Supporting materials for

Slow sand filtration of raw wastewater using biochar as an alternative filtration media

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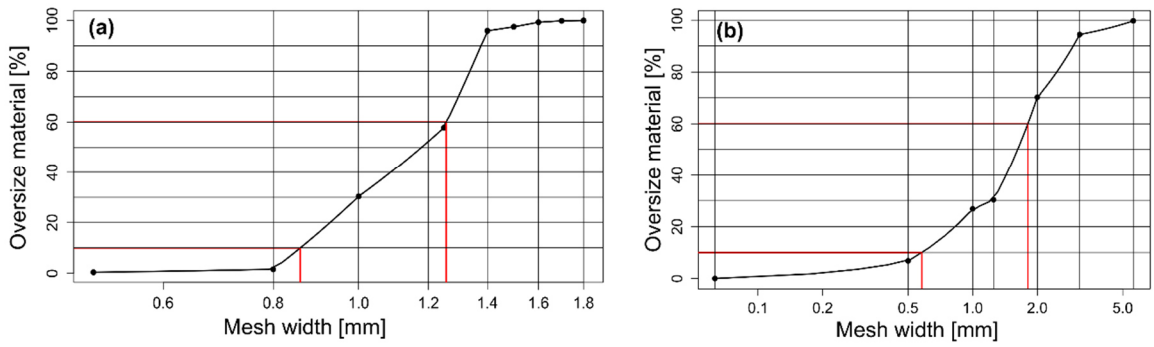

Figure S1. Grain size distribution of sand (a) and biochar (b).

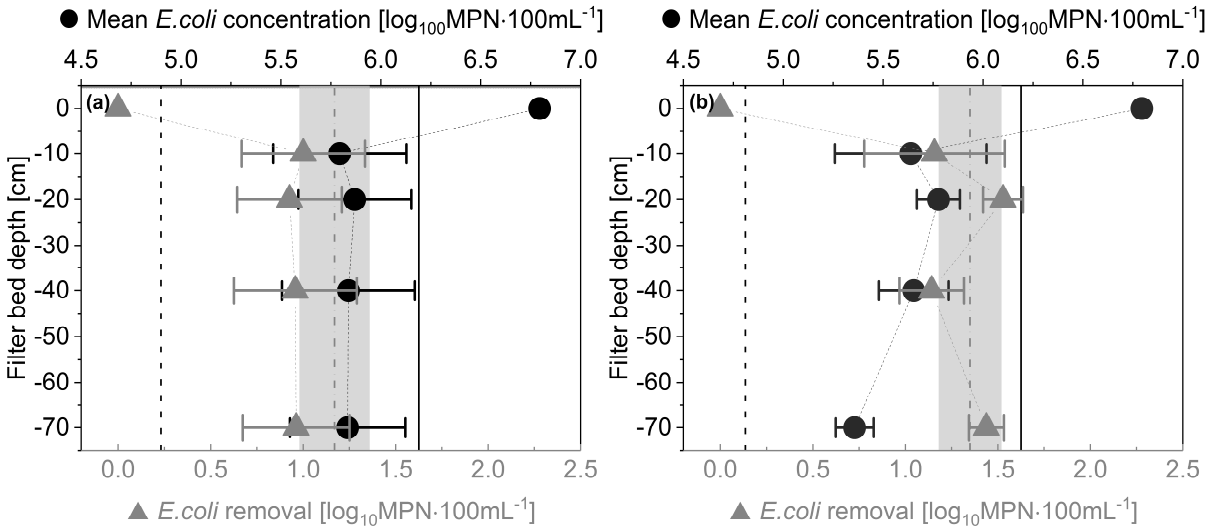

Figure S2. Mean *E. coli* concentration and removal over filter depth for sand (a) and biochar (b) filters (n = 3). 70 cm marks represent filter effluent and error bars represent standard deviation. Vertical grey dashed-dotted line represents mean *E. coli* removal rate over experimental time (n = 23) with 95 % confidence interval (grey area). Solid black line represents mean influent concentration and black dashed line the mean effluent concentration of *E. coli.*

