The microbial colonization of activated carbon block point-of-use (PoU) filters with and without chlorinated phenol disinfection byproducts

Electronic Supplementary Information

Contents:

1. Biofilm induction in *P. aeruginosa* strains with pentachlorophenol

Biofilm production by *P. aeruginosa* was quantified using a method described by O'Toole.[1] Overnight cultures of *P. aeruginosa* PAO1 and PA14 were diluted 1:100 in low salt LB medium and incubated with and w/o 100 μM of pentachlorophenol in 96-well microtiter plates for 20 hours at 37°C. Following incubation, cells in liquid cultures were removed and cells attached to the microtiter plate wells were stained with crystal violet. Subsequently, crystal violet retained in each well was solubilized in 30% acetic acid and quantified at 550 nm. Both *P. aeruginosa* PAO1 and PA14 produced more biofilm in the presence of pentachlorophenol.

Table S1. Biofilm quantity measured as optical density at OD550 nm.

Strain name and condition	PAO1 w/o PCP	PAO1 with PCP	PA14 w/o PCP	PA14 with PCP
Optical density at OD550 nm	1.8 ± 0.2	3.1 ± 0.2	0.5 ± 0.1	0.9 ± 0.1

2. PoU Manifold system setup and operation

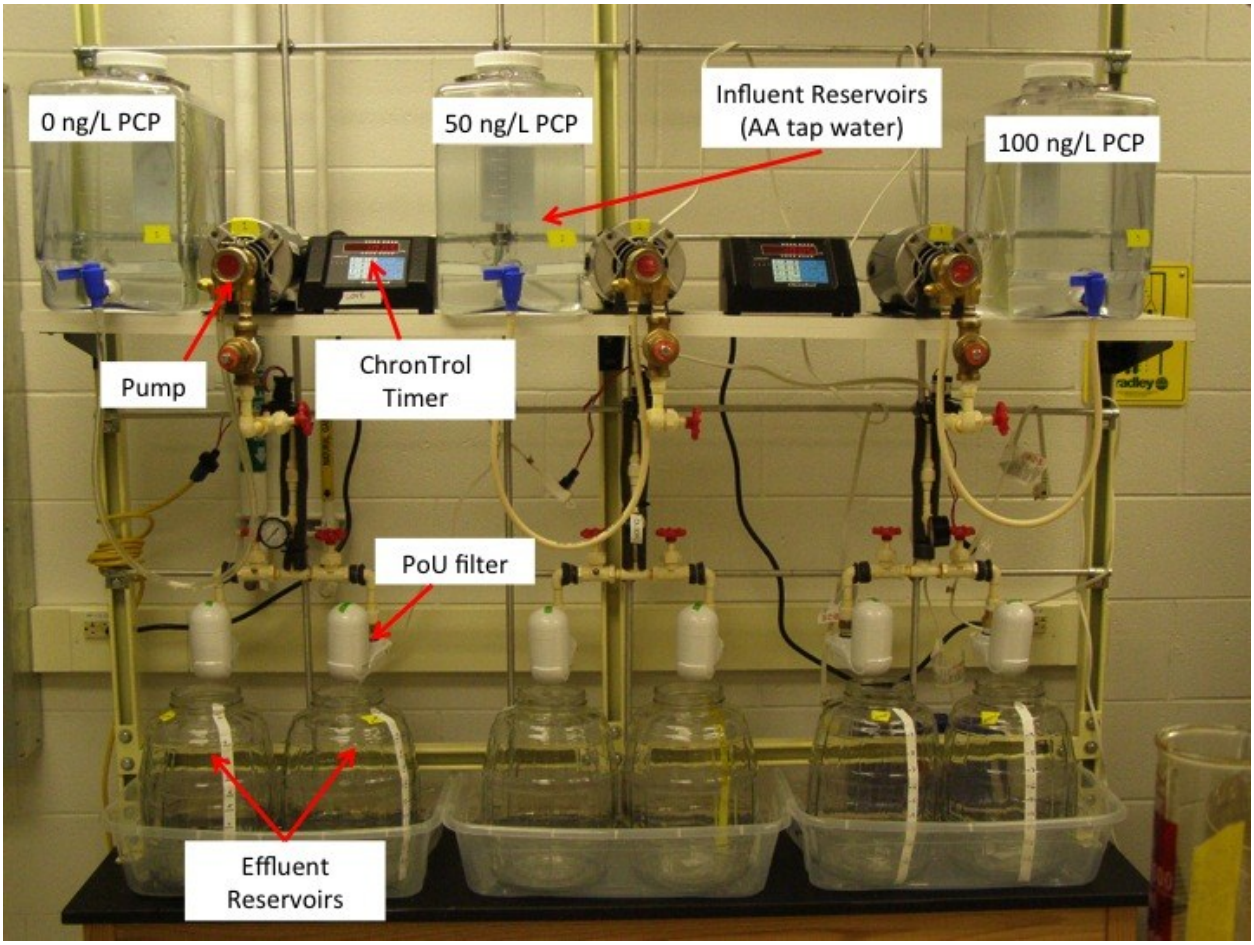


Fig. S1. Manifold system with influent reservoirs, duplicate PoU filters and effluent reservoirs.

As shown in Fig. S1, the general apparatus for the experiment consisted of a 20 L polycarbonate carboy connected by tubing to a brass 3/8 inch Procon rotary vane pump rated at 100 gallons per hour (gph) with a maximum pressure of 250 psi and a maximum temperature of 150˚ F. The pump was powered by a ¼ horsepower Marathon Electric motor rated at 1725 rpm and 60/50 Hz. The rotary vane pump was directly