 Wastewater preparation, storage and feeding

All column filters were fed using single-pass downflow. The feed consisted of raw wastewater, which was brought in from Uppsala wastewater treatment plant (Kungsängsverket) on a weekly basis. When needed, the concentration of PO<sub>4</sub>-P was modified (see section 1.2.3.3). The wastewater was stored in the fridge (4±2 °C) when brought from Kungsängsverket. Prior to each feeding, the refrigerated wastewater was homogenised and the required doses were then pumped from the refrigerated wastewater container to distribution containers placed at room temperature (20 °C). After acclimatisation to room temperature for 20 minutes, the wastewater doses were pumped from the distribution containers to the filters, using a peristaltic pump. When removal of *Salmonella* spp. was investigated (see section 1.2.3.2), the cultivated *Salmonella* was added to the distribution containers while the wastewater was acclimatising. The filters were fed 3-6 times a day depending on the HLR. Pumping and feeding of the filters were performed automatically and operated by digital timers.

### 1.2.5 Chemical and microbiological analysis

The inflow wastewater and the effluent from the different biochar filters were analysed to determine the COD and PO<sub>4</sub>-P, using Spectroquant® cell kits number 14772-14773 (COD) and 14543 (PO<sub>4</sub>-P) (Merck KGaA, Darmstadt, Germany). Concentrations were determined colorimetrically using a Nova 60 photometer (Merck KGaA). The pH was measured using WTW pH/ion 340i meter and the electrical conductivity (EC) was measured using a Condi 330i conductivity meter (WTW, Weilheim Germany).

The cultivation of *Salmonella* spp. was performed by incubating a single colony of *Salmonella* spp. in unselective microbial growth medium (nutrient broth, Swedish Veterinary Institute, Uppsala, Sweden) for 24±7 hours at 37 °C under continuous mixing of the bacteria and nutrient broth. For analysis of *E. coli* and *Salmonella* spp., 0.1 mL samples were taken from sterile tubes containing series of dilutions of inflow and effluents, and added to Petri dishes with Chromocult agar (VWR International, Sweden) for *E. coli* or XRD for *Salmonella* spp. The dishes were incubated at 37 °C for 24±5 hours and visible colonies of *E. coli* and *Salmonella* spp. were counted.

Removal efficiency (E) was calculated as the difference in concentration between inflow and outflow of the filters:

$$EE = 100 \frac{CC_{iiii} - CC_{oooooooo}}{CC_{iiii}} \quad (1)$$

where  $E$  is the removal efficiency (%),  $C_{in}$  is the concentration in the influent (mg L<sup>-1</sup>) and  $C_{out}$  is the concentration in the effluent (mg L<sup>-1</sup>).

Log<sub>10</sub> reduction in *E. coli* and *Salmonella* spp. was calculated as: Log<sub>10</sub>

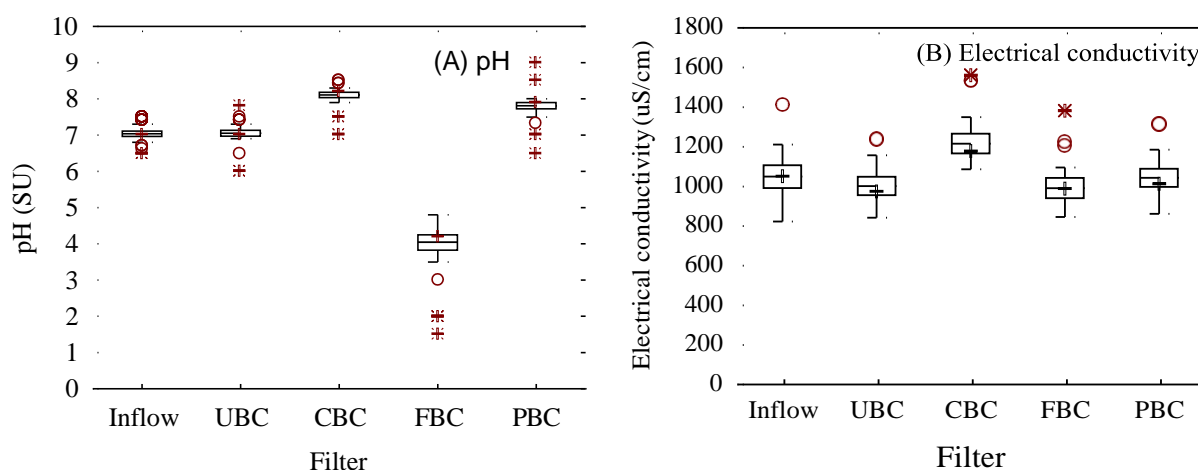
$$\text{reduction} = \text{Log}_{10}(C_{in}) - \text{Log}_{10}(C_{out}) \quad (2)$$

where  $C_{in}$  is the concentration of the microorganism in inflow and  $C_{out}$  is the concentration in the effluent (CFU mL<sup>-1</sup>). For the *Salmonella* spp.,  $C_{out}$  was the concentration in the effluent collected 24-48 hours after feeding the filters with the wastewater spiked with *Salmonella* spp.

## 1.3 RESULTS

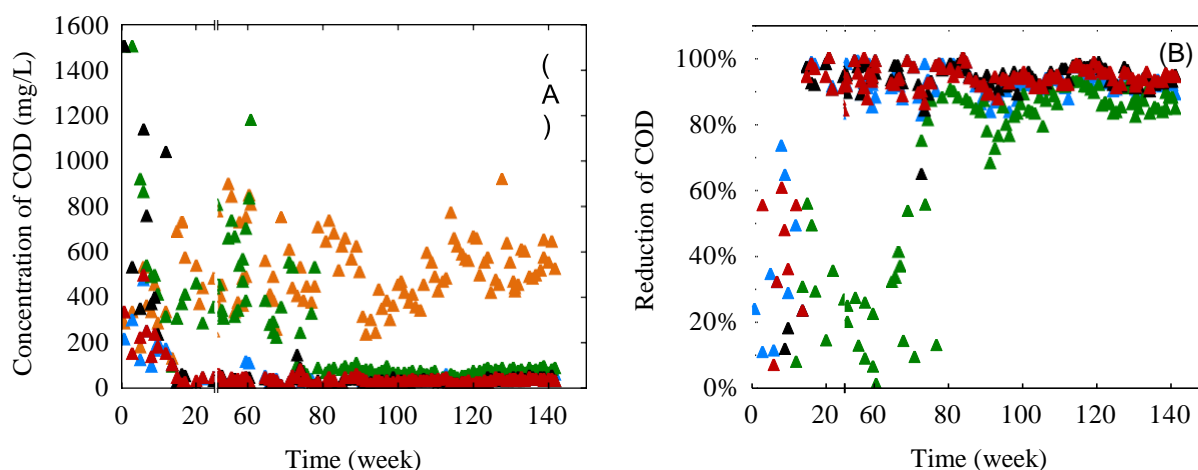
### 1.3.1 Removal of organic matter

The pH in the influent wastewater was neutral,  $7.02 \pm 0.27$ . The UBC filter effluent pH was just below neutral ( $7.06 \pm 0.30$ ), while effluents from CBC and PBC had higher pH ( $8.11 \pm 0.27$  and  $7.80 \pm 0.32$ , respectively) (Figure 2a). The largest pH change was observed for FBC, which had an average effluent pH of  $4.0 \pm 0.76$  over the experimental period. The EC in the inflow was  $1035 \pm 141 \mu\text{S cm}^{-1}$  and that in the effluents from the different filters varied within the range 980–1210  $\mu\text{S cm}^{-1}$  (Figure 2b). The effluent of CBC had the highest EC ( $1210 \pm 120 \mu\text{S cm}^{-1}$ ) and that of FBC and UBC had the lowest ( $980 \pm 130$  and  $990 \pm 120 \mu\text{S cm}^{-1}$ , respectively).



**Figure 2.** Boxplots showing A) pH and B) electrical conductivity (EC,  $\mu\text{S cm}^{-1}$ ) in the unimpregnated biochar filter (UBC), CaO-impregnated biochar filter (CBC), FeCl<sub>3</sub>-impregnated biochar filter (FBC) and biochar filter mixed with Polonite® (PBC). The middle line (–) of the box is the mean, the cross (+) is the median, the box is the confidence interval, the whiskers are the non-outlier range and the red dots are the outliers and extreme values.

During the 34 months of operation, the average COD concentration in the influent wastewater was  $573 \pm 151 \text{ mg L}^{-1}$ . During the first 2–3 weeks of start-up, the filters FBC and CBC showed the most fluctuating removal of COD (Figure 3). The effluents of FBC and CBC were initially turbid, but the turbidity disappeared after wash-out of the particles remaining after pyrolysis (Figure 3). The COD removal also stabilised after 3 weeks for all filters except CBC, for which removal of COD fluctuated. After 80 weeks of operation, the CBC started to show stable and high removal of COD. However, the CBC effluent continued to have a pale yellow colour, which persisted over the whole course of the experiment. During the last 53 weeks of operation, removal of PO<sub>4</sub>-P was high for all filters, including CBC, and COD removal was  $92 \pm 3$ ,  $87 \pm 4$ ,  $94 \pm 2$  and  $94 \pm 2$  % for UBC, CBC, FBC and PBC, respectively, with effluent concentration ranging from  $29 \text{ mg L}^{-1}$  (in FBC) to  $72 \text{ mg L}^{-1}$  in CBC (Figure 3).



**Figure 3.** A) Concentration of chemical oxygen demand (COD;  $\text{mg L}^{-1}$ ) as a function of time during the 129-week experiment in the influent (orange) and in effluent from the untreated biochar filter (UBC; blue), CaO-impregnated biochar filter (CBC; green),  $\text{FeCl}_3$ -impregnated biochar filter (FBC; black) and biochar filter mixed with Polonite® (PBC; red). B) Percentage COD removal in the effluent from UBC (blue), CBC (green), FBC (black) and PBC (red) filters during the 129 weeks of the experiment.

### 1.3.2. Retention of phosphate in impregnated biochar

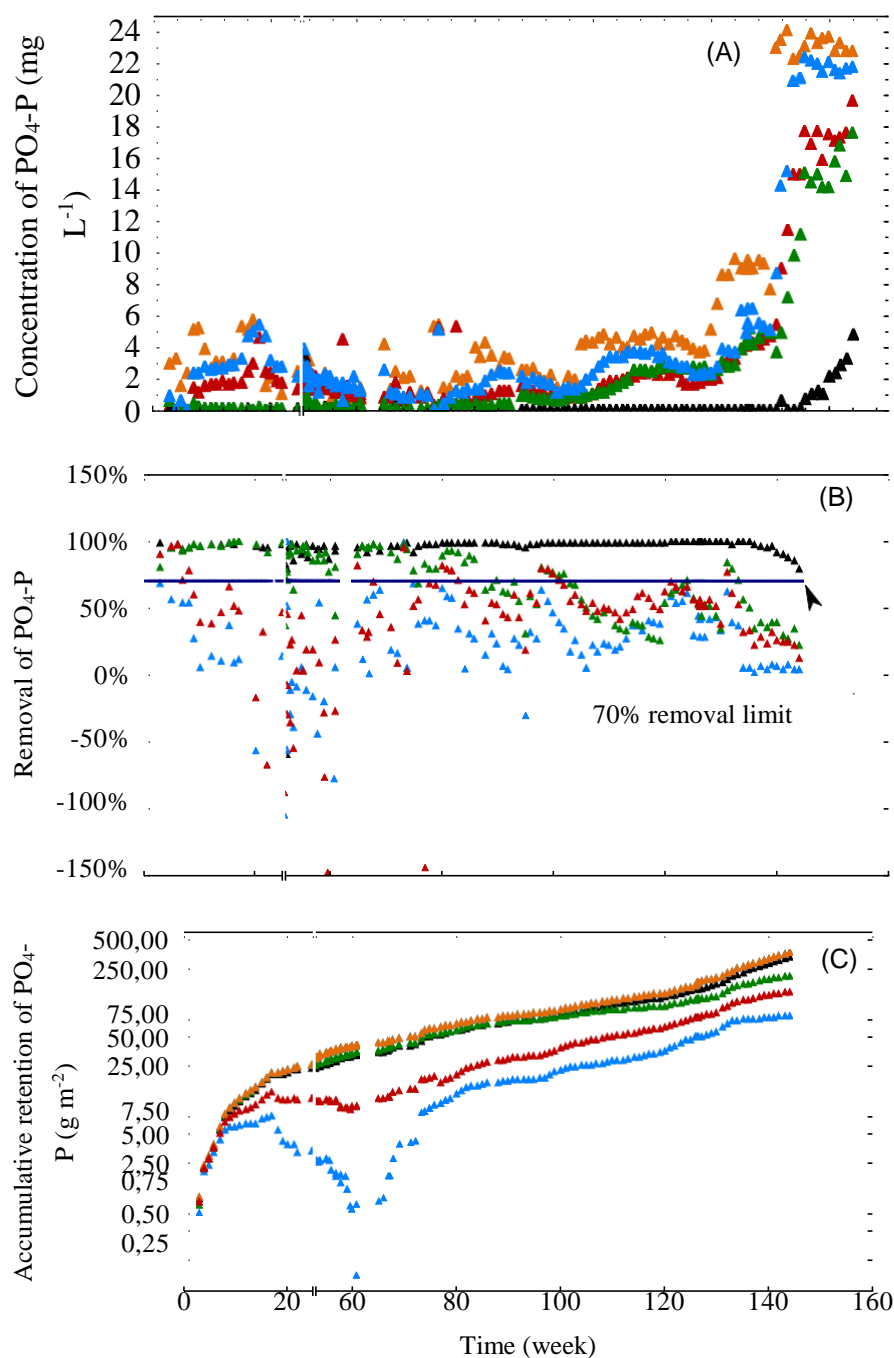
The concentration of  $\text{PO}_4\text{-P}$  in the raw wastewater displayed a relatively wide variation (range  $1\text{--}6 \text{ mg L}^{-1}$ ) (Figure 4). During start-up (27 weeks), the UBC and PBC filters removed  $17 \pm 59 \%$  and  $49 \pm 42 \%$ , respectively, of  $\text{PO}_4\text{-P}$  from the inflow, which had a  $\text{PO}_4\text{-P}$  concentration of  $3.25 \pm 1.37 \text{ mg L}^{-1}$ . The FBC and CBC filters had high start-up performance and removed  $97 \pm 2 \%$  and  $95 \pm 5 \%$ , respectively of  $\text{PO}_4\text{-P}$  during that period (Figure 4). When filter operation was interrupted by the 20-week pause, the performance of all filters was disturbed immediately after the restart and  $\text{PO}_4\text{-P}$  was leached from the filters. The highest effluent concentration of  $\text{PO}_4\text{-P}$  measured during the restart period was  $3.92 \text{ mg L}^{-1}$ , in the effluent of UBC (inflow contained  $0.65 \text{ mg PO}_4\text{-P L}^{-1}$ ). Two-three weeks after the restart, all filters recovered their pre-pause performance except UBC, which leached phosphate for an additional three weeks. The average  $\text{PO}_4\text{-P}$  removal rate from the filters during the restart period was  $-61 \pm 150 \%$  for UBC,  $-33 \pm 88 \%$  for PBC,  $74 \pm 28 \%$  for CBC and  $40 \pm 143 \%$  for FBC (the negative sign indicates release of  $\text{PO}_4\text{-P}$ ).

After 143 weeks of operation, the removal of  $\text{PO}_4\text{-P}$  (averaged over the last 14 weeks of operation) was  $15 \pm 18 \%$  for UBC,  $46 \pm 19 \%$  for CBC,  $94 \pm 6 \%$  for FBC and  $34 \pm 17 \%$  for PBC. The  $\text{PO}_4\text{-P}$  concentration measured during the final 14 weeks of operation was  $23.2 \pm 0.5 \text{ mg L}^{-1}$  for the inflow and  $19.7 \pm 4.1$ ,  $12.4 \pm 4.4$ ,  $1.4 \pm 1.4$  and  $15.2 \pm 4.0 \text{ mg L}^{-1}$  for UBC, CBC, FBC and PBC, respectively. In total, the filters received  $369.9 \text{ g PO}_4\text{-P m}^{-2}$  during 143 weeks of operation (Figure 4). The amount of  $\text{PO}_4\text{-P}$  retained in the filters over the same period was 82.1, 212.2, 326.5 and  $146.2 \text{ g m}^{-2}$  in UBC, CBC, FBC and PBC filters (Figure 4B). The adsorption capacity of the different biochar filters was 1.5, 3.9, 5.8 and  $2.6 \text{ mg per g}_{\text{biochar}}$  for UBC, CBC, FBC and PBC, respectively.

Only UBC seemed to have reached its full  $\text{PO}_4\text{-P}$  retention capacity after 143 weeks of operation. This is apparent from the low removal ( $<5\%$ ) of  $\text{PO}_4\text{-P}$  towards the end of the period



(15-23%) by the end. The FBC filter still had 79% removal by the end of the experimental period. However, its ultimate capacity was probably reached within a few weeks, as removal showed a decline thereafter in FBC.



**Figure 4.** A) Concentration of phosphate-phosphorus ( $\text{PO}_4\text{-P}$ ) in influent, (B) removal of  $\text{PO}_4\text{-P}$  in filters and C) accumulative amount of  $\text{PO}_4\text{-P}$  retained in the filters, expressed in  $\text{mg m}^{-2}$ , from start until end of the experiment in unimpregnated biochar filter (UBC; blue), CaO-impregnated biochar filter (CBC; green),  $\text{FeCl}_3$ -impregnated biochar filter (FBC; black) and biochar filter mixed with Polonite© (PBC; red). The orange line in Figure 4B shows the cumulative amount of  $\text{PO}_4\text{-P}$  applied to the filters in  $\text{g PO}_4\text{-P m}^{-2}$ .

### 1.3.3. Phosphate removal in response to changes in hydraulic loading rate

The concentration of  $\text{PO}_4\text{-P}$  under different HLRs was within the range 2.04-2.99  $\text{mg L}^{-1}$ , with no significant differences between HLRs (Table 3). The PLR applied to the filters during the HLR trial was  $167\pm174$ ,  $152\pm80$  and  $271\pm178$   $\text{mg m}^2 \text{ day}^{-1}$ , respectively, for HLR 56, 74 and  $112 \text{ L m}^{-2} \text{ day}^{-1}$ . The PLR applied during HLR-112 was thus significantly larger than that applied during HLR-56 and HLR-74. Of the filters tested, FBC showed the highest removal of  $\text{PO}_4\text{-P}$  (96%) and was the least sensitive to the increase/decrease in HLR, followed by CBC (77-95%) (Figure 5). UBC was the least efficient in  $\text{PO}_4\text{-P}$  removal (-9-46%) and least robust to changes in HLR.