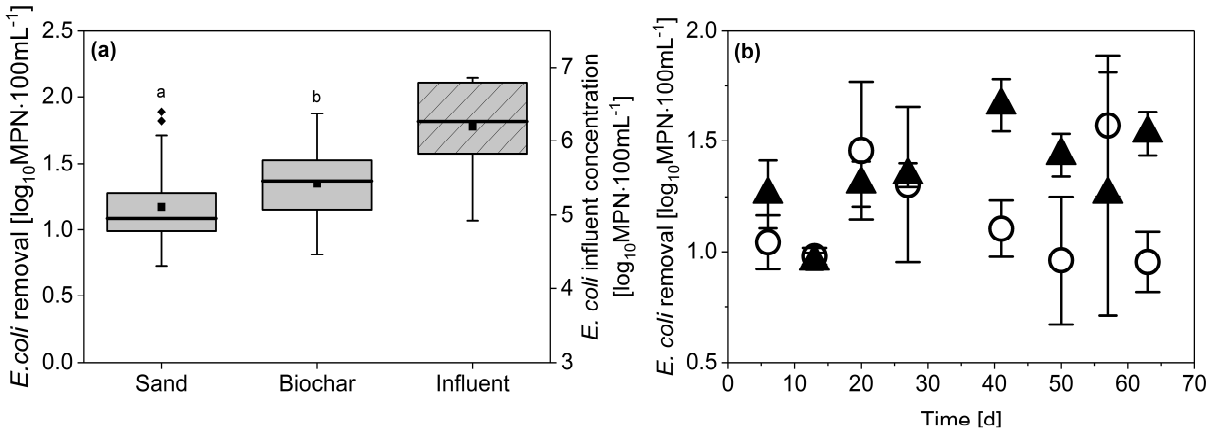

Figure S3. Boxplots of mean $\log_{10}$ removal of *E. coli* through sand and biochar filtration and influent concentration (a). Whiskers represent 1.5 interquartile range and mean values are presented as black squares. Letters indicate a significant difference of the mean (p <0.05) after post-hoc analysis. Mean removal rates of *E. coli* over experimental time for sand (circles) and biochar (black triangle) over experimental time (b). Error bars represent standard deviations.

Table S1. Properties of used filter materials. Specific surface area of sand was calculated according to Wichern et al.[1]

Parameter	Unit	Sand	Biochar
Max. diameter (d_{max})	mm	1.8	> 5.6
Effective diameter (d_{10})	mm	0.86	0.58
d_{60}	mm	1.26	1.81
Uniformity coefficient (U)		1.47	3.12
Porosity	%	40	59
HRT_{min}*	h	12	14.8
HRT_{max}**	h	27.6	30.4
Bulk density	g cm^{-3}	1.53	0.12
Specific surface area (BET)	m^2 g^{-1}	< 0.01	500
Specific surface area (BET)	m^2 m^{-3}	$< 1.53 \cdot 10^4$	$6.13 \cdot 10^7$
Surface area (BET) per filter bed	m^2	< 18	72,000

*with a minimum headwater level of 30 cm

**with a maximum headwater level of 102 cm

Table S*2*. Chemical characteristics of biochar, which was used in AnBF.

Parameter	Unit	Biochar
Ash	%	1.4
C	%	80.0
N	%	0.5
O	%	8.1
H	%	1.3
H/C	Molar ratio	0.19
O/C	Molar ratio	0.08
P	$mg \cdot kg^{-1}$	2,686
Al	$mg \cdot kg^{-1}$	654
Fe	$mg \cdot kg^{-1}$	745
K		17,978
Mg	$mg \cdot kg^{-1}$	2,350
Na	$mg \cdot kg^{-1}$	886

Table S3. Chemical characteristics of sand, which was used in AnBF.