connected to a ½ inch CASH ACME brass pressure regulator that allowed inlet pressure up to 400 psi and outlet pressure adjustable between 10 and 70 psi (maximum temperature 180 °F). This was set to approximately 42 psi on each rig to simulate household water pressure. Brass size adapters were in place to adjust between the 3/8 inch pump and the ½ in pressure regulator. Following the pressure regulator, ½ inch CPVC manual flow regulator valve was in place to moderate the flow to the overall system. Half inch CPVC was then placed to connect to the ½ inch 120VAC pilot operated ALCON brand normally closed

(NC) solenoid valves which controlled the flow. The solenoid valves could handle a maximum pressure of 150 psi and a maximum temperature of 120 ˚F. Half inch CPVC again followed the solenoid valve, at one point branching off to the pressure gauge as shown in Fig. S1. The flow was then split with a ½ inch CPVC T-connector and each branch contained a manual flow regulator valve because it was found that there was a great degree of hydraulic variation among the PoU filters. Finally a brass ½ inch CPVC to hose fitting was placed on the end and a number of faucet adapters were put into place to match one of four fittings that came with the faucet filter. Teflon tape and/or CPVC cement was used at each connection point as needed. Finally the PoU filter was attached to its proprietary fitting. The whole apparatus was affixed to the metal grid with zip ties for stability.

3. Characteristics of commercial PoU filter

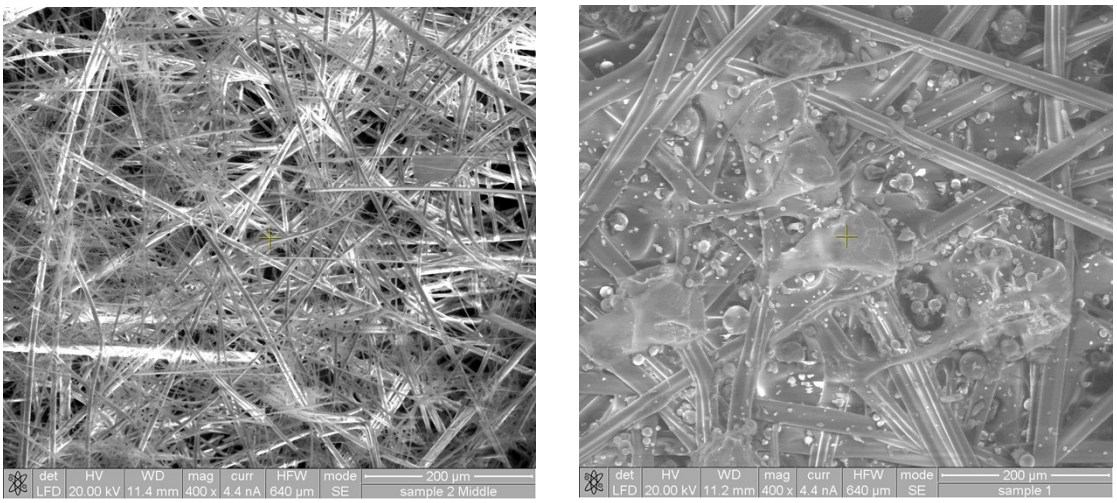
3.1 Physical characteristics of activated carbon block

Table S2. Physical characteristics of commercial PoU activated carbon block*.