In the UBC filter, the average percentage  $\text{PO}_4\text{-P}$  removal was lower at HLR-74 than HLR-56 and HLR-112, and the filter performed best when HLR was  $112 \text{ L m}^{-2} \text{ day}^{-1}$  (Table 3, Figure 5A). The average negative removal in UBC during HLR-74 ( $-9\pm100$  %) indicated leaching of  $\text{PO}_4\text{-P}$  from the filter. The time series for UBC showed that the decline in  $\text{PO}_4\text{-P}$  removal mostly occurred when the HLR was increased from 56 to  $74 \text{ L m}^{-2} \text{ day}^{-1}$ , with  $\text{PO}_4\text{-P}$  removal declining from 13% to -60% (Figure 5A). Another decline in removal (from 46 to 27 %) was observed when the HLR decreased from 112 to  $74 \text{ L m}^{-2} \text{ day}^{-1}$ . The average daily amount of  $\text{PO}_4\text{-P}$  retained in the UBC filter was similar for HLR-56 and HLR-74, but higher for HLR-112 (19, 30 and  $134 \text{ mg m}^{-2} \text{ day}^{-1}$ , respectively). The effluent concentration of  $\text{PO}_4\text{-P}$  for UBC was 2.53, 1.64 and  $1.24 \text{ mg L}^{-1}$  for HLR 56, 74 and  $112 \text{ L m}^{-2} \text{ day}^{-1}$ , respectively.

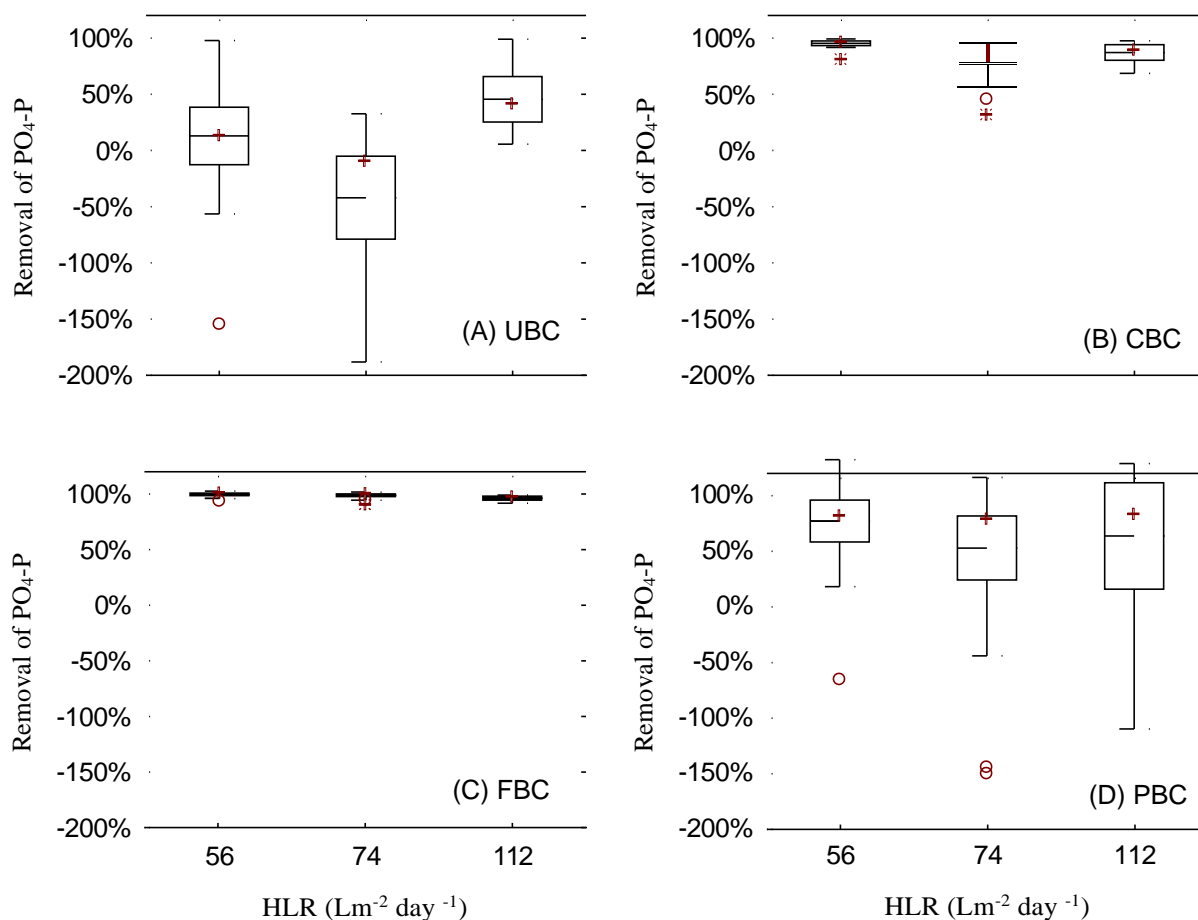
In the CBC, the  $\text{PO}_4\text{-P}$  removal at HLR-74 ( $77\pm17$  %) was lower than measured for HLR-56 and HLR-112 ( $95\pm4$  % and  $87\pm10$  %, respectively) (Table 3, Figure 5). Increasing the HLR from 74 to  $112 \text{ L m}^{-2} \text{ day}^{-1}$  slightly enhanced  $\text{PO}_4\text{-P}$  removal. The  $\text{PO}_4\text{-P}$  time series in CBC showed a decline (from 87% to 71%) in  $\text{PO}_4\text{-P}$  removal when the HLR decreased from 112 to  $74 \text{ L m}^{-2} \text{ day}^{-1}$  (Figure 4B). The amount of  $\text{PO}_4\text{-P}$  retained in the CBC filter was 149, 127 and  $255 \text{ mg m}^{-2} \text{ day}^{-1}$  and the effluent concentration was 0.12, 0.41 and  $0.24 \text{ mg L}^{-1}$  for HLR 56, 74 and  $112 \text{ L m}^{-2} \text{ day}^{-1}$ , respectively. The average percentage of removal of  $\text{PO}_4\text{-P}$  in the FBC filter was influenced by HLR (96% for all HLRs) (Table 3, Figure 5C). The amount of  $\text{PO}_4\text{-P}$  retained in the FBC filter was 140, 143 and  $257 \text{ mg m}^{-2} \text{ day}^{-1}$  and the effluent concentration was 0.1, 0.06 and  $0.07 \text{ mg L}^{-1}$  for HLR 56, 74 and  $112 \text{ L m}^{-2} \text{ day}^{-1}$ , respectively. The trend in  $\text{PO}_4\text{-P}$  removal in the PBC filter in response to changes in HLR was similar to that observed for UBC, but the levels of removal were higher in PBC ( $42\pm40$ ,  $18\pm78$  and  $29\pm76$ % for HLR-56, HLR-74 and HLR-12 respectively) (Figure 5D, Table 3). The average amount of  $\text{PO}_4\text{-P}$  retained in the PBC filter was 63, 68 and  $88 \text{ mg m}^{-2} \text{ day}^{-1}$  and the effluent concentration was 1.63, 1.16 and  $1.64 \text{ mg L}^{-1}$  for HLR 56, 74 and  $112 \text{ L m}^{-2} \text{ day}^{-1}$ , respectively.

**Table 3.** Concentrations of phosphate-phosphorus ( $\text{PO}_4\text{-P}$ ) in the influent and effluent,  $\text{PO}_4\text{-P}$  retention ( $\text{mg m}^{-2} \text{ day}^{-1}$ ) and  $\text{PO}_4\text{-P}$  removal in the unimpregnated biochar filter (UBC), CaO-impregnated biochar filter (CBC),  $\text{FeCl}_3$ -impregnated biochar filter (FBC) and biochar filter mixed with Polonite® (PBC) at different hydraulic loading rates (HLR). PLR = phosphorus loading rate

	Period	No. of weeks	HLR ( $\text{L m}^{-2} \text{ day}^{-1}$ )	Concentration of $\text{PO}_4\text{-P}$ ( $\text{mg L}^{-1}$ )	PLR and $\text{PO}_4\text{-P}$ retention ( $\text{mg m}^{-2} \text{ day}^{-1}$ )	Removal of $\text{PO}_4\text{-P}$ (%)
Inflow	HLR-56	30	56	$2.99 \pm 1.33$	$167 \pm 174^1$	
	HLR-74	30	74	$2.04 \pm 1.08$	$152 \pm 80^2$	
	HLR-112	10	112	$2.43 \pm 1.60$	$271 \pm 178$	
UBC	HLR-56	30	112	$2.53 \pm 1.36$	$19 \pm 102^1$	$13 \pm 53$
	HLR-74	30	74	$1.64 \pm 0.47$	$30 \pm 71^2$	$-9 \pm 100$
	HLR-112	10	112	$1.24 \pm 0.14$	$134 \pm 169$	$46 \pm 28$
CBC	HLR-56	30	74	$0.12 \pm 0.12$	$149 \pm 93^1$	$95 \pm 4$
	HLR-74	30	112	$0.41 \pm 0.29$	$127 \pm 80^2$	$77 \pm 17$
	HLR-112	10	74	$0.24 \pm 0.14$	$255 \pm 187$	$87 \pm 10$
FBC	HLR-56	30	112	$0.10 \pm 0.07$	$140 \pm 86^1$	$96 \pm 2$
	HLR-74	30	74	$0.06 \pm 0.04$	$143 \pm 77^2$	$96 \pm 3$
	HLR-112	10	112	$0.07 \pm 0.04$	$257 \pm 174$	$96 \pm 3$
PBC	HLR-56	30	74	$1.63 \pm 0.93$	$63 \pm 101^1$	$42 \pm 40$
	HLR-74	30	112	$1.16 \pm 0.66$	$68 \pm 99^2$	$18 \pm 78$
	HLR-112	10	74	$1.64 \pm 1.93$	$88 \pm 218$	$29 \pm 67$

<sup>1</sup> This loading period included the start-up and restart of the filter after reaching the stable state.

<sup>2</sup> Average loading conditions during the periods HLR74-1 and HLR74-2.



**Figure 5.** Boxplots showing the removal of phosphate-phosphorus ( $\text{PO}_4\text{-P}$ , %) in A) unimpregnated biochar filter (UBC), B) CaO-impregnated biochar filter (CBC), C)  $\text{FeCl}_3$ -impregnated biochar filter (FBC) and D) biochar filter mixed with Polonite® (PBC) for different hydraulic loading rates (HLR).

The middle line of the box is the mean, the cross is the median, the box is the confidence interval, the whiskers are the non-outlier range, and the red dots are the outliers and extreme values.

### 1.3.4 Response of filters to increasing phosphate loading rates

The average PLR during this trial was  $194 \pm 64$ ,  $324 \pm 27$ ,  $652 \pm 64$  and  $1723 \pm 39$   $\text{mg m}^2 \text{ day}^{-1}$  and the average concentration of  $\text{PO}_4\text{-P}$  in the inflow wastewater for these PLRs was  $2.6 \pm 0.86$ ,  $4.34 \pm 0.37$ ,  $8.76 \pm 0.86$  and  $23.16 \pm 0.53$   $\text{mg L}^{-1}$ , respectively. The HLR applied to the filters during this trial was  $74 \text{ L m}^{-2} \text{ day}^{-1}$ .

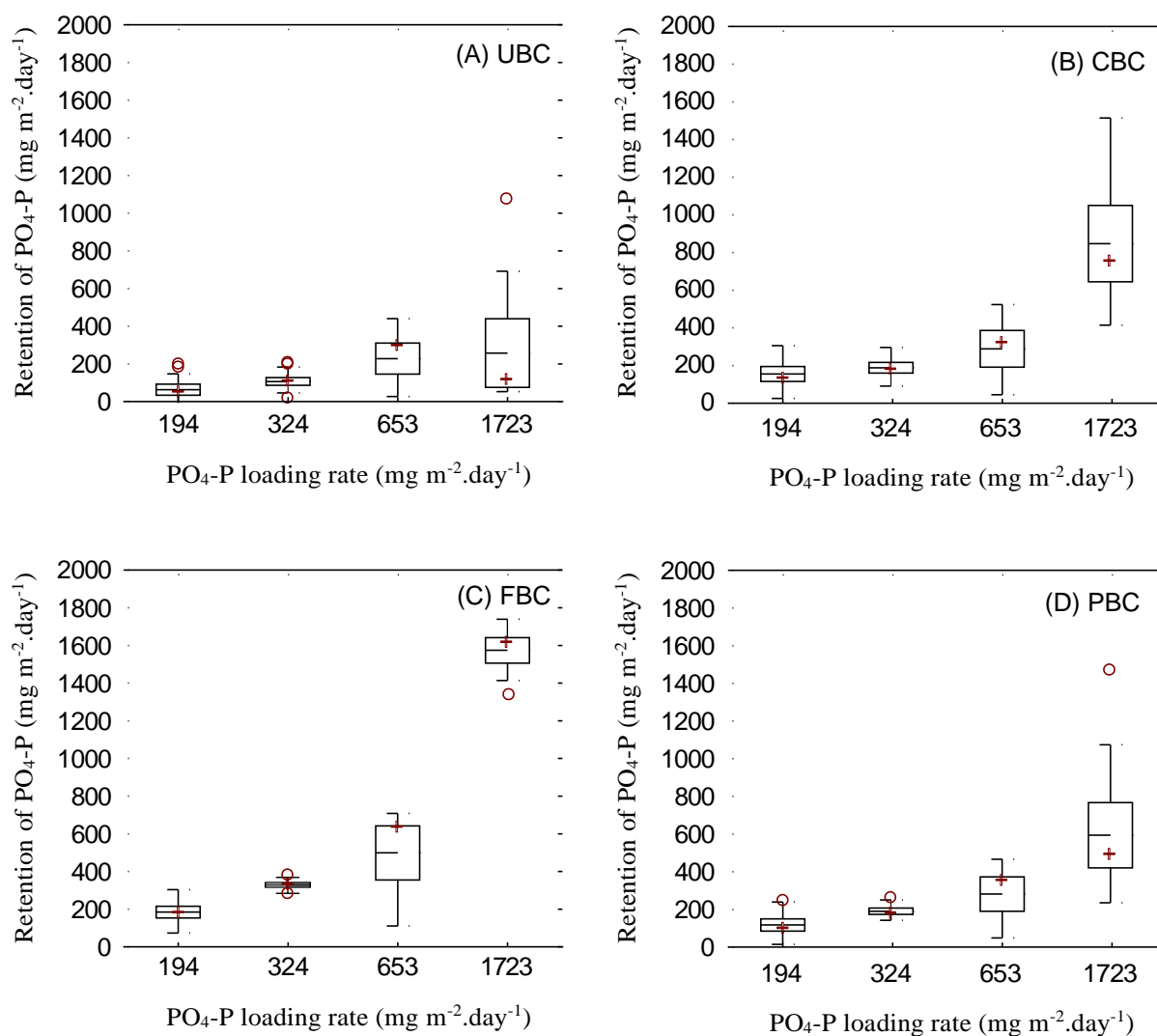
Among the filters, FBC (99%) showed the highest removal of  $\text{PO}_4\text{-P}$  at PLR-194 to PLR-1723, followed by CBC. FBC performance was robust, with low sensitivity to the increase in PLR. CBC and PBC showed comparable response to PLR in terms of  $\text{PO}_4\text{-P}$  removal and retention (Figure 6). The UBC filter showed the poorest capacity in removing  $\text{PO}_4\text{-P}$  from the wastewater at low and high PLR (Figure 6). Its highest retention capacity was  $286 \pm 76 \text{ mg m}^{-2} \text{ day}^{-1}$ , measured at PLR-653.

The stepwise increase in PLR from PLR-194 to PLR-652 enhanced the average removal and retention of  $\text{PO}_4\text{-P}$  in UBC (27% at PLR-194 to 44% at PLR-652), but the average removal declined from 44% at PLR-652 to 15% at PLR-1723. The average  $\text{PO}_4\text{-P}$  retention in UBC increased from  $62 \text{ mg m}^{-2} \text{ day}^{-1}$  at PLR-194 to  $257 \text{ mg m}^{-2} \text{ day}^{-1}$  at PLR-1723. The average concentration of  $\text{PO}_4\text{-P}$  in the effluent of UBC increased in response to the increase in PLR (Figure 6A). As regards  $\text{PO}_4\text{-P}$  removal in UBC over time, intermediate removal ( $\approx 60\%$ ) was observed at the beginning of PLR-194, PLR-652 and PLR-1723 and it then declined over time. The lowest  $\text{PO}_4\text{-P}$  removal in UBC was 4% with an effluent concentration of  $21.75 \text{ mg L}^{-1}$ , and it was measured at the end of PLR-1723 (week 14) (Figure 7A).