Parameter	Unit	Sand
Na_2O	% $w \cdot w^{-1}$	0.09
MgO	% $w \cdot w^{-1}$	0.02
Al_2O_3	% $w \cdot w^{-1}$	1.3
SiO_2	% $w \cdot w^{-1}$	97.6
K_2O	% $w \cdot w^{-1}$	0.87
CaO	% $w \cdot w^{-1}$	0.02
TiO_2	% $w \cdot w^{-1}$	0.024
Fe_2O_3	% $w \cdot w^{-1}$	0.067
P_2O_5	% $w \cdot w^{-1}$	< 0.01

Table S4. cLSM settings

Parameters	Settings
Extinction (Alexa Fluor 488; EPS)	488 nm
Emission (Alexa Fluor 488; EPS)	490 – 550 nm
Extinction (SYTO60; total cell count)	633 nm
Emission (SYTO60; total cell count)	640 – 700 nm
z-stack slice distance	2 µm
Picture area	465 x 465 µm
Scanner speed	200 hz
Picture resolution	1024 x 1024 pixel
Voxel size	0.455 x 0.455 x 2.0 µm

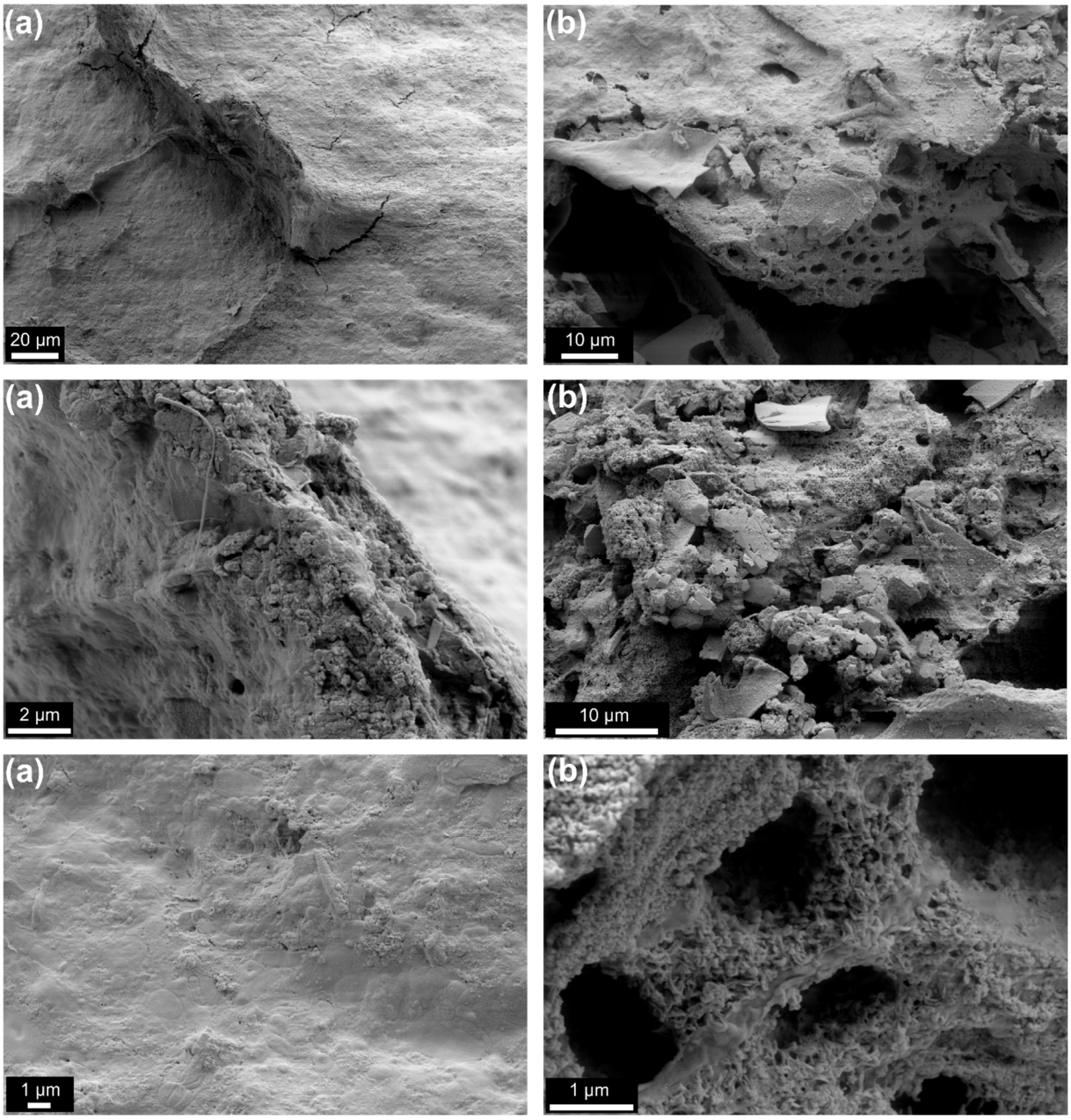

Figure S4. Scanning electron images of sand (a) and biochar (b) filter material after 70 days at 10 ± 2.5 cm.