Filter No.	Brunauer-Emmett-Teller (BET) Surface Area, m^2/g	Pore Size, Å	Porosity
1	578 (20)	22.1 (0.8)	0.0621 (0.0002)
2	661 (26)	21.8 (0.02)	0.0666 (0.0026)
3	548 (26)	22.1 (0.6)	0.0540 (0.0013)

*Standard deviations are shown in the parenthesis

3.2 SEM image of fabric membrane



(a) (b)

Fig. S2. SEM image of fabric membrane.

Scanning electron micrographs of the two layers of the PoU filter fabric that make up the components extracted in biomass and community analyses. Both micrograph images are shown at the same magnification and the scale bar represents 200 µm. (a) Glass fiber filter layers that faced influent side. (b) Polymeric fiber filter layer that faced the activated carbon block.

4. Table of the water volumes processed for PoU filters

Table S3. Total volumes of water processed for PoU filters.

PCP treatment (ng/L)		Total volume of water processed (L)
0	Replicate 1	485
	Replicate 2	494
50	Replicate 1	505
	Replicate 2	562
100	Replicate 1	484
	Replicate 2	583
Average		519

5. Image of used fabric membranes

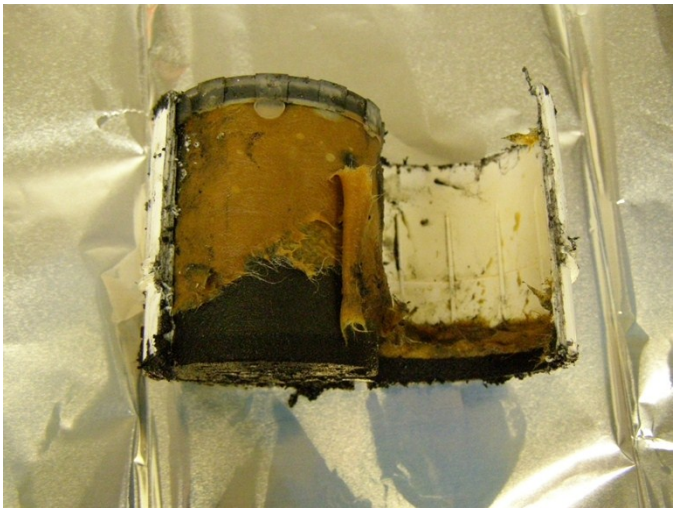


Fig. S3. PoU filter disassembly.

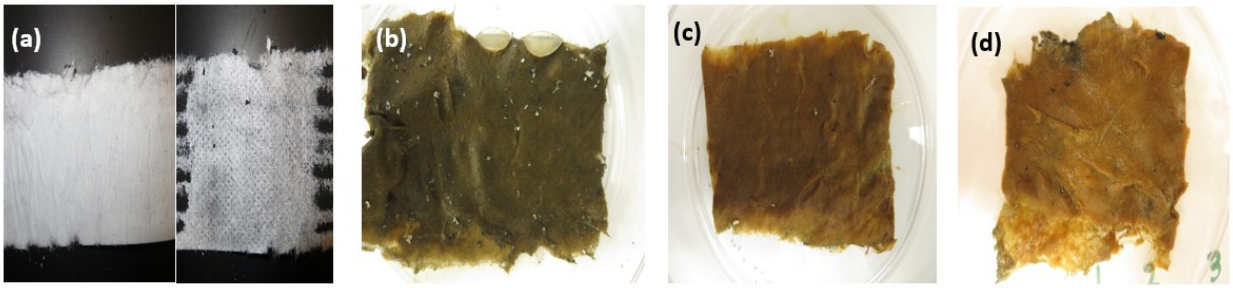


Fig. S4. Representative images of fabric membranes. (a) Clean fabric that faced influent side (left) and activated carbon block (right); and used fabrics that were sacrificed after 67-day operation spiked with (b) 0 ng/L PCP; (c) 50 ng/L PCP; and (d) 100 ng/L PCP, respectively. The control fabric was dark grey while the fabrics of all filters exposed to PCP were medium to light tan.