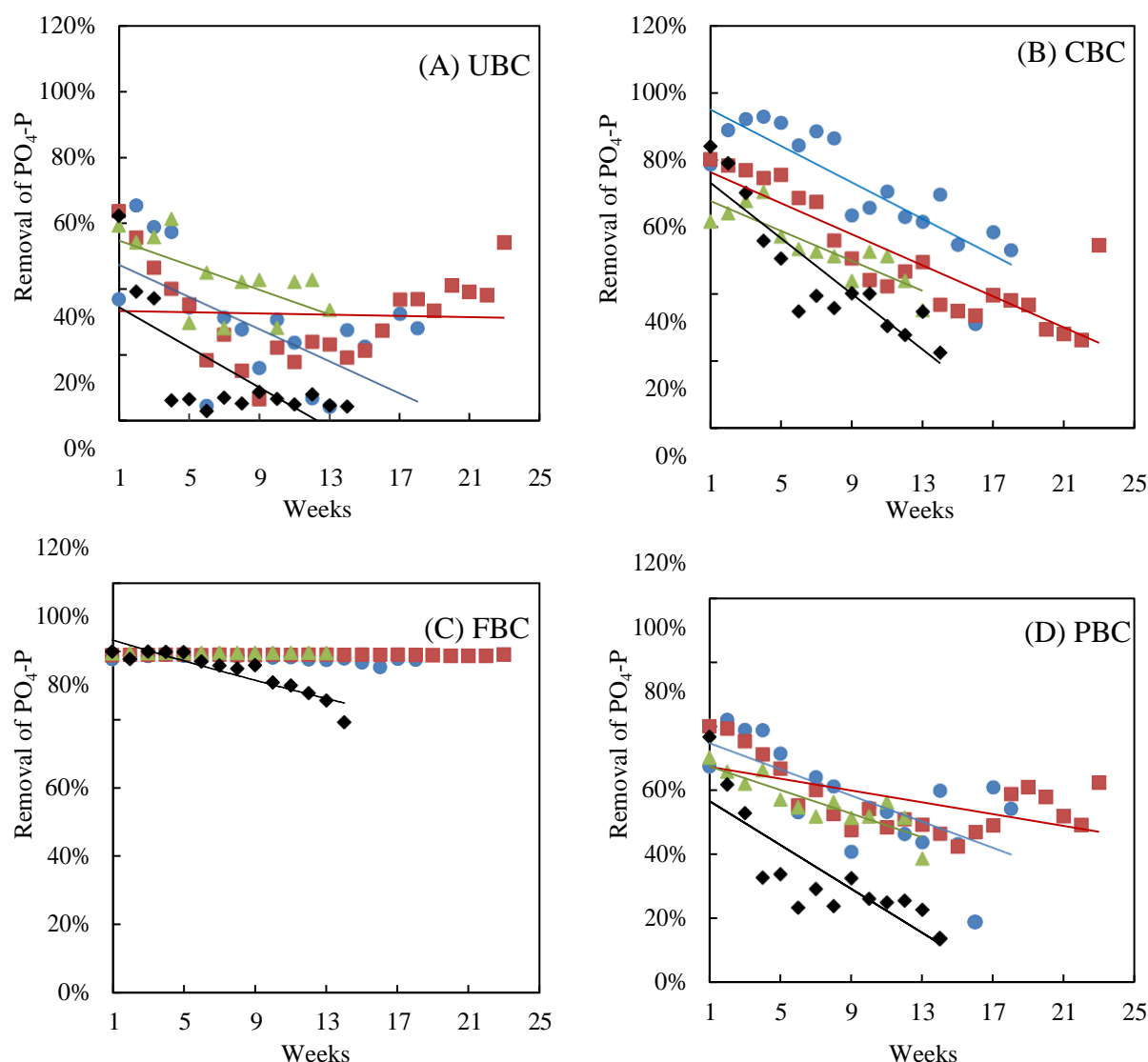
The average removal of  $\text{PO}_4\text{-P}$  in CBC decreased from 72% at PLR-194 to  $\approx 50\%$  at PLR-324, PLR-324 and PLR 1723. The average amount of  $\text{PO}_4\text{-P}$  retained in CBC increased from  $155 \pm 80$  to  $833 \pm 351 \text{ mg m}^2 \text{ day}^{-1}$  as the PLR increased stepwise from 194 to  $1723 \text{ mg m}^2 \text{ day}^{-1}$ . The concentration of  $\text{PO}_4\text{-P}$  in the effluent of CBC increased in response to the increase in PLR (Table A1, Figure 6B). At the beginning of each PLR trial,  $\text{PO}_4\text{-P}$  removal started high ( $\approx 80\%$ ) and then declined with time to reach the lowest removal (23%) at the end of PLP-1723, with an effluent concentration of  $17.6 \text{ mg L}^{-1}$  (Figure 7B).

Unlike all other filters, FBC showed high average removal of  $\text{PO}_4\text{-P}$  ( $>99\%$ ) during all PLRs until week 5 in PLR-1723, when the removal started to decline. The average retention of  $\text{PO}_4\text{-P}$  in FBC increased from 185 to  $1574 \text{ mg m}^{-2} \text{ day}^{-1}$  in response to the increase in PLR from 194 to  $1723 \text{ mg m}^{-2} \text{ day}^{-1}$  and was the highest among all filters (Table A1, Figure 6C). During all PLR trials, the average concentration of  $\text{PO}_4\text{-P}$  in the effluent of FBC was stable at  $<0.05 \text{ mg L}^{-1}$  except for PLR-1723, when the concentration started to increase. By the end of PLR-1723, the  $\text{PO}_4\text{-P}$  concentration in the effluent was  $4.74 \text{ mg L}^{-1}$ . The variations in  $\text{PO}_4\text{-P}$  removal over time were very low in FBC during PLR-194 to PLR-652. However, five weeks after the start of PLR-1723, the removal started to decline and reached 79% in week 14 of PLR-1723, giving  $4.74 \text{ mg PO}_4\text{-P L}^{-1}$  in the effluent (Figure 7C).

The average removal of  $\text{PO}_4\text{-P}$  in PBC was around 57% during PLR-194 to PLR-653, but it declined drastically in PLR-1723 to reach 34%. The average retention of  $\text{PO}_4\text{-P}$  in PBC increased from 118 to 588  $\text{mg m}^{-2} \text{ day}^{-1}$  in response to the increase in PLR from 194 to 1723  $\text{mg m}^{-2} \text{ day}^{-1}$ . As in filter CBC, the removal of  $\text{PO}_4\text{-P}$  started slightly high (70-80%) at the beginning of each of the PLR trials and then declined with time. The lowest removal (14%) was at PLR-1723, with an effluent concentration of 19.65  $\text{mg PO}_4\text{-P L}^{-1}$  (Figure 7D).



**Figure 6.** Boxplots of A) retention of phosphate-phosphorus ( $\text{PO}_4\text{-P}$ ,  $\text{mg m}^{-2} \text{ day}^{-1}$ ) in unimpregnated biochar filter (UBC), (B) CaO-impregnated biochar filter (CBC), (C)  $\text{FeCl}_3$ -impregnated biochar filter (FBC) and (D) biochar filter mixed with Polonite© (PBC) in response to increasing phosphate loading rate (PLR). The middle line (—) of the box is the mean, the cross (+) is the median, the box is the confidence interval, the whiskers are the non-outlier range, and the red dots are the outliers and extreme values.



**Figure 7.** Percentage removal of phosphate-phosphorus ( $\text{PO}_4\text{-P}$ ) in (A) unimpregnated biochar filter (UBC), (B) CaO-impregnated biochar filter (CBC), (C)  $\text{FeCl}_3$ -impregnated biochar filter (FBC) and (D) biochar filter mixed with Polonite© (PBC) as a function of time during PLR-194 (●), PLR-324 (■), PLR-652 (▲) and PLR-1723 (◆).

### 1.3.5 Removal of *Escherichia coli* and *Salmonella* spp. and response to changes in hydraulic loading rate

The concentration of *E. coli* in the inflow wastewater was 3.9-4.75  $\log_{10}$  CFU mL (Table 4). On average, effluent of CBC and UBC contained the highest number of *E. coli* colonies, while effluent of FBC and PBC contained the lowest number (Table 4). The average removal of *E. coli* in all filters was within the range 2.92-3.69  $\log_{10}$  units. With respect to effects of HLR on removal of *E. coli*, HLR-112 decreased removal in FBC and PBC filters and the effluent contained higher numbers of *E. coli* compared with HLR-74 (Table 4). After spiking with *Salmonella* spp., the wastewater contained 4.33-7.73  $\log_{10}$  CFU mL<sup>-1</sup> (Table 4). On average, the greatest reduction in *Salmonella* spp. was observed in PBC and FBC (3.99-4.25  $\log_{10}$  units), while it was lower in CBC and UBC (2.8-3.3  $\log_{10}$  units). HLR-112 had significant effects on removal of *Salmonella* spp. in PBC and FBC and more colonies were observed in the effluent

of these filters than with HLR-56 and HLR-74 (Table 4). Following addition of *Salmonella* spp.-spiked wastewater at HLR-56, the effluent of all filters was clear of *Salmonella* within 5

days. At HLR-74, some of the colonies leached only from the UBC filter for 14 days after addition and *Salmonella* spp. concentration was  $3.9 \log_{10} \text{CFU mL}^{-1}$  on day 14. At HLR-112, only FBC leached *Salmonella* spp. for 14 days after spiking and the effluent contained  $10 \text{CFU mL}^{-1}$  at day 14.

**Table 4.** Concentrations ( $\log_{10}$  units) of *Escherichia coli* and *Salmonella* spp. in inflow wastewater and in effluent of unimpregnated biochar filter (UBC), CaO-impregnated biochar filter (CBC),  $\text{FeCl}_3$ -impregnated biochar filter (FBC) and biochar filter mixed with Polonite® (PBC) at hydraulic loading rate HLR-56, HLR-74 and HLR-112

	<i>E. coli</i>			<i>Salmonella</i> spp.		
	HLR-56	HLR-74	HLR-112	HLR-56	HLR-74	HLR-112
Inflow	$3.90 \pm 0.41$	$3.93 \pm 0.46$	$4.75 \pm 0.76$	$4.33 \pm 1.21$	$6.55 \pm 0.21$	$7.73 \pm 0.51$
PBC	$0.61 \pm 0.98$	$0.04 \pm 0.00$	$0.88 \pm 1.15$	$0.04 \pm 0.00$	$0.04 \pm 0.00$	$3.74 \pm 0.44$
FBC	$0.04 \pm 0.00$	$0.04 \pm 0.00$	$1.60 \pm 0.43$	$0.04 \pm 0.00$	2.30	$3.50 \pm 0.08$
CBC	$0.61 \pm 1.13$	$1.23 \pm 1.03$	$1.37 \pm 0.27$	2.70	$3.75 \pm 0.39$	$3.31 \pm 0.19$
UBC	$0.65 \pm 1.22$	$1.90 \pm 0.79$	$1.57 \pm 0.94$	2.70	1.53	$3.74 \pm 0.25$

## 1.4 DISCUSSION

### 1.4.1 Removal of organic matter

After start-up and during the entire period of the study, the UBC, FBC and PBC filters efficiently removed organic matter, represented by COD, from the wastewater (removal rate 90-96%). In contrast, the CBC filter showed fluctuating removal of COD during the first 80 weeks of the experiment and a pale yellow colour was evident in the effluent. All biochar filters were produced by impregnating pine bark with a relevant chemical and then heating the mixture at 350-400 °C for 3.5 hours without oxygen. It appears that impregnating the bark with CaO formed an insulating layer on the bark particles and therefore the heat distribution, and hence the pyrolysis process in CBC, was not as complete as it was for UBC and FBC. This is shown by the yellow colour released in the effluent of CBC, indicating organic acids from bark. Previous studies have shown that bark used for onsite wastewater treatment releases a yellow colour deriving from natural organic acids and tannins (Dalahmeh *et al.*, 2012; Ribé *et al.*, 2009). Previous studies also report that biologically active organic matter (BOD) is totally removed by bark filters (>98%). Therefore, we concluded that the CBC material removed all the organic matter present in the wastewater, but added natural organic matter from the bark itself. To investigate the removal of organic matter from wastewater, it would have been more appropriate to measure BOD or total organic carbon (TOC).

Over the course of the experiment, the filters received on average  $39 \pm 12 \text{ g COD m}^{-2} \text{ day}^{-1}$ , which is high compared with the OLR recommended by the Swedish Environmental Protection Agency (SEPA) of  $5\text{-}6 \text{ g COD m}^{-2} \text{ day}^{-1}$  (Naturvårdsverket, 2006). Despite the high OLR, long-term COD removal in UBC, FBC and PBC was high (94-96%), without showing any sign of clogging or deterioration of performance (weeks 20-143). This indicates that biochar filters can be designed for high OLR without compromising the quality of the effluent water in terms of organic matter removal. Such capacity means that biochar filters have 5- to 6-fold lower filtration capacity than conventional sand filters.

According to the Swedish Agency for Marine and Water Management, the effluent from onsite wastewater treatment systems should achieve a minimum reduction of 90% in organic matter for all areas (sensitive and non-sensitive to wastewater discharge) (Havs- och vattenmyndigheten, 2016). All filters tested complied with this effluent quality requirement.

#### **1.4.2 Retention of phosphate in impregnated biochar and response to loading conditions**

In filters designed for phosphorus removal, the capacity of the filter to retain  $\text{PO}_4\text{-P}$  depends on pH and on the availability of surfaces rich in metal oxides such as aluminium, iron and calcium oxides (Arias *et al.*, 2001; Hylander *et al.*, 2006). Biochar filters impregnated with calcium (CBC filter) and iron (FBC filter) showed better removal of  $\text{PO}_4\text{-P}$  than unimpregnated biochar filters (UBC and PBC). The  $\text{PO}_4\text{-P}$  adsorbed to the FBC and CBC filters reacted with iron and calcium minerals to form strong precipitates or surface complexes. The FBC filter maintained low pH (<4.5 SU) during the entire experimental period, indicating continuous formation of iron oxide in the FBC and hence enhanced  $\text{PO}_4\text{-P}$  adsorption to these iron oxides. This was confirmed by X-ray diffraction tests of FBC surfaces after 100 weeks of operation, which showed a high content (26%) of akaganeite (a chloride-containing iron (III) oxide-hydroxide mineral). This showed success in achieving long-lasting impregnation of biochar using  $\text{FeCl}_3$ . In contrast, in the CBC filter with its higher pH, the  $\text{PO}_4^{3+}$  ions formed complexes with calcium ions, and  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{Ca}_4\text{H}(\text{PO}_4)_3 \cdot 3\text{H}_2\text{O}$  could have been formed at the beginning of filter operations (US EPA, 2002). The adsorption of  $\text{PO}_4\text{-P}$  in CBC deteriorated with time. When the surface of the biochar was examined using XRD after 100 weeks of operation, calcium oxides were not among the minerals deposited on the biochar. This shows that the impregnation of biochar with CaO was reversed during operation of the filter. The PBC showed less retention of  $\text{PO}_4\text{-P}$  than FBC and CBC, despite its high content of anorthite and orthoclase, which are known to bind phosphate. The amount of Polonite© mixed with the biochar to produce PBC was only 30% of its weight and this was probably not enough to efficiently remove  $\text{PO}_4\text{-P}$  from the wastewater. The UBC filter showed poor retention of  $\text{PO}_4\text{-P}$  and it is likely that the minor removal (5-50%) during the experiment was due to  $\text{PO}_4^{3+}$  assimilation in bacterial biofilm, and to weak adsorption to silica and aluminium found in the quartz, albite and microcline deposited on the surface of UBC. These minerals were likely to originate from soil particles in wastewater.

Increasing the HLR from 56 to 74  $\text{L m}^{-2} \text{ day}^{-1}$  decreased retention of  $\text{PO}_4\text{-P}$  in UBC and PBC. In fact, the UBC experienced breakthrough during HLR-74 and  $\text{PO}_4\text{-P}$  desorbed/washed out from the filter (Figure 4). The PBC did not show breakthrough when the HLR increased, but the removal rate of  $\text{PO}_4\text{-P}$  decreased substantially (from 42% to 18%). High hydraulic loads increase the infiltration rate and thereby reduce the exchanges between mobile water in macropores and water retained in micropores (Boller *et al.*, 1993). When the HLR increases, the contact time between  $\text{PO}_4\text{-P}$  and the filter media decreases and less adsorption occurs. For CBC, the first increase in HLR (HLR-56 to HLR-74) enhanced the removal of  $\text{PO}_4\text{-P}$  due to enhanced surface wetting and therefore more  $\text{PO}_4\text{-P}$  reached calcium oxides hiding in biochar particles. However, when HLR-74 increased to HLR-112, the removal of  $\text{PO}_4\text{-P}$  declined due

to wash-out of retained  $\text{PO}_4\text{-P}$ . Calcium is also likely to have been washed out when the HLR increased and hence less  $\text{PO}_4\text{-P}$  could be bound to calcium oxides. This was confirmed by the XRD analysis after HLR-112, in which we could not detect calcium oxides in the CBC material. The FBC was not influenced by the HLR, because  $\text{PO}_4\text{-P}$  was mostly removed by chemical adsorption, in which the bonds between the  $\text{PO}_4$  and iron oxides are strong. Leaching of  $\text{PO}_4\text{-P}$  in this form will be triggered by *e.g.* changes in pH or oxidation-reduction (redox) potential. During the entire HLR trials, the pH in the FBC was about 4 SU, with very low fluctuations.

In all filters except FBC, the average concentration of  $\text{PO}_4\text{-P}$  in the effluent increased in response to increasing PLR and the retention of  $\text{PO}_4\text{-P}$  in UBC, CBC and PBC was concentration- or PLR-dependent (Figures 4 and 6). This was due to UBC, CBC and PBC developing new equilibrium conditions each time the applied PLR changed. In addition, more  $\text{PO}_4\text{-P}$  was retained, which indicates that the absolute optimum adsorption capacity of these filters was not reached. Other factors might also explain this behaviour, *e.g.* when the PLR increased more bacterial growth occurred in the filters and thus more phosphate was assimilated. At the beginning of each of the PLR trials, the concentration of  $\text{PO}_4\text{-P}$  in effluent of UBC, CBC and PBC started low and then increased with time, which indicated that  $\text{PO}_4\text{-P}$  removal was high at the beginning of each PLR trial and increased thereafter (Figure 7). However, this was due to old water with low levels of  $\text{PO}_4\text{-P}$  draining from the filters for several days after the onset of the new PLR trial. The hydraulic retention times were not determined for these filters.

In contrast, FBC maintained low  $\text{PO}_4\text{-P}$  concentrations ( $<0.05 \text{ mg L}^{-1}$ ) in effluent at high and low PLR ( $194\text{-}1723 \text{ g m}^{-2} \text{ L}^{-1} \text{ day}^{-1}$ ). This indicates that the adsorption capacity of the FBC material was mainly limited by chemical adsorption of  $\text{PO}_4$  onto iron oxides available on the surface of the filter. The removal of  $\text{PO}_4\text{-P}$  declined in FBC at PLR-1723 and this was accompanied by an increase in pH from 4-4.5 to 5.0-5.4 SU), which showed that the iron oxides in FBC had started to be consumed after formation of  $\text{FeOOH-PO}_4$ . Siwek *et al.* (2019) reported that, with increasing pH, the sorption affinity of iron compounds to phosphate ions drops, whereas solubility of their combinations with phosphorus increases. The same study reported that the optimal pH for creating durable complexes of  $\text{FeOOH-PO}_4$  is below 5-7.

In general, phosphate retention capacity was highest in FBC and lowest in UBC, with the filters following the order  $\text{FBC} > \text{CBC} > \text{PBC} > \text{UBC}$ , with overall  $\text{PO}_4\text{-P}$  retention of 326.49, 212.18, 146.16 and  $82.12 \text{ g m}^{-2}$ , respectively. Based on the retention capacities, it was possible to approximately estimate the service life of the FBC filter for different concentrations of  $\text{PO}_4\text{-P}$  in wastewater (Table 5). The service life defined here is the length of period during which a filter can be successfully used to remove  $>70\%$  of  $\text{PO}_4\text{-P}$  from wastewater. The 70% value was chosen because the Swedish Agency for Marine and Water Management requires 70% removal of  $\text{PO}_4\text{-P}$  in areas classified as non-sensitive recipients (Havs- och vattenmyndigheten, 2016). Theoretically, having a low concentration ( $<2.6 \text{ mg L}^{-1}$ ) of  $\text{PO}_4\text{-P}$  in wastewater would allow the FBC filter to serve for 55 months (4.6 years) and CBC to serve for 12 months, before their  $\text{PO}_4\text{-P}$  removal declines to  $<70\%$ . The wastewater in onsite systems usually contains high concentrations ( $6\text{-}12 \text{ mg L}^{-1}$ ) of  $\text{PO}_4\text{-P}$ . If a FBC filter is used to treat this water without any pre-treatment, its service life might decline to 16 months, after which it might fail to meet the

70% PO<sub>4</sub>-P removal limit. The service life of UBC and PBC could not be estimated for the 70% removal limit because these filters did not achieve efficient removal right from the start of filter operations.