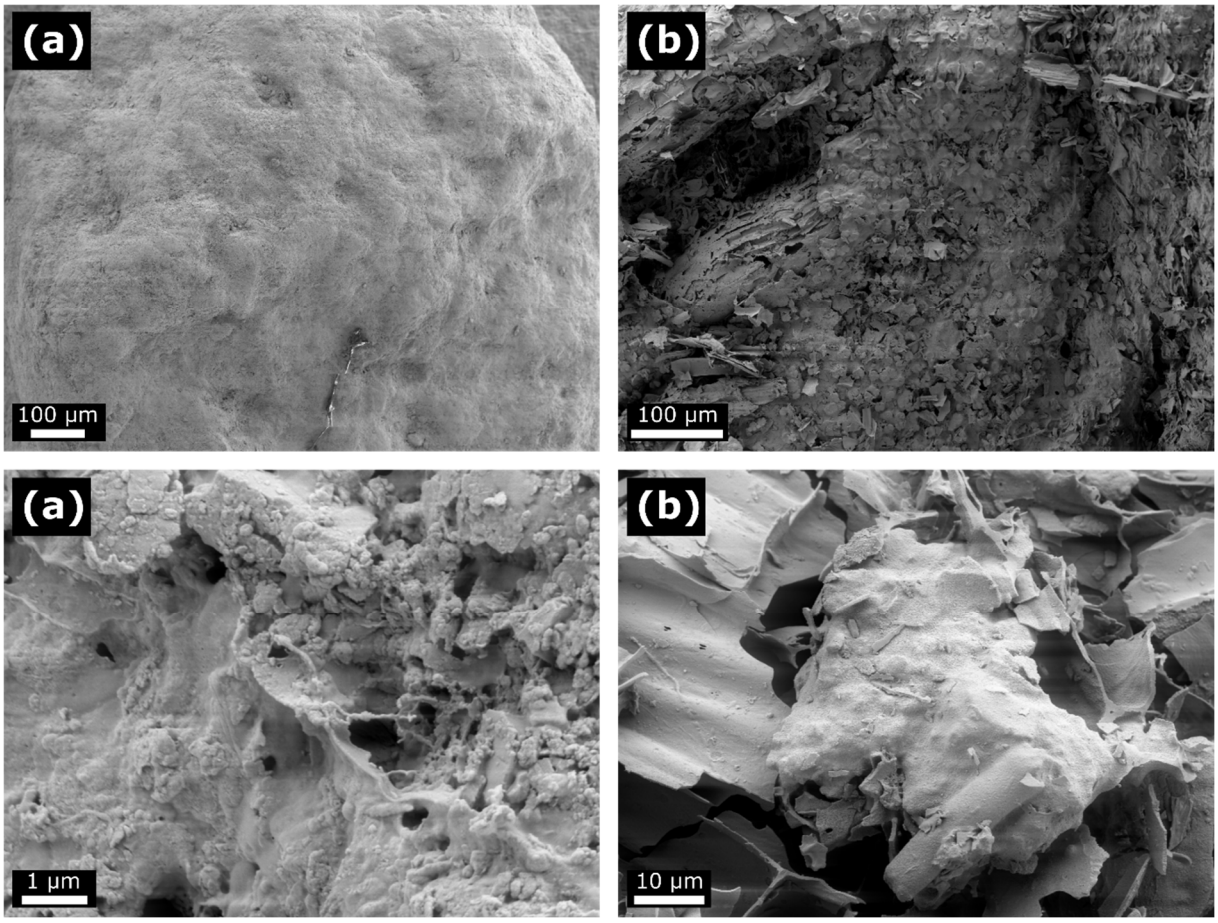

Figure S5. Scanning electron images of sand (a) and biochar (b) filter material after 70 days at 30 ± 2.5 cm.

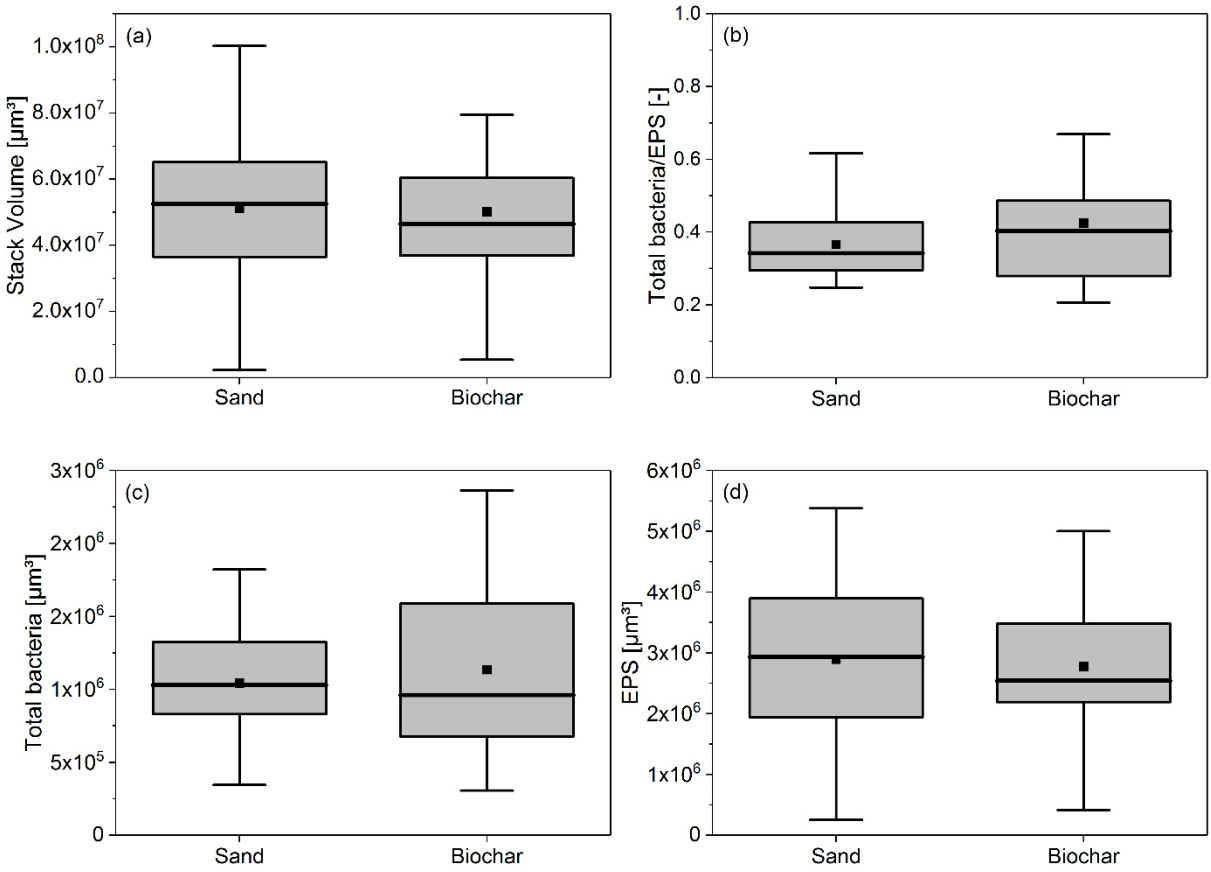

Figure S6. Results from cLSM microscope analysis of sand (n = 31) and biochar (n = 25) particles. Average stack volume (a) of scans, total bacteria/EPS ration (b), Volume of total bacteria (c) and Volume of EPS- Glycoprotein (d) .

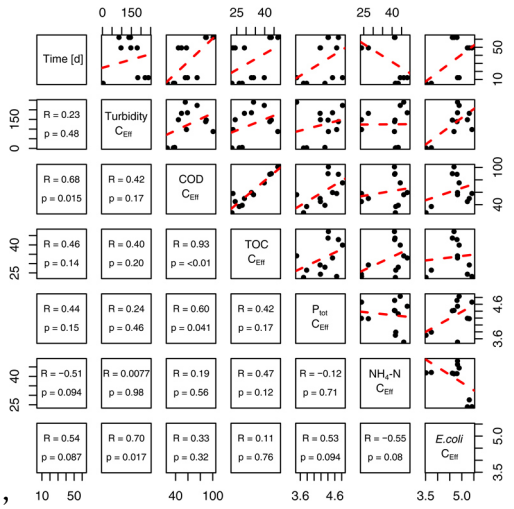

Figure S7a. Correlation analysis for effluent concentrations of biochar filters for Tubidity [FNU]; COD, TOC, P_{tot} and NH_4-N [mg·L^{-1}] and *E. coli* [$\log_{10}$MPN·100mL^{-1}] over time.