**Table 5.** Estimated service life<sup>1</sup> (in months) of unimpregnated biochar filter (UBC), CaO-impregnated biochar filter (CBC), FeCl<sub>3</sub>-impregnated biochar filter (FBC) and biochar filter mixed with Polonite® (PBC) under different phosphorus loading rates (PLR) at a fixed hydraulic loading rate of 74 L m<sup>-2</sup>

Concentration of PO <sub>4</sub> -P (mg L <sup>-1</sup> )	PLR (mg m <sup>-2</sup> day <sup>-1</sup> )	Service life (months)			
		UBC	CBC	FBC <sup>2</sup>	PBC
2.6	194	–	12	55	–
4.4	324	–	7	33	–
8.8	652	–	4	16	–
23.2	1723	–	1	6	–

<sup>1</sup> The service life was estimated as the overall PO<sub>4</sub>-P retention (mg m<sup>-2</sup>) divided by PLR (mg m<sup>-2</sup>). The overall PO<sub>4</sub>-P retention was estimated as the cumulative amount of PO<sub>4</sub>-P retained in the filters until the filters removed ≤70% of the applied PO<sub>4</sub>-P. The CBC filter reached this limit after 1.65 years and FBC reached it after 2.75 years.

### 1.4.3 Removal of *Escherichia coli* and *Salmonella* spp. and response to changes in hydraulic loading rates

Generally, FBC showed the best removal of *E. coli* at HLR-56 and HLR-74 (3.9 log<sub>10</sub> units reduction) and *Salmonella* spp. (4.2–4.3 log<sub>10</sub> units reduction). The effluent of the FBC did not contain any *E. coli* at these HLR, which indicates good inactivation of *E. coli* in FBC. During the microbial analysis, the pH in FBC filter was around 3 SU. It is likely that the low pH in the FBC inactivated *E. coli* and *Salmonella* spp. In a previous study, Lee *et al.* (2015) concluded that low pH (2.5 SU) effectively enhances inactivation of *E. coli* O157:H7 and *Salmonella* Typhimurium, but their study included heat treatment. Due to their mineral content (iron in FBC and aluminium in PBC), it is expected that FBC and PBC have positively charged surfaces. The negatively charged *E. coli* and *Salmonella* spp. are hydrophobic and the presence of positively charged surfaces in FBC and PBC is likely to have enhanced their adsorption in these filters. In positively charged filter media, reductions in viruses and bacteria of 90–>99% have been reported (Gupta & Chaudhuri, 1995; Chaudhuri & Sattar, 1990; Chaudhuri & Sattar, 1986; Prasad & Malay, 1989). The CBC filter was impregnated with calcium, which could ideally cause CBC to carry positive charge on its surfaces, but there were indications that the calcium impregnation was reversed and some calcium washed out, which left the CBC with much less positive charge than FBC. This explains the low removal of the bacteria in the CBC. Performance of UBC in removal of *E. coli* and *Salmonella* spp. was comparable to that of CBC.

The addition of *Salmonella* spp. to the wastewater was intended to mimic the performance of the filter during episodic contamination of wastewater with this pathogen and the influence of HLR on its removal. At the initial of HLR-56, PBC and FBC removed *Salmonella* spp. effectively (1 CFU mL<sup>-1</sup> in the effluent) within 5 days, and no further washout of the microorganism was observed. However, *Salmonella* spp. leached for 14 days from UBC at HLR-74 and FBC at HLR-112. The samples collected 21 and 28 days after spiking with *Salmonella* spp. showed that all effluents were clear of the microorganism. It is difficult to draw

conclusions based on these results, but protective measures, *e.g.* wearing gloves and mask and washing hands, are recommended for at least 3 weeks when handling filter materials after outbreaks of disease.

## 1.5. CONCLUDING REMARKS

- All impregnated filters showed efficient removal of organic matter from wastewater and a low risk of clogging due to accumulation of organic matter or biofilm.
- Iron-impregnated filter material (FBC) showed the best performance in phosphorus removal from wastewater in onsite systems. FBC has high adsorption capacity of phosphate and is able to achieve high retention over long periods ( $\approx 4$  years), especially if wastewater contains  $< 3 \text{ mg PO}_4\text{-P L}^{-1}$ . This filter was robust and was not sensitive to changes in hydraulic loading rate or phosphorus loading rate. The FBC filter produced water with very low levels of *E. coli* and *Salmonella* spp. at HLR  $< 74 \text{ L m}^{-2} \text{ day}^{-1}$ .
- It is recommended that iron-impregnated biochar be used in a separate phosphorus filter module (post-treatment step) after organic matter and solids are removed from wastewater. In this approach, the adsorption sites on the impregnated biochar material will be saved for phosphate removal.

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## 2. COMBINED VERTICAL-HORIZONTAL FLOW BIOCHAR FILTER FOR ENHANCED NITROGEN REMOVAL

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### 2.1 BACKGROUND

Total nitrogen is removed in vertical soil filters partly by adsorption of ammonium ( $\text{NH}_4^+$ ), but the main removal is achieved by nitrification-denitrification in biofilm (Pell, 1991) or ANAMOX (Erler *et al.*, 2008). Nitrification is performed by nitrifying bacteria (aerobic and autotrophic bacteria), which derive their energy from oxidation of  $\text{NH}_4^+$  to nitrite ( $\text{NO}_2$ ) in a first step and then further to nitrate ( $\text{NO}_3$ ) (Bassin *et al.*, 2012). In a second stage, facultative bacteria called denitrifying bacteria reduce  $\text{NO}_3$  or other nitrogen oxides to form nitrous oxide ( $\text{N}_2\text{O}$ ) and nitrogen gas ( $\text{N}_2$ ) under anaerobic conditions, in a process called denitrification (Brar *et al.*, 1978). During denitrification,  $\text{NO}_3$  is used as a source of oxygen for respiration. Denitrifying bacteria are heterotrophic, which means that they need an organic carbon source to remove nitrogen.

Wastewater production in households varies on a daily, weekly and seasonal basis, which leads to variability in organic loading rate (OLR) and hydraulic loading rate (HLR) to the wastewater treatment system. Under peak conditions, this can lead to a temporary breakdown of the infiltration system, so-called episodic failure (Beal *et al.*, 2008). Thus, it is necessary for the infiltration bed to have the capacity to withstand variations in HLR and OLR and maintain resilient and steady treatment performance. Consequently, successful design requires knowledge about the capacity of the particular filter material to buffer high variations in water flow and organic loading. To avoid clogging or breakthrough conditions, it is necessary to balance the hydraulic and organic loading against the material properties of the filter (Torrens *et al.*, 2009). This requires knowledge about the capacity of the particular filter material to buffer high variations in water flow and organic loading.

The combined vertical-horizontal flow biochar filter system developed in this project was designed to include two sections (Figure 8). The first of these was an aerobic vertical flow filter (VFF) section in which the wastewater percolated through the biochar media in unsaturated mode. The unsaturation mode meant that the pores in the media had access to air, which would encourage nitrification of  $\text{NH}_4\text{-N}$ . The second section in the system was a horizontal flow filter (HFF), in which biochar was saturated with water and had little access to air. This enabled anaerobic conditions to enhance the denitrification process, and hence nitrogen removal from the wastewater. In this section, we describe the performance of the combined vertical-horizontal flow biochar filter system (VFF-HFF system) in terms of organic matter and nitrogen removal during an operating period of 126 weeks (2.42 years), with special focus on the long-term removal, *i.e.* during the last 52 weeks of operation. In addition, we discuss the effects of HLR on nitrification in VFF and denitrification in HFF. Finally, we briefly describe removal of *E. coli* and *Salmonella* spp. in the filters and the effects of HLR on removal of these microorganisms.

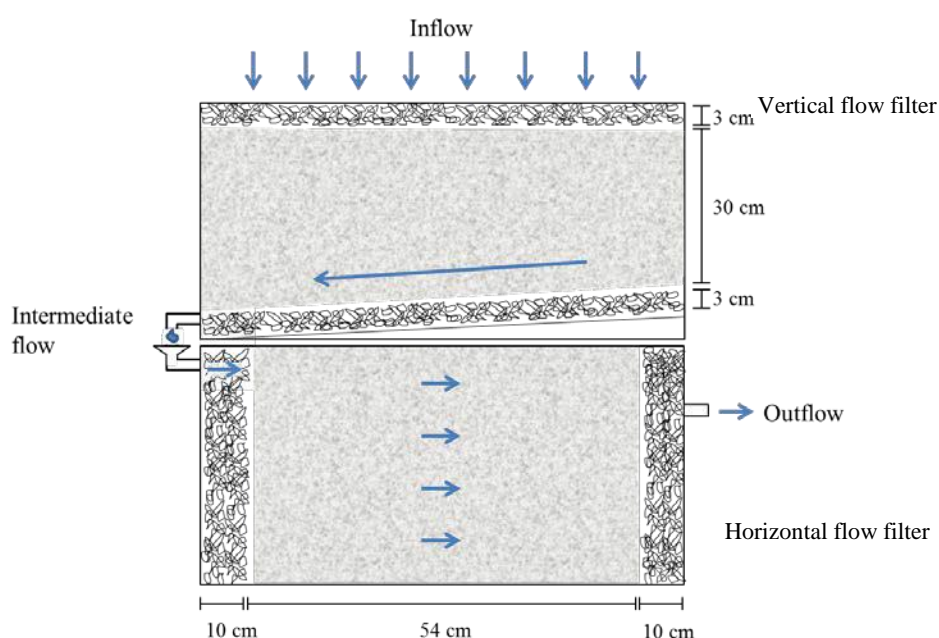
## 2.2 MATERIALS AND METHODS

### 2.2.1 Experimental set-up

A biochar filter with an aerobic vertical flow section (VFF) combined with an anaerobic horizontal flow section (HFF) was installed and filled with biochar originating from a mixture of pine and spruce wood biomass obtained from Vindelkol AB (Vindelkol, 2017). The HFF and VFF sections comprised two 70-L boxes, each measuring 74 cm × 29 cm × 40 cm (height × width × depth), placed on top of each other. In the VFF, a 3-cm drainage layer with slope 1.5:60 (*i.e.* 2.5%) was prepared with coarse biochar (8-16 mm particle diameter) at the bottom. The section was then filled up to 30 cm with biochar with particle size varying between 2.5 and 5 mm. A second 3-cm layer of coarse biochar was placed on top of the main filter to prevent clogging on the surface.

The HFF biochar filter was prepared by filling the box with coarse biochar (25-40 mm particle diameter) in two 10-cm layers at the inlet and outlet sides. The remaining 54 cm of the section was then filled with biochar (1.6-2.5 mm diameter). The depth of the biochar in the HFF was 30 cm. The outlet of the HFF was located 4 cm lower than the inlet. Before the start of the experiment, the filter was gently washed with distilled water. During the experiment, pumps fed the filter with 1.5 L three times a day, at 9:00, 16:00 and 01:00 (total of 4.5 L day<sup>-1</sup>), which gave a flow of around 21 L m<sup>-1</sup> day<sup>-1</sup> (64 L m<sup>-3</sup> day<sup>-1</sup>).

The filters were fed with real wastewater brought from Uppsala WWTP (Kungsängsverket). Preparation, storage and feeding of wastewater to the filters were as described in section 1.2.4.



**Figure 8.** Combined aerobic vertical flow filter (VFF) and anaerobic horizontal flow filter (HFF) unit for nitrogen removal from wastewater. The material in the filter was biochar made from hardwood biomass. Diagram taken from previous reports in this series (Dalahmeh, 2018) (Stenström, 2017).

## 2.2.2 Operating conditions of the filters

The filters were tested over an operating period of 126 weeks, starting from October 2016 until end of March 2019. During the operating period, the removal of pollutants by the filters was investigated in response to different loading conditions mimicking start-up, steady state, operational pauses and variable HLR.

### 2.2.2.1 Start-up, pause and restart period

Removal of pH, Tot-N, NH<sub>4</sub>-N, NO<sub>3</sub>-N and COD in the biochar filters was investigated during the start-up period of 25 weeks of operation. During this period, the filters were loaded at a HLR of 16-23 L m<sup>-2</sup> day<sup>-1</sup>, an OLR of 11.0±4.5 g COD m<sup>-2</sup> day<sup>-1</sup> and a nitrogen loading rate (NLR) of 773±228 mg Tot-N m<sup>-2</sup> day<sup>-1</sup>. After that, the filters were paused for 26 weeks and then reloaded with wastewater under similar loading rates as in the start-up phase for a period of 6 weeks. The pause in operation was planned to mimic discontinuity of wastewater flows due to vacation by household members or technical problems leading to stops in filter operation.

### 2.2.2.2 Changing hydraulic loading rate

The response of VFF and HFF filters to changing hydraulic loading conditions was investigated with respect to removal of Tot-N, NH<sub>4</sub>-N, NO<sub>3</sub>-N, COD *E. coli* and *Salmonella* spp. During this trial, the HLR was first increased from 23 to 31 L m<sup>-2</sup> day<sup>-1</sup> and then to 39 L m<sup>-2</sup> day<sup>-1</sup>. Thereafter, the HLR was decreased again to 31 L m<sup>-2</sup> day<sup>-1</sup>. Since the COD in the inflow fluctuated during the experiment, the effect of changes in OLR on the overall removal of Tot-N in the entire system was investigated by creating linear regression models. The filters received wastewater at the planned HLRs without modifying the quality of the wastewater for all wastewater parameters except for *Salmonella* spp. Detectable colonies of *Salmonella* spp. were not found in the raw wastewater and therefore cultivated *Salmonella* spp. was added to the wastewater used to feed the filters to reach a final concentration of 10<sup>6</sup>-10<sup>8</sup> CFU mL<sup>-1</sup> in the wastewater (see section 1.2.3.2). The OLRs and NLRs applied during the HLR trial are summarised in Table 6.

**Table 6.** Loading conditions in the changing hydraulic loading rate (HLR) study. OLR = organic loading rate, NLR = nitrogen loading rate. Values shown are mean± SD

ID	Duration (week)	HLR (L m <sup>-2</sup> day <sup>-1</sup> )	OLR (g COD m <sup>-2</sup> .day <sup>-1</sup> )	NLR (mg Tot-N m <sup>-2</sup> .day <sup>-1</sup> )	Parameters investigated
HLR-23 <sup>1</sup>	31	23	11.0±4.8	878±284	Tot-N, NH <sub>4</sub> -N, NO <sub>3</sub> -N, COD, pH, EC, <i>Salmonella</i> spp. and <i>E. coli</i>
HLR31-1 <sup>2</sup>	12	31	14.4±8.3	2323±187	Tot-N, NH <sub>4</sub> -N, NO <sub>3</sub> -N, COD, pH and EC
HLR-39	12	39	18.9±5.9	1403±203	Tot-N, NH <sub>4</sub> -N, NO <sub>3</sub> -N, COD, pH, EC, <i>Salmonella</i> spp. and <i>E. coli</i>
HLR31-2 <sup>3</sup>	46	31	15.5±4.2	1206±269	

<sup>1</sup> This loading period included the start-up and restart of the filter after reaching stable state.

<sup>2</sup> Prior to this loading period, the filters received wastewater at 23 L m<sup>-2</sup> day<sup>-1</sup>.

<sup>3</sup> Prior to this loading period, the filters received wastewater at 39 L m<sup>-2</sup> day<sup>-1</sup>.

### 2.2.3 Sampling and analyses

Samples of influent and effluent from the VFF and HFF units were collected once a week and analysed for pH, EC, Tot-N, NH<sub>4</sub>-N, NO<sub>3</sub>-N, COD, *Salmonella* spp. and *E. coli* (Table 2). The incoming wastewater and the filter effluents were analysed using Spectroquant® cell kits number 14772-14773 for determination of COD, 00683 for NH<sub>4</sub>, 09713 for NO<sub>3</sub> and 14963 for Tot-N (Merck KGaA, Darmstadt, Germany). Concentrations were determined colorimetrically using a Nova 60 photometer (Merck KGaA). The pH was measured using WTW pH/ion 340i meter and the electrical conductivity (EC) was measured using a Condi 330i conductivity meter (WTW, Weilheim Germany). The cultivation of *Salmonella* spp. was performed by incubating a single colony of *Salmonella* spp. in unselective microbial growth medium (nutrient broth, Swedish Veterinary Institute, Uppsala, Sweden) for 24±7 hours at 37 °C under continuous mixing of the bacteria and nutrient broth. For analysis of *E. coli* and *Salmonella* spp., 0.1 mL samples were taken from sterile tubes containing series of dilutions of influent and effluents, and added to Petri dishes with Chromocult agar (VWR International, Sweden) for *E. coli* or XRD for *Salmonella* spp. The dishes were incubated at 37 °C for 24±5 hours and visible colonies of *E. coli* and *Salmonella* spp. were counted.

Removal efficiency (E) was calculated as the difference in concentration between influent and effluent of the filters:

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

where  $E$  is the removal efficiency (%),  $C_{in}$  is the concentration in influent (mg L<sup>-1</sup>) and  $C_{out}$  is the concentration in effluent (mg L<sup>-1</sup>).

Log<sub>10</sub> reduction in *E. coli* and *Salmonella* spp. was calculated as: Log<sub>10</sub>

$$\text{reduction} = \text{Log}_{10}(C_{in}) - \text{Log}_{10}(C_{out}) \quad (2)$$

where  $C_{in}$  is the concentration of the microorganism in influent and  $C_{out}$  is the concentration in effluent (CFU mL<sup>-1</sup>). For *Salmonella* spp.,  $C_{out}$  was the concentration in the effluent collected 24-48 hours after feeding the filters with the wastewater spiked with *Salmonella* spp.