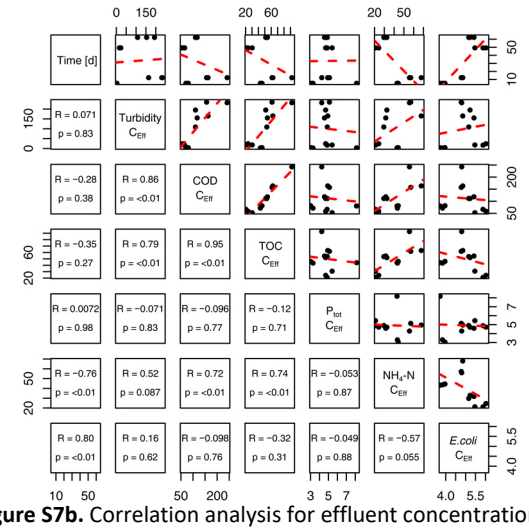

Figure S7b. Correlation analysis for effluent concentrations of sand filters for Tubidity [FNU]; COD, TOC, P_{tot} and NH_4-N [mg·L^{-1}] and *E. coli* [$\log_{10}$MPN·100mL^{-1}] over time.

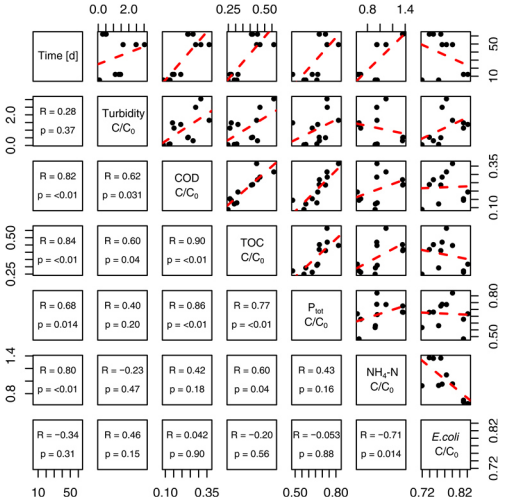

Figure S7c. Correlation analysis for normalized (C·C_0^{-1}) concentrations of biochar filters, using influent (C_0) and effluent concentrations (C) of parameters over time.

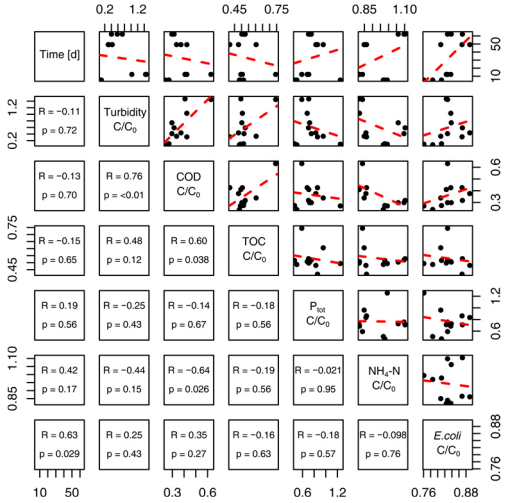

Figure 7d. Correlation analysis for normalized (C·C_0^{-1}) concentrations of sand filters, using influent (C_0) and effluent concentrations (C) of parameters over time.

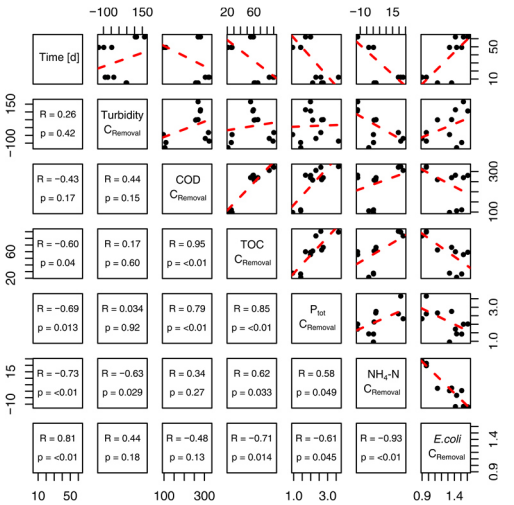

Figure S7e. Correlation analysis for removal efficiency of biochar filters for Tubidity [FNU]; COD, TOC, P_{tot} and NH_4-N [mg·L^{-1}] and *E. coli* [$\log_{10}$MPN·100mL^{-1}] over time.

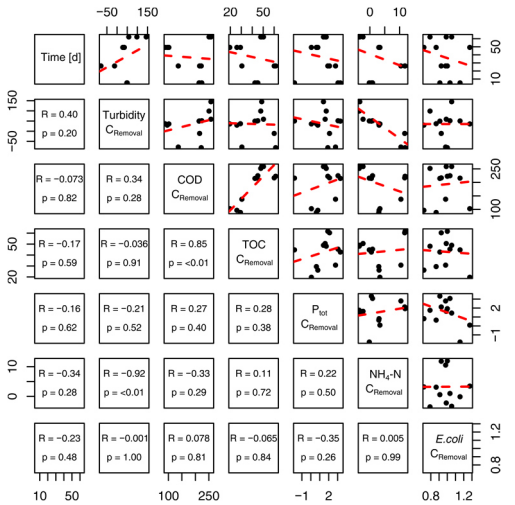

Figure S7f. Correlation analysis for removal efficiency of sand filters for Tubidity [FNU]; COD, TOC, P_{tot} and NH_4-N [mg·L^{-1}] and *E. coli* [$\log_{10}$MPN·100mL^{-1}] over time.

Figure S7. Correlation analysis between selected parameters over experimental time (n=12), for biochar (7a, 7c, 7e) and sand (7b, 7d, 7f) filter, using linear regression. The Pearson correlation coefficient between the individual parameters is represented as "R" with the corresponding *p*-value.

References

1. Wichern, M., Lindenblatt, C., Lübken, M. & Horn, H. Experimental results and mathematical modelling of an autotrophic and heterotrophic biofilm in a sand filter treating landfill leachate and municipal wastewater. *Water Res.* **42**, 3899–3909 (2008).