Nitrification and denitrification efficiency was calculated as:

$$N_{eff} = 100 \frac{[NO_3-N]_{VFF} - [NO_3-N]_{inflow}}{[NO_3-N]_{inflow}} \quad (3)$$

where NO<sub>3</sub>-N<sub>VFF</sub> is the concentration of nitrate-nitrogen in the effluent from the vertical flow filter, NO<sub>3</sub>-N<sub>inflow</sub> is the concentration of nitrate-nitrogen in the incoming wastewater and Tot-N<sub>inflow</sub> is the total nitrogen in the incoming wastewater.

and

$$D_{eff} = 100 \frac{[NO_3-N]_{HFF} - [NO_3-N]_{inflow}}{[NO_3-N]_{inflow}} \quad (4)$$

where NO<sub>3</sub>-N<sub>HFF</sub> is the concentration of nitrate-nitrogen in the effluent from the horizontal flow



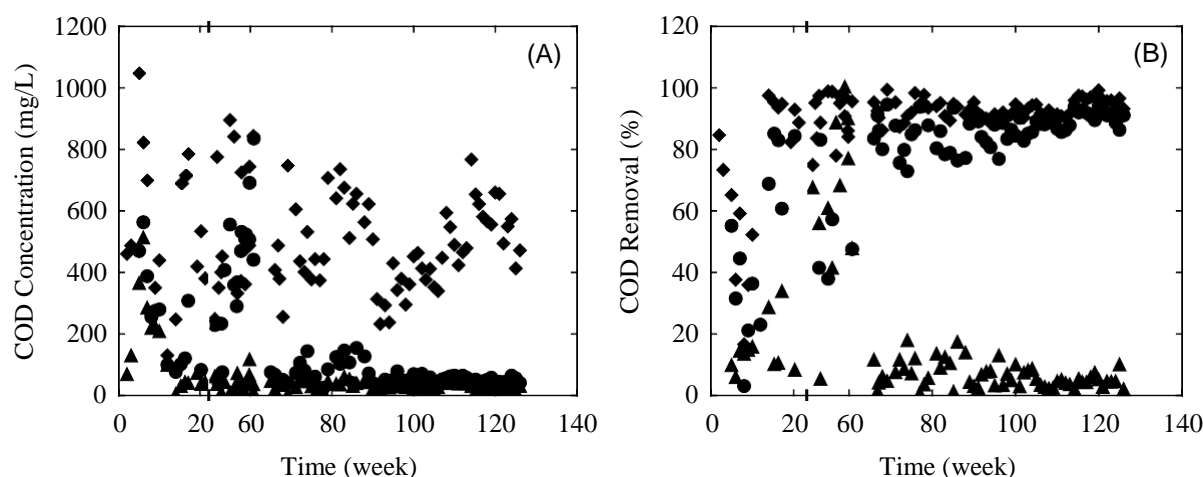
## 2.3 RESULTS

### 2.3.1 Removal of organic matter

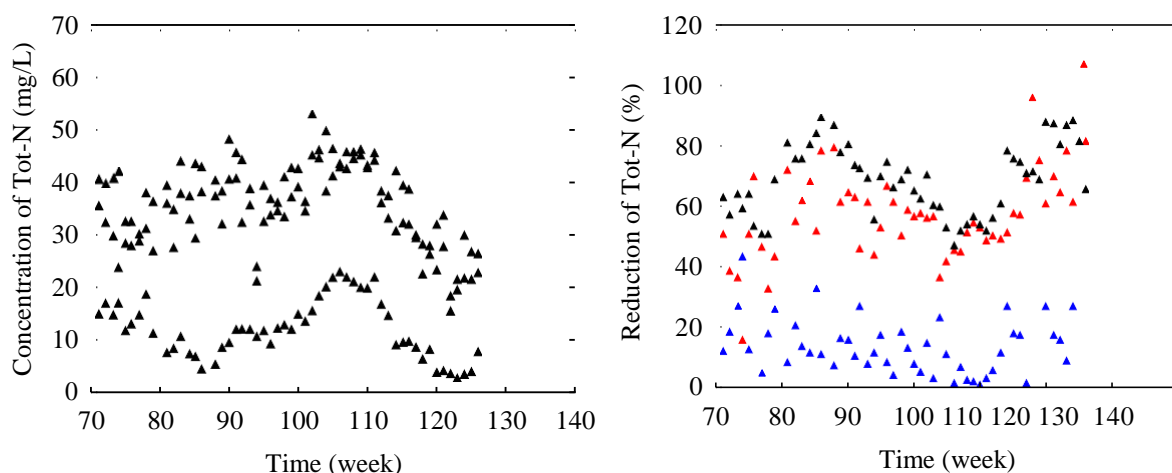
Over the whole experimental period (126 weeks), the average concentration of COD was  $144 \pm 171 \text{ mg L}^{-1}$  in the VFF and  $39 \pm 33 \text{ mg L}^{-1}$  in the HFF (Figure 9). On average, the removal of COD in the entire system during the 126-week period was high ( $91 \pm 11 \%$ ) (Figure 9), with most of the removal ( $72 \pm 25 \%$ ) occurring in the VFF. The long-term removal of COD in the entire system, measured as average removal during the last 52 weeks of operation, was  $93 \pm 3 \%$  with most of the removal ( $87 \pm 5 \%$ ) occurring in the VFF. The effluent of VFF and HFF contained  $61 \pm 21$  and  $30 \pm 12 \text{ mg COD L}^{-1}$ , respectively. Changing the HLR from 23 to 31 to  $39 \text{ L m}^{-2} \text{ day}^{-1}$  did not influence COD removal in the VFF and HFF and the overall removal of the system varied within 90-93% with the different HLRs ( $p > 0.05$ ).

### 2.3.2 Long-term removal of nitrogen and effects of hydraulic loading rate

The long-term performance of the filters is presented as performance in nitrogen removal, nitrification and denitrification during the last 52 weeks of the experiment. Concentration of Tot-N showed frequent fluctuations and the average during the last 52 weeks of the experiment was  $38 \pm 8 \text{ mg L}^{-1}$ , with maximum concentration of  $53 \text{ mg L}^{-1}$  and minimum concentration of  $18 \text{ mg L}^{-1}$ . The average concentration of Tot-N in the effluent of the entire system, *i.e.* effluent from the HFF, was  $12 \pm 6 \text{ mg L}^{-1}$  with minimum concentration of  $2.8 \text{ mg/L}$  and maximum concentration of  $23 \text{ mg L}^{-1}$  (Figure 10). The long term removal of Tot-N in the entire system was  $71 \pm 12 \%$ , with maximum removal of 89.5% and minimum removal of 47% (Figure 10). In total, 60% of the measurements made during the last 52 weeks showed  $\geq 70\%$  removal of Tot-N, and 44% showed Tot-N removal  $\geq 80\%$ . Most of the total Tot-N removal was achieved by the HFF, with an average removal of  $61 \pm 14 \%$ .

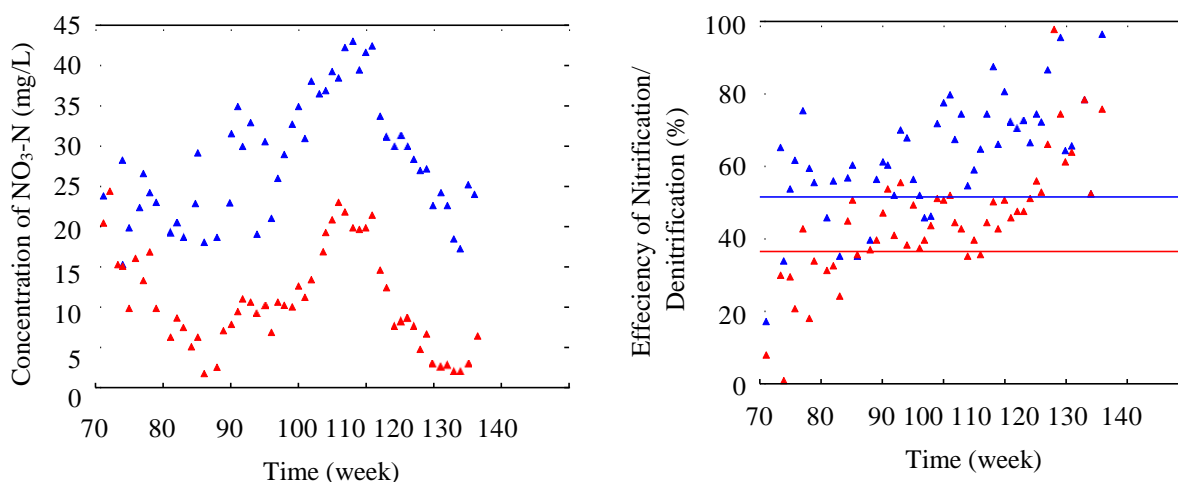


**Figure 9.** Scatter plots of A) concentration of chemical oxygen demand (COD) in the inflow (♦) and effluent of the vertical flow filter (●) and horizontal flow filter (▲) during 129 weeks of operation. B) Percentage removal of COD in the vertical flow filter (●), horizontal flow filter (▲) and entire system (vertical + horizontal filters; ♦).



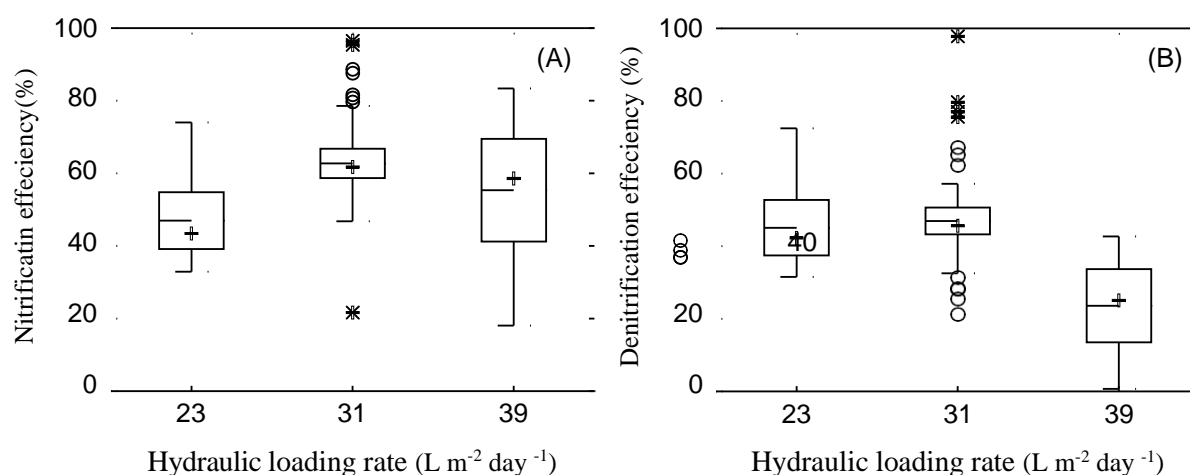
**Figure 10.** Scatter plots of (left) concentration of total nitrogen (Tot-N) in the inflow (▲) and effluent of the vertical flow filter (▲) and horizontal flow filter (▲) during the last 52 weeks of operation at HLR of  $31 \text{ L m}^{-2} \text{ day}^{-1}$ , and (right) percentage removal of Tot-N in the vertical flow filter (▲), horizontal flow filter (▲) and entire system (vertical + horizontal filters; ▲).

The long-term removal of ammonium ( $\text{NH}_4\text{-N}$ ) was  $93 \pm 2 \%$ , with almost all the removal occurring in the VFF. The respective concentration of  $\text{NH}_4\text{-N}$  in effluent from VFF and HFF was  $4.5 \pm 4.3 \text{ mg L}^{-1}$  and  $< 2.0 \pm 0.1 \text{ mg L}^{-1}$ . The nitrification in the VFF and denitrification in the HFF showed increasing trends during the entire period of the experiment, including the last 52 weeks (Figure 11). The long-term average nitrification efficiency was  $65 \pm 15 \%$ , with maximum efficiency of 97%. The long-term denitrification efficiency was  $49 \pm 14 \%$ , with maximum efficiency of 98%. The effluent from VFF and HFF showed periodic variations represented by a decreasing trend in nitrate ( $\text{NO}_3\text{-N}$ ) concentration followed by an increasing trend (Figure 11). The average concentration of  $\text{NO}_3\text{-N}$  in the effluent of VFF and HFF was  $29.3 \pm 7.5$  and  $10.7 \pm 6.0 \text{ mg L}^{-1}$ , respectively.



**Figure 11.** Scatter plots of (left) concentration of nitrate ( $\text{NO}_3\text{-N}$ ) in effluent of the vertical flow filter (▲) and horizontal flow filter (▲) during the last 52 weeks of the experiment at HLR  $31 \text{ L m}^{-2} \text{ day}^{-1}$  and (right) efficiency of nitrification in the vertical flow filter (▲) and denitrification in the horizontal flow filter and in the entire system (vertical + horizontal filters; ▲).

The statistical analysis showed that stepwise changes in the HLR from 23 to 39 L m<sup>-2</sup> day<sup>-1</sup> affected the performance of the VFF and HFF with respect to nitrification efficiency, denitrification efficiency and removal of Tot-N in the entire system ( $p < 0.05$ ) (Figure 12). Increasing the HLR from 23 to 31 L m<sup>-2</sup> day<sup>-1</sup> increased the nitrification efficiency from 46 to 62 %. However, when the HLR increased from 31 to 39 L m<sup>-2</sup> day<sup>-1</sup>, the denitrification efficiency decreased from 46 to 25 %. The stepwise increase in HLR from 23 to 31 and then 39 L m<sup>-2</sup> day<sup>-1</sup> decreased the overall removal of Tot-N in the entire system from 81% to 71% to 59% ( $p < 0.05$ ). With respect to the effects of OLR, statistical analysis showed a significant positive relationship with removal of Tot-N in the entire VFF and HFF system ( $p < 0.05$ ). In other words, removal of Tot-N increased when OLR increased and *vice versa*.

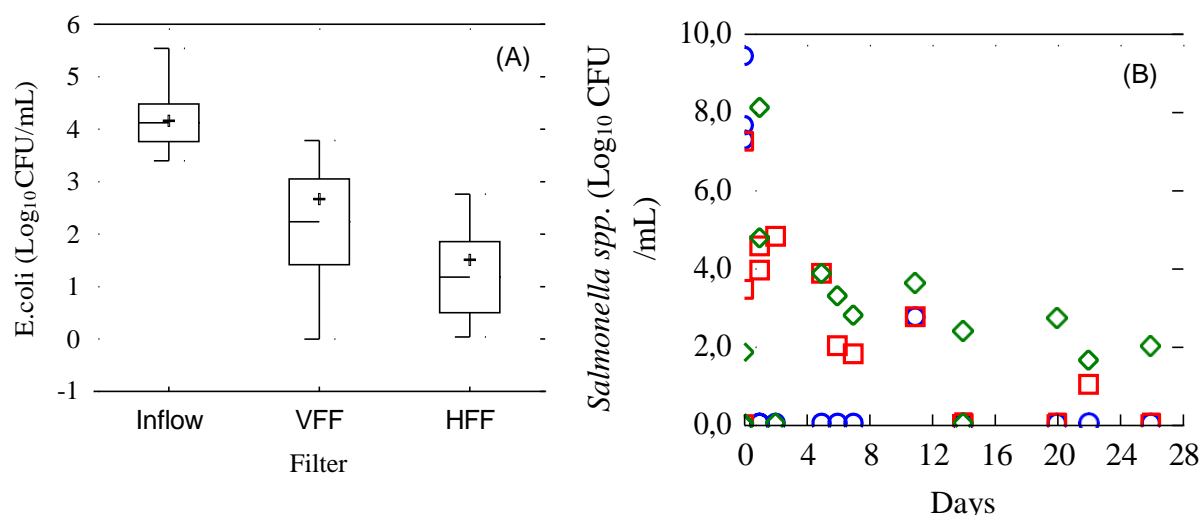


**Figure 12.** Boxplots of (A) nitrification efficiency in the vertical flow filter (VFF), (B) denitrification efficiency in the horizontal flow filter (HFF) and (C) overall removal of total nitrogen (Tot-N) in the entire system (combined VFF and HFF sections). The middle line (—) of the box is the mean, the cross (+) is the median, the box is the confidence interval, the whiskers are the non-outlier range and the red dots are the outliers and extreme values.

### 2.3.3 Pathogen removal

The concentration of *E. coli* in the inflow was 3.9-4.75 log<sub>10</sub> CFU mL<sup>-1</sup> (see Table 4). On average, effluent of VFF contained slightly lower numbers of *E. coli* (3.39±1.29 log<sub>10</sub> CFU mL<sup>-1</sup>) than effluent of HFF (2.76±1.05 log<sub>10</sub> CFU mL<sup>-1</sup>), but the difference was not statistically significant (Figure 13A). On average, the reduction in *E. coli* in VFF was 1.53 log<sub>10</sub> units, while the overall reduction in the entire system was 2.95 log<sub>10</sub> units. With increasing HLR from 23 to 31 and then 39 L m<sup>-2</sup> day<sup>-1</sup>, no significant effects were observed on the performance of the VFF and the HFF in terms of *E. coli* removal. The VFF achieved a 1.09-2.1 log<sub>10</sub> units reduction ( $p > 0.05$ ) and the HFF achieved a 2.48- 3.39 log<sub>10</sub> units reduction ( $p > 0.05$ ) at the HLR values investigated.

After spiking with *Salmonella* spp., the wastewater contained 7.29-9.40 log<sub>10</sub> CFU mL<sup>-1</sup>. The VFF and the HFF did not effectively remove *Salmonella* spp., but rather attenuated the microorganisms and slowly released them over time (Figure 13B). Following the addition of *Salmonella* spp. to the wastewater at HLR-23, the effluent of HFF released *Salmonella* spp. for 20, 5 and 21 days at HLR 23, 31 and 39 L m<sup>-2</sup> day<sup>-1</sup>, respectively, and the effluent contained 1.6 log<sub>10</sub> CFU mL<sup>-1</sup> at HLR 39 L m<sup>-2</sup> day<sup>-1</sup>.



**Figure 13.** (A) Boxplots of *Escherichia coli* concentration in the inflow and in effluent of the vertical flow filter (VFF) and horizontal flow filter (HFF). The middle line (–) of the box is the mean, the cross (+) is the median, the box is the confidence interval and the whiskers are the non-outlier range. B) Concentration of *Salmonella* spp. in the inflow (blue) and in effluent of HFF (green) and VFF (red) following spiking of the wastewater with *Salmonella* spp.

## 2.4 DISCUSSION

### 2.4.1 Removal of organic matter

Generally, the combined system of VFF and HFF achieved efficient removal of organic matter. After it reached stable state, the long-term removal of organic matter was  $93 \pm 3$  %. According to general recommendations by the Swedish Agency for Marine and Water Management for small and onsite wastewater treatment systems (HVMFS 2016: 17), the removal of organic material ( $BOD_7$ ) should be at least 90% for all such plants (Havs- och vattenmyndighetens, 2016). We did not measure  $BOD_7$  but, based on a  $BOD_7:COD$  ratio of 0.43, the combined VFF and HFF system achieved more than 90% removal of  $BOD_7$ .

Despite its shallow depth (30 cm), the VFF removed 87% of COD, *i.e.* most of the organic matter removal in the system, and did not show any sign of clogging or failure. The biodegradation of the organic matter under aerobic conditions led to this high removal of COD in the VFF. It should be pointed out the inflow was distributed over the whole area of the VFF and that the filter received raw wastewater on an intermittent basis (4 times per day). The uniform distribution of the wastewater probably led to better utilisation of the infiltration area and the intermittent loading gave the biofilm on the biochar particles the chance to consume the applied organic matter before the next loading, which prevented accumulation of organics and hence clogging. In the HFF, only a minor proportion of the COD was removed. We believe that the COD removed in the HFF was consumed by the denitrifying bacteria, which reduced the oxidised nitrogen to nitrogen gas. According to Daigger (2014), the amount of COD required to reduce nitrate to nitrogen is about  $2.85 \text{ mg O}_2 \text{ mg}^{-1} \text{ N}$ . The effluent of VFF contained on average  $29 \text{ mg NO}_3\text{-N L}^{-1}$ , which required about  $83 \text{ mg COD L}^{-1}$  to be available for denitrification. The effluent of the VFF and HFF contained  $61 \pm 21$  and  $30 \pm 12 \text{ mg COD L}^{-1}$ , respectively. The change in HLR from 23 to 31 to  $39 \text{ L m}^{-2} \text{ day}^{-1}$  did not influence the efficiency of the system in COD removal. It is likely that the changes in the HLR were not so large as to

have a significant influence, but we also speculate that the HFF worked as a buffering zone and it used its capacity (biofilm) to degrade additional organic matter which escaped from the VFF without treatment.

### 2.4.2 Long-term removal of nitrogen

On a long-term basis, the combined VFF-HFF system achieved good removal of Tot-N ( $71 \pm 12$  %) (Figure 10). During the last 52 weeks of operation, only one measurement showed Tot-N removal  $< 50$ %. Combining the anaerobic VFF with an anaerobic stage (HFF) provided a suitable environment for nitrification-denitrification and hence enhanced nitrogen removal significantly. In fact, after stabilisation of the combined HFF-VFF system, most of the nitrogen removal ( $61 \pm 14$  %) occurred in the HFF (saturated section) and only a small proportion of Tot-N removal ( $10 \pm 12$  %) occurred in the VFF. This indicates that addition of a saturated flow filter, *i.e.* horizontal flow, is crucial to achieving acceptable levels of nitrogen removal from wastewater. However, the VFF was still a critical component of the system, since without it nitrification would have not been achieved. This was proven by the good nitrification capacity in the VFF ( $65 \pm 15$  %). Due to nitrification, most of the  $\text{NH}_4\text{-N}$  in the wastewater ( $34.2 \pm 9.1 \text{ mg L}^{-1}$ ) was removed ( $93 \pm 2$  %), which left only  $4.5 \pm 4.3 \text{ mg L}^{-1}$  in the effluent from the VFF. In parallel with  $\text{NH}_4\text{-N}$  removal, the VFF effluent showed an increase in  $\text{NO}_3\text{-N}$  concentration ( $29.3 \pm 7.5 \text{ mg L}^{-1}$ ). The  $\text{NO}_3\text{-N}$  concentration decreased in the HFF, which showed that nitrification and denitrification took place in the system. The nitrification and denitrification efficiencies started low, but both continued to improve over time even during the last 52 weeks of filter operation (the system operated for 126 weeks). Nitrifying bacteria are autotrophic and use inorganic carbon sources, *e.g.*  $\text{CO}_2$ , to obtain energy. This causes slow growth rate of nitrifying bacteria (Bassin *et al.*, 2012). In addition, other factors such as temperature, pH, concentration of organic matter and concentration of  $\text{NH}_4$  affect the growth of nitrifying bacteria (Bassin *et al.*, 2012). It seemed that conditions favouring the growth of denitrifying bacteria (*i.e.* development of anoxic zones and anaerobic biofilm) were developing over time in the biochar particles in the HFF and therefore the denitrification efficiency increased (Figure 11).

According to the Swedish Agency for Marine and Water Management, effluent from onsite wastewater treatment systems should show a minimum reduction of 50% for nitrogen if the surrounding area is classified as sensitive (Havs- och vattenmyndigheten, 2016). The combined vertical-horizontal flow filter system achieved on average  $71 \pm 12$  % removal of Tot-N, which is in good compliance with the regulations.

### 2.4.3 Effects of hydraulic loading rate on nitrogen removal

Increasing the HLR from  $23$  to  $31 \text{ L m}^{-2} \text{ day}^{-1}$  increased the nitrification efficiency from 46 to 62 %. The specific surface of the biochar used in the system was large ( $200 \text{ m}^2 \text{ g}^{-1}$ ). Thus, when the HLR was increased more surfaces were reached by the wastewater and hence more contact between nitrifying bacteria and  $\text{NH}_4$  occurred, which led to increased nitrification. Another explanation is that HLR  $23 \text{ L m}^{-2} \text{ day}^{-1}$  was low and not all the infiltrative surface of the VFF was used. Thus, when the HLR was increased, the infiltrative surface of the biochar was effectively utilised and therefore nitrification was enhanced. An increase in HLR usually leads to washout of  $\text{NH}_4\text{-N}$  without nitrification owing to shortening contact time between nitrifying

bacteria and  $\text{NH}_4\text{-N}$  (Dalahmeh *et al.*, 2014b). Increasing the HLR from 31 to 39  $\text{L m}^{-2} \text{ day}^{-1}$  decreased the denitrification efficiency, which was reflected as high concentrations of  $\text{NO}_3\text{-N}$  in the effluent from the HFF ( $16.1 \pm 4.0 \text{ mg L}^{-1}$ ) compared with inflow to the HFF ( $23.7 \pm 4.8 \text{ mg L}^{-1}$ ). In parallel with the decrease in denitrification efficiency, the overall removal of Tot-N in the system decreased. This is because at HLR-39, more nitrate escaped from the HFF before it was reduced to  $\text{N}_2$  by the bacteria. Most likely, the hydraulic retention time in the HFF decreased in response to the increase in HLR. Despite the decline, the system still removed  $59 \pm 7 \%$  of Tot-N, which still acceptable and in line with the 50% nitrogen removal recommended by the Swedish Agency for Marine and Water Management for areas with a sensitive recipient (Havs- och vattenmyndigheten, 2016).

#### 2.4.4 Pathogen removal

The overall reduction in *E. coli* in the VFF and HFF system was  $2.95 \log_{10}$  units, compared with  $3.9\text{-}4.75 \log_{10} \text{ CFU mL}^{-1}$  in the inflow. This means that substantial numbers of *E. coli* were present in the effluent of the system. Removal of *E. coli* in the VFF and HFF was only achieved by filtration and straining in the pores of the biochar, and conditions for inactivation of bacteria (*e.g.* high or low pH, high temperature) were not present. Similarly, the VFF and HFF did not achieve effective removal of *Salmonella* spp., but rather attenuated the concentration of the microorganism and released it gradually. It should be pointed out that the levels of *Salmonella* spp. spiked in the wastewater were very high ( $7.29\text{-}9.40 \log_{10} \text{ CFU mL}^{-1}$ ) compared with those expected to be found in wastewater. Previous studies report that wastewater can contain  $0.001\text{-}100 \text{ CFU mL}^{-1}$  (WHO, 2006), which is much lower than the level in the present study. However, if the wastewater contains up to  $3.0 \log_{10} \text{ CFU mL}^{-1}$ , a combined VFF and HFF biochar system might be able to remove *Salmonella* spp. and the effluent would be considered safe for disposal.

### 2.5 CONCLUDING REMARKS

- The long-term performance of the system was satisfactory in terms of organic matter and nitrogen removal ( $>90\%$  and  $>70\%$ , respectively). No signs of clogging or deterioration of either organic matter or Tot-N were observed during the 126 weeks of operation.
- Despite the variation in hydraulic loading, the system still achieved high removal of organic matter (90-93%) and nitrogen ( $>50\%$  removal), which showed robustness of the combined VFF and HFF filters.
- The combined VFF and HFF system achieved substantial removal rates of *E. coli* and *Salmonella* spp. ( $3.0 \log_{10}$  units), but the safety of the effluent will depend on the initial concentration of the microorganisms in the inflow. At higher levels of microbial contamination, playing or bathing in water close to points where the effluent is released should be avoided. Protective measures, *e.g.* wearing gloves and mask and washing hands, are recommended for at least 3 weeks when handling filters after cases of infection.
- In addition, the design of the filter system with respect to depth:width:length of the horizontal flow system has not yet been investigated and more research is needed on this aspect.



### 3. REMOVAL OF PHARMACEUTICALS AND PER- AND POLYFLUORINATED ALKYL SUBSTANCES (PFASs)

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#### 3.1 BACKGROUND

Onsite sewage facilities (OSSFs) have recently been recognised as a significant source of hazardous micropollutants such as pharmaceutically active compounds (PhACs) and polyfluorinated alkyl substances (PFASs) (Ejhed *et al.*, 2011; Hav- och vattenmyndigheten, 2016). Conventional OSSF treatment techniques, such as sand filter and soil infiltration treatment systems, have been proven to be only partially efficient or even inefficient in removal of different types of organic micropollutants, including PhACs. For example, Gros *et al.* (2016) reported intermediate to low removal of PhACs in soil beds used for OSSF (*e.g.* 46% removal for ibuprofen, 86% for diclofenac, 44% for metoprolol and 73% for caffeine). Blum *et al.* (2017) reported insignificant (<10%) removal of *e.g.* acetaminophen in soil beds. Matamoros *et al.* (2009) found that well-functioning sand filters achieved removal efficiency of >80% for the PhACs salicylic acid, ibuprofen and carboxy-ibuprofen, but failed to remove carbamazepine, diclofenac and ketoprofen. In addition, Gros *et al.* (2016) reported  $\Sigma$ PFAS concentrations of 18-52 ng L<sup>-1</sup> in the influent of OWTSS and 57 ng L<sup>-1</sup> in grey water. The same study found that effluent from soil beds showed higher  $\Sigma$ PFAS concentrations (33 ng L<sup>-1</sup>) than the influent (18 ng L<sup>-1</sup>), indicating degradation of PFAS precursors. A purification step for micropollutants is generally lacking in OWTSS (Blum *et al.*, 2017).

Thus a better understanding of the effects of biofilm on removal of PFASs via adsorption and bioadsorption processes is essential for the development of biochar filter-based OWTSS to achieve concurrent removal of organic matter, nutrients and micropollutants such as PFASs. Besides adsorption, biofilms can play an important role in biodegradation of certain PhACs. In fact, adsorption and biodegradation through co-metabolism in biologically active carbon has been used for removal of metoprolol in a study which found that easily degradable organic substances such as acetate enhanced the removal rate (Abromaitis *et al.*, 2016). In contrast, Saravia and Frimmel (2008) showed that the presence of organic matter reduced adsorption of the PhAC carbamazepine on activated carbon, due to competitive adsorption between the carbamazepine and organic matter. Synergetic removal of PhACs, organic matter and nutrients in biochar filters in a system in which a combination of adsorption and biodegradation processes is utilised and sustained by self-biogenesis of the filter is a novel technology which can be developed for OSSF treatment. While the biodegradation mechanism itself can be similar in different filter systems and media, the combination of biofilm-enhanced degradation and adsorption will vary widely depending on the type and characteristics of the media used and the physicochemical properties of the micropollutant (Ahmed *et al.*, 2017; Dalahmeh *et al.*, 2014a; Sun *et al.*, 2016).

In this section, we discuss the potential of biochar filters for onsite wastewater treatment in terms of micropollutant removal. Specifically, we describe the effects of biodegradation, adsorption and a combination of these two processes on the removal of PhACs and PFAS from

wastewater using biochar filters and assess the efficiency of biochar filters with/without active biofilm in removal of PhACs and PFAS from wastewater.

## **3.2 MATERIAL AND METHODS**

### **3.2.1 Experimental set-up**

Removal of the target PFASs and PFAS was investigated in four different biochar (BC) filter treatments: BC-no-biofilm, BC-active-biofilm, BC-inactive-biofilm, sand-active-biofilm, compared with sand alone (Figure 14). The experiments were performed over a period of 22 weeks in acrylic column filters (diameter 5 cm; height 60 cm) filled with biochar (four treatments) and one sand column. Wastewater from the influent of pre-sedimentation tanks at Uppsala's municipal wastewater treatment plant (Kungsängsverket), Sweden, was spiked with PhACs and PFASs. The biochar filter without biofilm (BC-no-biofilm) was used to assess adsorption of PhACs and PFASs onto the biochar surface. The biochar filter with active biofilm (BC-active-biofilm) was used to investigate concurrent biological degradation and adsorption (bioadsorption) of PhACs and PFASs onto biochar. The biochar filter with inactivated biofilm (BC-inactive-biofilm) was used to assess the removal of PFASs by adsorption onto surfaces of dead biofilm (dead bacteria and extracellular materials from dead biofilm) and organic deposits (solids from wastewater) besides the biochar surface. In addition, biological degradation of PhACs and PFASs by active biofilm treatment was investigated in sand (Sand-active-biofilm).

### **3.2.2 Biofilm development and inactivation**

The BC-no-biofilm and filter blank were not fed with any liquid during the first 5 weeks of the experiment. From week 6 until week 22, the BC-no-biofilm was fed with pure MilliQ<sup>®</sup> water spiked with PhACs and PFASs and 10 mL of 1500 sodium azide mg L<sup>-1</sup> (NaN<sub>3</sub>) to inhibit development of biofilm. In BC-active-biofilm, BC-inactive biofilm and Sand-active-biofilm, the biofilm was first established by supplying the filters with wastewater for a period of 5 weeks. After the initial 5 weeks, the biofilm in BC-active-biofilm and Sand-active-biofilm was kept active by supplying the filters with wastewater spiked with PhACs and PFASs until the end of the experiment (week 22). In BC-inactive-biofilm, the biofilm was inactivated after 5 weeks by heating the filters at 70 °C for 3 hours in week 6 and week 9. From week 6 until week 22, BC-inactive biofilm received wastewater spiked with PFASs, to which 10 mL of sodium azide solution (1500 mg NaN<sub>3</sub> L<sup>-1</sup>) was continuously added to inhibit re-establishment of active biofilm (Figure 14).

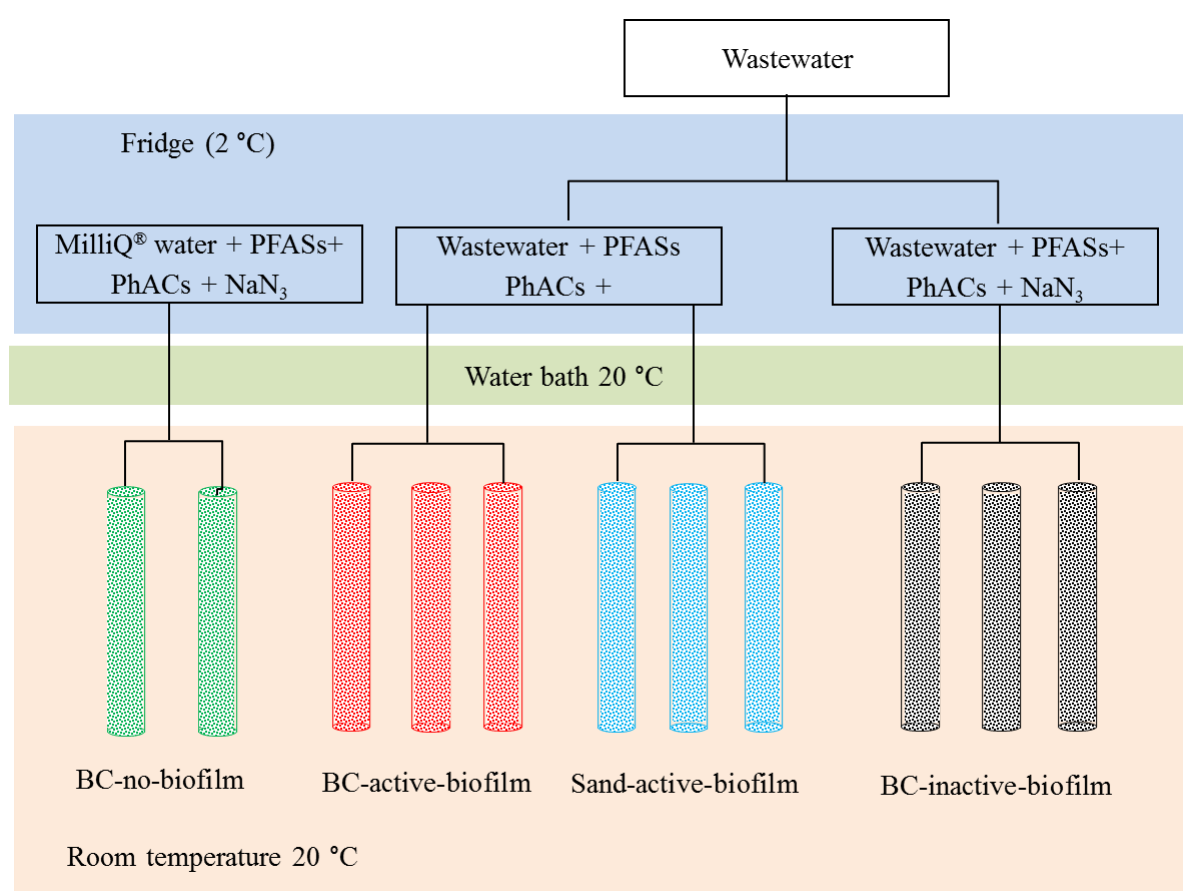
### **3.2.3 Wastewater preparation, feeding and sampling**

To feed the filters in treatments with active and inactive biofilm, municipal wastewater was collected weekly from the influent of the pre-sedimentation tanks at Kungsängsverket and inoculated with a standard mixture to give a final concentration of 3-5 µg L<sup>-1</sup> of 13 target PFASs and 10 µg L<sup>-1</sup> of four target PhACs (Table 7). Similarly, the MilliQ<sup>®</sup> water used to feed the BC-no-biofilm filters was spiked with the same PhACs and PFAS target compounds at the same final concentrations. A portion of wastewater inoculated with PhACs and PFASs was used to feed the biochar and sand filters with active biofilm (BC-active-biofilm and Sand-active-

biofilm) (Figure 14). Another portion was inoculated with sodium azide ( $1500 \text{ mg L}^{-1}$ ) and used to feed the biochar filters with inactivated biofilm (BC-inactive-biofilm). All filters received their relevant feed using a single-pass downflow regime at HLR of  $50 \text{ L m}^{-2} \text{ day}^{-1}$  over a period of 22 weeks. In daily sampling, 50 mL of influent and column effluent ( $71 \pm 5 \text{ mL}$ ) from each filter were collected in amber glass bottles and cooled to  $0-2 \text{ }^{\circ}\text{C}$ . After a 7-day period, the influent and effluent samples from each column were pooled in amber glass bottles to a single sample and stored at  $-18 \text{ }^{\circ}\text{C}$  until PhAC and PFAS analysis.

### 3.2.4 Extraction and analysis of PFASs

The PhACs and PFASs were extracted and analysed according to methods described in Dalahmeh et al. (2018); Dalahmeh et al. (2019a).



**Figure 14.** Schematic diagram of the column experiments using biochar (BC) with active biofilm, inactive biofilm and no biofilm, sand with active biofilm and a glass bead filter as a filter blank (T5,  $n = 1$ ). PhACs = pharmaceutically active compounds. Diagram taken from (Dalahmeh et al., 2018).

**Table 7.** List of the pharmaceutically active compounds (PhACs) and polyfluorinated alkyl substances (PFASs) investigated in this study and their properties and corresponding internal standards (IS)

Acronym	Compound (Therapeutic effects)	Chemical Formula	MW (g mol <sup>-1</sup> )	$pK_a$	Log $K_{oc}$	Log $K_{ow}$
<b>Pharmaceuticals (PhACs)</b>						
Met	Metoprolol ( $\beta$ -blocker)	C <sub>15</sub> H <sub>25</sub> NO <sub>3</sub>	267.37	9.57 <sup>j</sup>	1.79	1.88
CBZ	Carbamazepine (Antidepressant)	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O	236.27	7.0	3.59	2.45
Ran	Ranitidine (Anti-ulcer agent)	C <sub>13</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub> S	314.41	8.08	4.44	0.27
Caff	Caffeine (Stimulant)	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	194.19	10.4	1.00	-0.07
<b>Polyfluorinated alkyl substances (PFASs)</b>						
PFBS (C <sub>4</sub> )	Perfluorobutane sulfonate	C <sub>4</sub> F <sub>9</sub> SO <sub>3</sub> <sup>-</sup>	299.05	0.14	2.5	3.9
PFHxS (C <sub>6</sub> )	Perfluorohexane sulfonate	C <sub>6</sub> F <sub>13</sub> SO <sub>3</sub> <sup>-</sup>	399.07	0.14	2.7	5.17
PFOS (C <sub>8</sub> )	Perfluorooctane sulfonate	C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup>	499.09	0.14	3.34	6.43
FOSA (C <sub>8</sub> )	Perfluorooctane sulfonamide	C <sub>8</sub> F <sub>17</sub> SO <sub>2</sub> NH <sub>2</sub>	499.12	5.56	n.a.	5.62
PFBA (C <sub>3</sub> )	Perfluorobutanoate	C <sub>3</sub> F <sub>7</sub> CO <sub>2</sub> <sup>-</sup>	213.04	0-1	n.a.	2.82
PFPeA (C <sub>4</sub> )	Perfluoropentanoate	C <sub>4</sub> F <sub>9</sub> CO <sub>2</sub> <sup>-</sup>	263.05	-0.10	1.95	3.43
PFHxA (C <sub>5</sub> )	Perfluorohexanoate	C <sub>5</sub> F <sub>11</sub> CO <sub>2</sub> <sup>-</sup>	313.06	2-3	1.91	4.06
PFHpA (C <sub>6</sub> )	Perfluoroheptanoate	C <sub>6</sub> F <sub>13</sub> CO <sub>2</sub> <sup>-</sup>	363.07	-0.20	2.19	4.67
PFOA (C <sub>7</sub> )	Perfluorooctanoate	C <sub>7</sub> F <sub>15</sub> CO <sub>2</sub> <sup>-</sup>	413.08	2-3	2.31	5.3
PFNA (C <sub>8</sub> )	Perfluorononanoate	C <sub>8</sub> F <sub>17</sub> CO <sub>2</sub> <sup>-</sup>	463.09	-0.21	2.33	5.92
PFDA (C <sub>9</sub> )	Perfluorodecanoate	C <sub>9</sub> F <sub>19</sub> CO <sub>2</sub> <sup>-</sup>	513.10	-0.22	3.17	6.5
PFUnDA (C <sub>10</sub> )	Perfluoroundecanoate	C <sub>10</sub> F <sub>21</sub> CO <sub>2</sub> <sup>-</sup>	563.11	-0.22	3.30	7.15
PFDoDA (C <sub>11</sub> )	Perfluorododecanoate	C <sub>11</sub> F <sub>23</sub> CO <sub>2</sub> <sup>-</sup>	613.12	-0.22	n.a.	7.77

n.a. = not available.

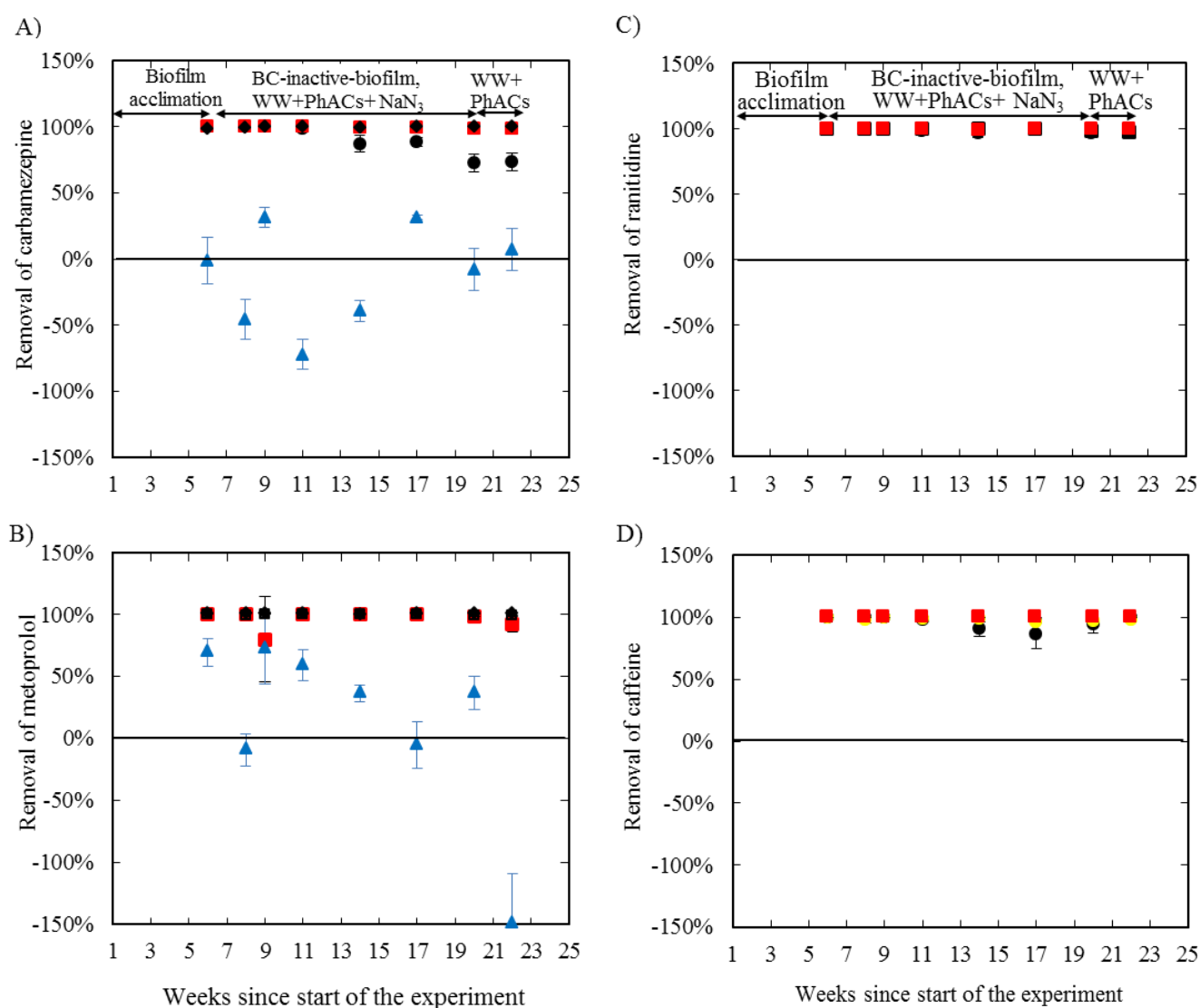
### 3.3 RESULTS

#### 3.3.1 Removal of PhACs

The average concentration of metoprolol in the influent in the different treatments ranged from 1900 to 3100 ng L<sup>-1</sup>. The BC-active-biofilm and BC-no-biofilm filters showed efficient and consistent removal of metoprolol during the experiment, achieving mean removal of >99% and an effluent concentration of 24±2 ng L<sup>-1</sup> (Figure 15). The BC-inactive-biofilm filter displayed a high overall removal rate of 98±3 %. The Sand-active-biofilm filters showed fluctuations in removal of metoprolol, with the highest removal (73±29 %) measured in week 9 and the lowest removal (36±7 %) measured in week 14. Metoprolol was released in the effluent of the sand filter in weeks 8, 17 and 22, with a peak concentration of 660±110 ng L<sup>-1</sup> in week 22.

The average concentration of ranitidine in influent to the different treatments varied from 3200±860 to 7200±1500 ng L<sup>-1</sup>. The average removal of ranitidine in the different biochar treatments ranged from 98 to 99% (Figure 15C). In week 22, the concentration of ranitidine in the effluent of the BC-inactive-biofilm filter was 74±10 ng L<sup>-1</sup>, corresponding to a removal rate of 98%. The removal of ranitidine in the Sand-active-biofilm filter varied between 96% and 99% over time, with the lowest removal rate of 96% (corresponding to 116±19 ng L<sup>-1</sup> in the effluent) observed at the end of the experiment.

The concentration of caffeine in influent to the different treatments varied between  $3800 \pm 720$  and  $4700 \pm 3200$  ng L<sup>-1</sup>. Efficient overall removal of caffeine, with average rates of 96-99%, was observed during the experiment in all filters except BC-inactive-biofilm. In these filters, breakthrough of caffeine occurred in weeks 13 to 17, which resulted in an effluent concentration of 270-560 ng L<sup>-1</sup> (Figure 15D). However, the removal efficiency increased thereafter for the BC-inactive-biofilm filters to the end of the experiment, when the effluent concentration was 25 ng L<sup>-1</sup>, demonstrating 99% removal.



**Figure 15.** Capacity of the biochar (BC)-based BC-active-biofilm (■), BC-inactive-biofilm (●) and BC-no-biofilm (♦) and of Sand-active biofilm (▲) for removal of (A) carbamazepine, (B) metoprolol, (C) ranitidine and (D) caffeine during the 22-week experiment. Symbols and error bars represent mean  $\pm$  standard deviation. PhACs = pharmaceutically active compounds. Diagram taken from (Dalahmeh et al., 2018).

### 3.3.2 Removal of PFASs

In BC-no-biofilm, the removal efficiency of PFASs with a long perfluorocarbon chain, such as PFBA (C<sub>3</sub>), PFPeA (C<sub>4</sub>), PFHxA (C<sub>5</sub>), PFHpA (C<sub>6</sub>) and PFBS (C<sub>4</sub>), was on average lower than 60%, with PFBA (C<sub>3</sub>) showing the lowest removal ( $20 \pm 30$  %) (Figures 16 and 17). The removal

efficiency of PFASs with a long perfluorocarbon chain, such as C<sub>7</sub>-C<sub>11</sub> PFCAs and C<sub>6</sub>, C<sub>8</sub> PFSA and FOSA (C<sub>8</sub>), was high and ranged between 90±10 % and 99±2 %. C<sub>7</sub>-C<sub>11</sub> PFCAs and C<sub>6</sub>, C<sub>8</sub> PFSA and FOSA (C<sub>8</sub>) showed consistently stable removal efficiencies from week 6 until week 22 of the experiment (Figures 16-18).

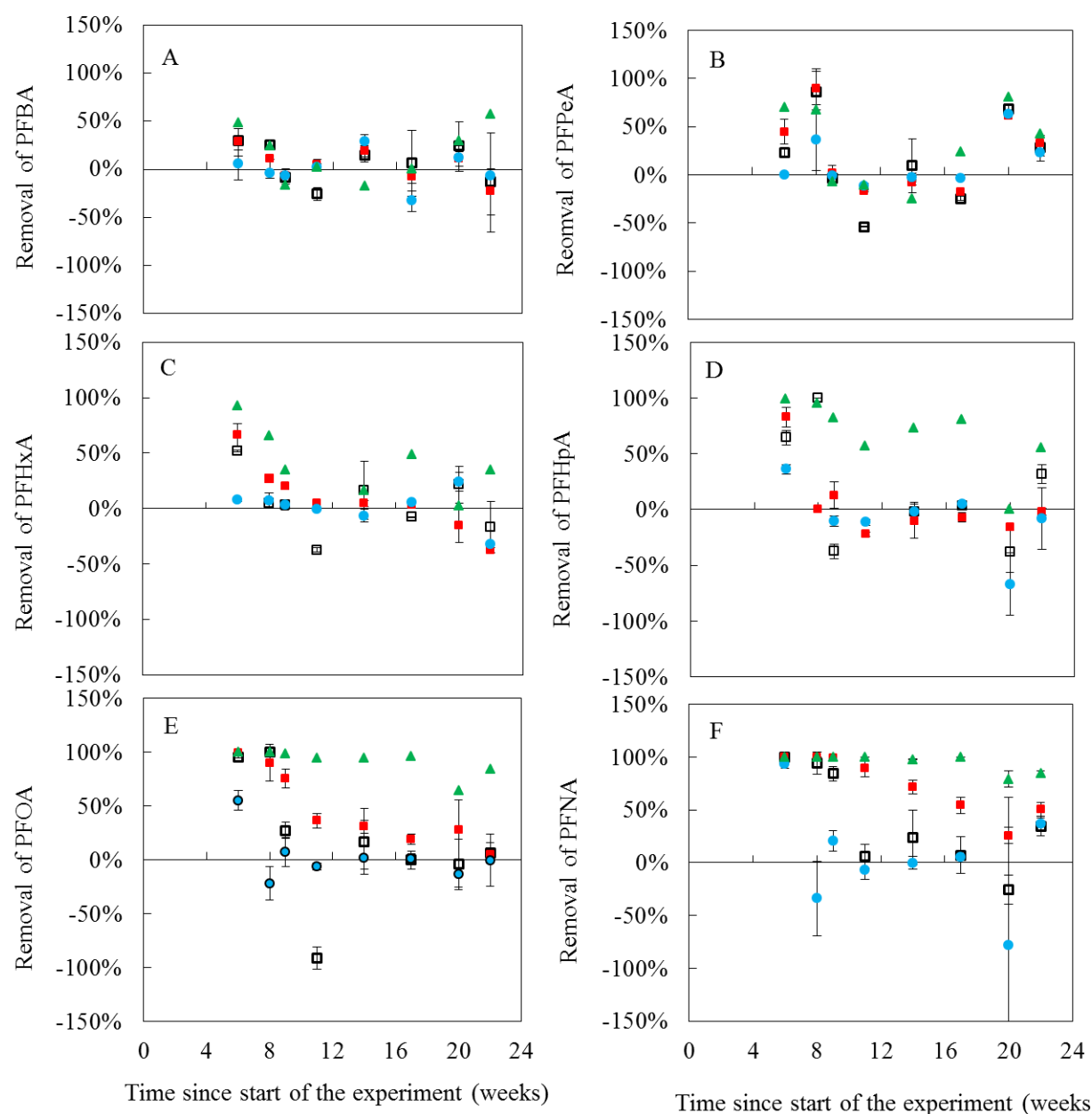
In BC-active-biofilm, the low removal efficiency of PFBA (C<sub>3</sub>), PFPeA (C<sub>4</sub>), PFHxA (C<sub>5</sub>) and PFBS (C<sub>4</sub>) and the high removal (90-99%) of the longer-chained PFASs, PFDA (C<sub>9</sub>), PFUnDA (C<sub>10</sub>), PFDoDA (C<sub>11</sub>), PFOS (C<sub>8</sub>) and FOSA (C<sub>8</sub>) were not significantly different from those observed for BC-no-biofilm ( $p>0.05$ ). However, the removal of PFOA (C<sub>7</sub>), PFNA (C<sub>8</sub>) and PFHxS (C<sub>6</sub>) in BC-active-biofilm ranged between 5-50% during week 22 and was lower than that estimated for BC-no-biofilm (75-90%) (Figures 16 and 17).

BC-inactive-biofilm showed poor removal of all PFASs (-20-70%) except PFUnDA (C<sub>10</sub>), PFDoDA (C<sub>11</sub>) and FOSA (C<sub>8</sub>), with removal efficiencies of around 90% (Figures 17 and 18). Similarly to in BC-no-biofilm and BC-active-biofilm, mean removal of PFUnDA (C<sub>10</sub>), PFDoDA (C<sub>11</sub>) and FOSA (C<sub>8</sub>) was high (90±30 %, 90±10 % and 90±20 %, respectively). However, their removal efficiencies started to decrease in week 17 and reached 85±1 % for PFUnDA (C<sub>10</sub>), 70±6 % for PFDoDA (C<sub>11</sub>) and 86±3 % for FOSA (C<sub>8</sub>) in week 22.

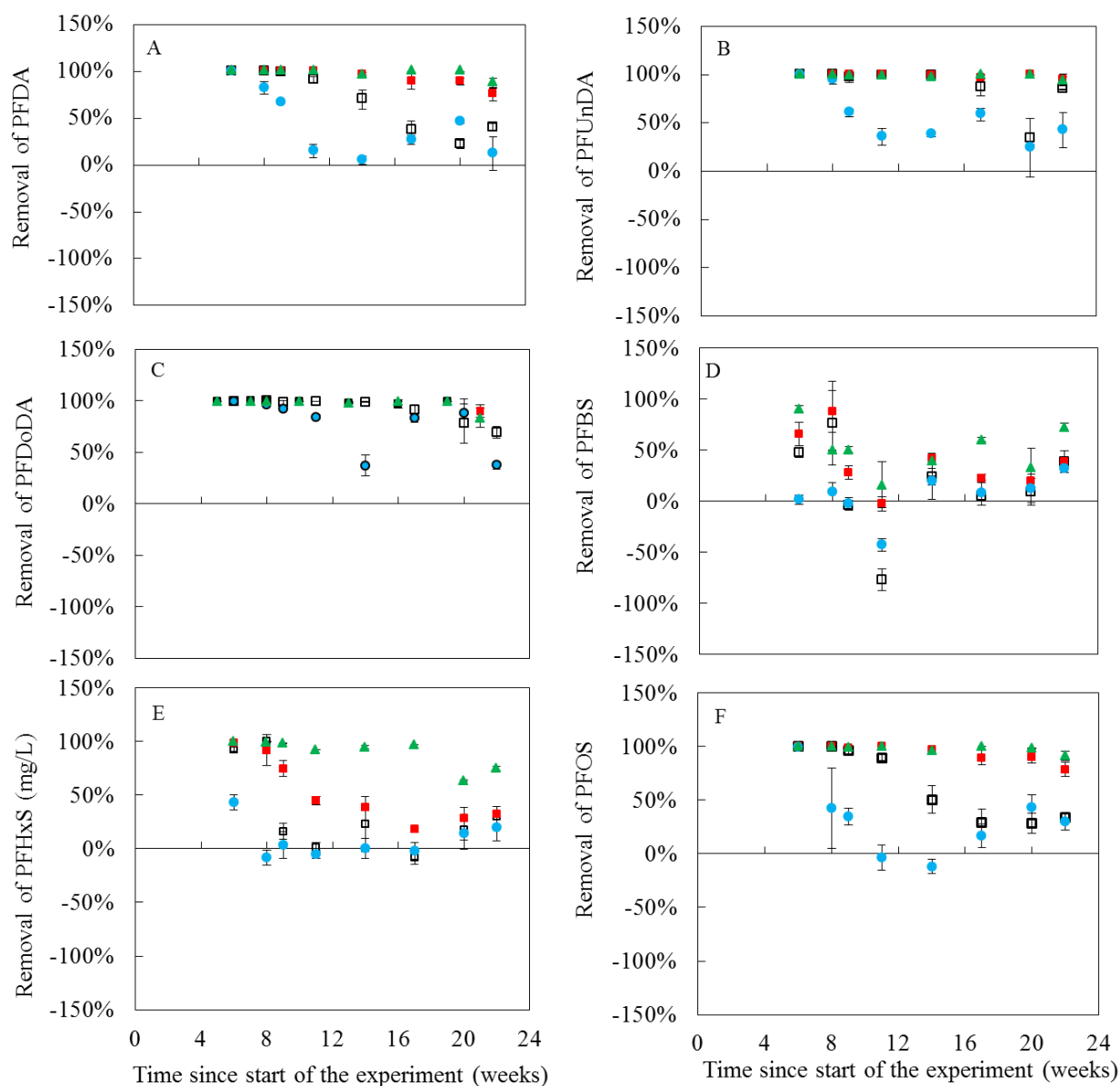
Sand-active-biofilm showed high mean removal only for FOSA (C<sub>8</sub>) (86±6 %), which was sustained throughout the weeks of the experiment (Figure 18). On the other hand, PFUnDA (C<sub>10</sub>) (60 ± 30%) and PFDoDA (C<sub>11</sub>) (70 ± 40%) had better mean removal than the rest of the target PFASs, but their removal efficiency declined with time (Figures 16 and 17).

The mass of PFASs adsorbed to the filter media over the period of experiment (22 weeks) is referred to the  $\Sigma$ PFAS adsorption capacity (expressed as ng  $\Sigma$ PFAS per g). BC-no-biofilm showed the highest  $\Sigma$ PFAS adsorption capacity (1200 ng g<sup>-1</sup>, 62%), while the Sand-active-biofilm showed the lowest (40 ng g<sup>-1</sup>, 17%). BC-active-biofilm adsorbed 600 ng  $\Sigma$ PFAS g<sup>-1</sup> and BC-inactive-biofilm adsorbed 500 ng  $\Sigma$ PFAS g<sup>-1</sup>.

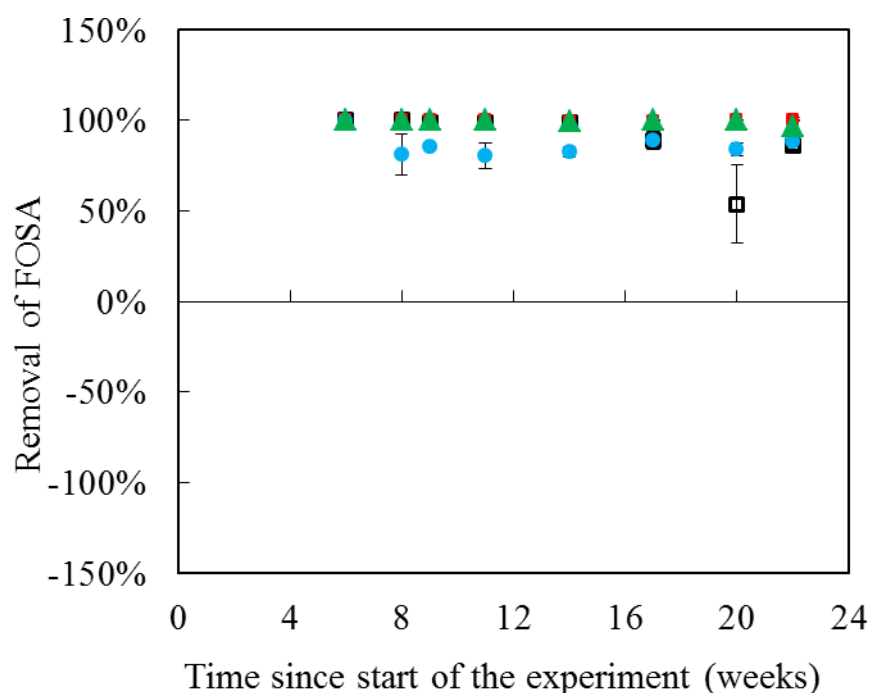
As regards the adsorption capacity of the individual PFASs in BC-no-biofilm, PFBA (C<sub>3</sub>) showed the lowest cumulative adsorption capacity (0 ng g<sup>-1</sup>) and PFOA (C<sub>7</sub>) and PFNA (C<sub>8</sub>) showed the highest (165±2.9 ng g<sup>-1</sup> or 90% adsorption and 168±1.3 ng g<sup>-1</sup> or 96% adsorption, respectively). The adsorption patterns of the individual PFASs in BC-active-biofilm and BC-inactive biofilm showed similar trends to those obtained for BC-no-biofilm, but the adsorption rates were lower for BC-active-biofilm and BC-inactive-biofilm than for BC-no-biofilm.



**Figure 16.** Efficiency of the biochar (BC)-based filters BC-no-biofilm (T1, ▲), BC-active-biofilm (T2, ■) and BC-inactive-biofilm (T3, □) and of Sand-active biofilm (T4, ●) in removal of A) PFBA, B) PFPeA, C) PFHxA, D) PFHpA, E) PFOA and F) PFNA during a 22-week experiment. Symbols and error bars represent mean  $\pm$  standard deviation.  $n=2$  for BC-no-biofilm,  $n=3$  for BC-active-biofilm, BC-inactive-biofilm and Sand-active-biofilm. Diagram taken from (Dalahmeh et al., 2018)(Dalahmeh et al., 2018)(Dalahmeh et al., 2018)(Dalahmeh et al., 2018)(Dalahmeh et al., 2018)(Dalahmeh et al., 2018)(Dalahmeh et al., 2018)Dalahmeh *et al.* (2019b).



**Figure 17.** Efficiency of the biochar (BC)-based filters BC-no-biofilm (T1, ▲), BC-active-biofilm (T2, ■) and BC-inactive-biofilm (T3, □) and of Sand-active biofilm (T4, ●) in removal of A) PFDA, B) PUnDA, C) PDoDA, D) PFBS, E) PFHxS and F) PFOS during a 22-week experiment. Symbols and error bars represent mean  $\pm$  standard deviation.  $n=2$  for BC-no-biofilm,  $n = 3$  for BC-active-biofilm, BC-inactive-biofilm and Sand-active-biofilm. Diagram taken from Dalahmeh *et al.* (2019b).



**Figure 18.** Efficiency of the biochar (BC)-based filters BC-no-biofilm (T1, ▲), BC-active-biofilm (T2, ■) and BC-inactive-biofilm (T3, □) and of Sand-active biofilm (T4, ●) in removal of perfluorooctane sulfonamide (FOSA) during a 22-week experiment. Symbols and error bars represent mean  $\pm$  standard deviation.  $n=2$  for BC-no-biofilm,  $n=3$  for BC-active-biofilm, BC-inactive-biofilm and Sand-active-biofilm. Diagram taken from Dalahmeh *et al.* (2019b).

### 3.4 DISCUSSION

#### 3.4.1 Removal of PhACs

Carbamazepine was removed efficiently in BC-no-biofilm and in BC-active-biofilm (Figure 15A). The removal mechanism was mainly adsorption to the surface of the biochar, as indicated by the high removal in BC-no-biofilm. The poor to no removal of carbamazepine in the Sand-active-biofilm filters (31% to -46%) indicated that carbamazepine is poorly biodegradable. Radjenovic *et al.* (2007) also report poor biodegradability of carbamazepine. However, due to the contribution of active biomass to pollutant removal, the lifetime of the adsorbent is longer. In the absence of a substantial biologically active biofilm, as in BC-inactive-biofilm, the adsorption of carbamazepine declined from 99 to 73 % after 14 weeks of treatment due to competition for adsorption surfaces between organic matter and PhACs.

The removal of metoprolol was efficient in all biochar treatments, mostly due to its high adsorption to the biochar surface (in BC-no-biofilm and BC-active-biofilm) or due to partitioning to solids available on the biochar surface (in BC-inactive-biofilm). Sorption of metoprolol to the biofilm also occurred in the Sand-active-biofilm filter, but to a lesser extent (36-73%) than in BC-active-biofilm (>95%). This is due to the fact that sand has a smaller specific surface area ( $0.14 \text{ m}^2 \text{ g}^{-1}$ ) than biochar ( $180 \text{ m}^2 \text{ g}^{-1}$ ). However, the removal of

metoprolol in Sand-active-biofilm observed in this study agrees with findings by Gros *et al.* (2016) showing removal of 42% in sand filter beds and 44% in biological mini-treatment plants.

Removal of ranitidine was efficient in all filters, including Sand-active-biofilm, demonstrating high biodegradation capacity of ranitidine. Rosal *et al.* (2010) reported 75% removal of ranitidine in a large-scale biological wastewater treatment plant. In contrast, Carucci *et al.* (2006) observed only low removal in a sequencing batch reactor under aerobic and anaerobic conditions and high ranitidine concentrations (2-3 mg L<sup>-1</sup>).

Removal of caffeine was efficient in all biochar filters and in Sand-active-biofilm, which suggests that biodegradation was an effective mechanism for removal of this substance. In contrast, the efficient removal of caffeine observed in BC-no-biofilm indicates dominance of adsorption in biochar filters. Batt *et al.* (2007) concluded that removal of caffeine, among other organic micropollutants in wastewater, is dependent on a combination of biological and physico-chemical treatment. Efficient removal of caffeine, as observed in the present study, has also been reported in slow sand filters (D'Alessio *et al.*, 2015). In contrast, Gros *et al.* (2016) reported lower removal rates of caffeine, with 81% in mini-biological treatment systems, 73% in soil infiltration systems and only 38% in large-scale treatment plants with biological treatment.

### 3.4.2 Adsorption of PFAS

PFASs are extremely resistant to biological degradation and adsorption was the main process governing removal of PFASs in all BC-no-biofilm, BC-active-biofilm and BC-inactive-biofilm. The low removal of short perfluorocarbon chain (C<7) substances (*e.g.* PFBA (C<sub>3</sub>), PFPeA (C<sub>4</sub>), PFHxA (C<sub>5</sub>), PFHpA (C<sub>6</sub>) and PFBS (C<sub>4</sub>) in all treatments can be explained by their highly hydrophilic nature, leading to high water solubility (Zaggia *et al.*, 2016). Ahrens *et al.* (2011) also showed that shorter-chain PFCAs (C<7) predominantly partition to the dissolved phase. Consequently, the removal of PFBA (C<sub>3</sub>), PFPeA (C<sub>4</sub>), PFHxA (C<sub>5</sub>), PFHpA (C<sub>6</sub>) and PFBS (C<sub>4</sub>) was low. It has also been observed previously that short-chain PFASs are replaced by longer-chain PFASs in anion exchange and GAC adsorbents, which can lead to low removal efficiency for shorter-chain PFASs (McCleaf *et al.*, 2017). Generally, shorter-chain PFASs are known to have low removal efficiencies in wastewater (Barmentlo *et al.*, 2015). In contrast, low water solubility (log *S* = -3.5 to -5.9), high log soil organic carbon-water partitioning coefficient (*K*<sub>oc</sub>) (3.1-3.3) and high log octanol-water partition coefficient (*K*<sub>ow</sub>) (5.9-7.7), which are associated with increasing perfluorocarbon chain length, resulted in higher removal via adsorption for C<sub>7</sub>-C<sub>11</sub> PFCA, C<sub>6</sub> and C<sub>8</sub> PFSA and FOSA than for their short-chain equivalents. In a sorption study, Ahrens *et al.* (2011) found that long-chain PFCAs (C<sub>7-11</sub>), PFSA (C<sub>6</sub> and C<sub>8</sub>) and FOSA (C<sub>8</sub>) were present in both the dissolved and solid phase (*i.e.* sediment). However, the same study found that long-chain PFCAs (C>11) were exclusively adsorbed to the solid phase. Similar results were observed in a study demonstrating increased log *K*<sub>oc</sub> values with increased perfluorocarbon chain length, *i.e.* higher sorption potential of longer-chain PFASs than their short-chain equivalents (Higgins & Luthy, 2006).

The presence of biofilm on the biochar in BC-active-biofilm did not influence the removal of either C<sub>3</sub>-C<sub>5</sub> PFCAs or C<sub>4</sub> PFSA. For the longer-chain PFASs PFDA (C<sub>9</sub>), PFUnDA (C<sub>10</sub>),

PFDODA (C<sub>11</sub>), PFOS (C<sub>8</sub>) and FOSA (C<sub>8</sub>), BC-active-biofilm showed high removal efficiencies (90-99%), but the values were not significantly different from those obtained for BC-no-biofilm ( $p>0.05$ ). These PFASs have high log  $K_{ow}$  (6.5-7.8) and high  $K_{oc}$  (3.17-3.34), which indicates a tendency to sorb to the organic phase and solid phase (Rayne & Forest, 2009). This means that PFDA (C<sub>9</sub>), PFUnDA (C<sub>10</sub>), PFDODA (C<sub>11</sub>) and PFOS (C<sub>8</sub>) are prone to attach to lipid-rich surfaces such as wastewater, which contains lipids (oil, fat and grease) and proteins. Thus, retention of the fat, oil and grease, together with the biofilm, provided surface area for adsorption of PFDA (C<sub>9</sub>), PFUnDA (C<sub>10</sub>), PFDODA (C<sub>11</sub>), PFOS (C<sub>8</sub>) and FOSA (C<sub>8</sub>).

The inactivation of biofilm treatment emphasised the importance of biofilm in clearing the surface of biochar of accumulated organic matter. This is shown by the low removal of most PFASs in BC-inactive-biofilm compared with BC-active-biofilm. The negative removal of some PFASs (*e.g.* PFBA (C<sub>3</sub>), PFPeA (C<sub>4</sub>), PFHxA (C<sub>5</sub>) and PFBS (C<sub>4</sub>)) in BC-inactive-biofilm indicates desorption of the compounds, which were initially bound in BC-inactive-biofilm (before the surface was saturated). Similarly to treatments BC-no-biofilm and BC-active-biofilm, BC-inactive-biofilm achieved high mean removal of PFUnDA (C<sub>10</sub>), PFDODA (C<sub>11</sub>) and FOSA (C<sub>8</sub>) but their removal efficiency started to decrease in week 17 and reached  $85\pm 1\%$  for PFUnDA (C<sub>10</sub>),  $70\pm 6\%$  for PFDODA (C<sub>11</sub>) and  $86\pm 3\%$  for FOSA (C<sub>8</sub>) in week 22. It is possible that the inactivation of the biofilm and the accumulation of the organic matter and suspended solids on the surface of BC-inactive-biofilm minimised the number and availability of free adsorption sites.

The poor removal of most PFASs in Sand-active-biofilm can be attributed to the poor adsorption capacity of the sand. Adsorption primarily depends on the available specific surface and porosity of the medium (Ratola *et al.*, 2003), which explains the high sorption to biochar, with specific surface  $184\text{ m}^2\text{ g}^{-1}$ , in comparison with sand, with specific surface  $0.2\text{ m}^2\text{ g}^{-1}$ . Sand has inactive surface properties (Jean *et al.*, 2004) and the available adsorptive surface of the sand-based filter was that of the biofilm, which is also limited by the small specific surface of the particles. Zhou *et al.* (2009) concluded that micropollutants such as pharmaceuticals can only be temporarily retained in sand, because of saturation of the active sites or loss of biofilm. The results obtained in this study are in agreement with findings in previous studies that sand filtration has little or no intrinsic capacity for removal of PFASs (Rostvall *et al.*, 2018).

### 3.5 CONCLUDING REMARKS

This study investigated the potential of biochar filters for onsite wastewater treatment as a replacement or complement for sand filters. The emphasis of the work was on effects of biodegradation, adsorption and a combination of these on removal of PhACs and PFAS from wastewater using biochar filters. From the results obtained for biochar filters with active, inactive and no biofilm and from sand filters with active biofilm, the following can be concluded:

- Biochar is an efficient medium for removal of carbamazepine and metoprolol, prominently by adsorption.

- Biochar and sand filters are equally efficient in removal of ranitidine and caffeine through adsorption, biodegradation and a combination of these.
- Biochar-no-biofilm is an efficient medium for removal of C<sub>7</sub>-C<sub>11</sub> PFCAs, C<sub>6</sub> and C<sub>8</sub> PFSA and FOSA (C<sub>8</sub>), primarily by adsorption.
- BC-active-biofilm and BC-inactive-biofilm have comparable capacity for removal of proteinophilic PFASs (*i.e.* C<sub>10</sub>-C<sub>11</sub> PFCAs and FOSA (C<sub>8</sub>)), through adsorption on the surface of biochar, biofilm and accumulated wastewater organic matter.

Biochar thus seems to be a promising medium for onsite wastewater treatment, especially for removal of PhACs and PFASs of perfluorocarbon chain length >C<sub>6</sub>. The best way to apply biochar filters is as a second-stage treatment (or post-treatment) step aimed at removal of PFASs after organic matter has been removed. In this treatment stage, PFASs will be predominantly removed by adsorption (as in the BC-no-biofilm treatment).

Biochar surfaces are finite and their adsorption capacity can be expected to deplete with time following continuous exposure to pollutants. More research is needed to evaluate the service life of biochar in respect to removal of micropollutants.



## ONGOING AND FUTURE RESEARCH

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Knowledge gained during our previous research was employed and integrated to develop compact biochar-based systems for onsite wastewater treatment, called multi-drawer biochar filters. The multi-drawer compact biochar filter is a cabinet composed of stacked drawers filled with different types of biochar, designed, produced and mixed or treated to achieve certain properties enabling removal of a specific type of pollutant. Three multi-drawer biochar filter units were installed in July 2019 in a single household in Gamla Uppsala and their performance is currently under investigation.

Transition to using biochar as filter materials with all requirements regarding technological development, market potential and organisational and regulatory aspects needs investigation. Life cycle assessment, economic cycle assessment and other assessments related to environmental impacts (*e.g.* reduction in greenhouse gases, carbon sink, renewable energy production) of biochar-based onsite wastewater treatment systems are needed.



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