6. Sample schedule and sample type

Water quality parameters including total alkalinity, total and dissolved organic carbon (TOC and DOC), specific UV absorbance (SUVA), pH, chlorophenols, free and total chlorine residual and heterotrophic plate count (HPC) were measured regularly in grab or composite samples as noted on the sample schedule in Table S4. For the two week composite sample, 150 mL aliquots were collected from each influent and effluent container daily, dechlorinated and acidified, and added to an amber composite glass jar for each sample location. Composited aliquots were collected daily for two weeks to create a composite sample for influent and effluent TOC and chlorophenol analysis.

Table S4. Sample schedule and sample type of water analysis.

Parameter	Frequency	Influent	Effluent	Sample type
Total alkalinity	Weekly	X		Grab
DOC	2 Weeks	X		Grab
SUVA	2 Weeks	X		Grab
pH	Weekly	X	X	Grab
Free and total Cl_2 residual	Weekly*	X	X	Grab
Chlorophenols	Daily	X	X	2-wk composite, acidified (pH 2)
TOC	Daily	X	X	2-wk composite, acidified (pH 2)
HPC	Weekly	X	X	Grab

*Effluent samples were analyzed for total Cl_2 residual twice during the experiment (at 30 and 52 days).

7. Chlorophenol analysis

Dichlorophenol, trichlorophenol, tetrachlorophenol, and pentachlorophenol were analyzed for and quantified using a solid phase extraction (SPE) and gas chromatography –electron capture detector (GC-ECD) method. The less chlorinated forms were analyzed in case they were formed as products of PCP degradation. The samples were concentrated by passing through a modified polystyrene divinyl benzene copolymer. The analytes were reverse-eluted with methanol and derivatized by acetic anhydride. The acetyl derivatives were extracted into hexane and then analyzed by GC-ECD.

One liter of water sample was spiked with 25 µl of 1 mg/L dimethylnitrobenzene (DMNB), which functioned as a surrogate. The SPE cartridge was styrene/divinyl benzene porous polymer packing available as Supelclean ENVI-Chrom P (500 mg/6 ml) (Supelco, Bellefonte, PA). It was pre-conditioned with 3 mL methanol two times and activated by 3 mL acidified (pH2) Milli-Q water three times. The cartridges were kept wet during condition, and an additional 3 mL more Milli-Q water was added as the flow rate was adjusted to maintain the cartridge wet as the sample began to load.

The one liter water sample spiked with surrogate was loaded at the flow rate of 4-6 ml per minute (about 3-4 drops per second). After the sample had finished loading, 20 mL of acidified Milli-Q water was used to rinse the sample bottle two times and was passed through the cartridge. Finally, the cartridges were dried for 25-30 minutes on the manifold with the vacuum on to remove residual water.

Since chlorophenols are nonpolar, they were strongly retained by the sorbent. The SPE cartridge was turned upside down during the elution process. This reverse-elution helps to remove the chlorophenols on the sorbent surface and minimizes the volume of solvent, which achieves better recovery. Thus, 3 ml of methanol was used to reverse-elute analytes at a flow rate of 0.5 ml/minute (approximately 1 drop every 3-4 seconds). Before the first 3 mL methanol finished passing through the cartridge, 2 mL more methanol was added for another elution. After the methanol passed through, the manifold port valves were fully opened to collect residual methanol. The eluent was stored below -10°C.

To improve the chromatographic detection of the analytes, the eluent was derivatized using acetic anhydride and extracted from methanol to hexane through the following steps. The methanol eluent was spiked with 2 ml of 5% potassium carbonate solution, 25 µL of 6 mg/L trichlorotoluene (TCT) as an internal standard, 2 mL hexane, and 400 µL of acetic anhydride in that order. The solution was vortexed for 40 s at 3000 rpm and let stand for 1 min. The top hexane phase was extracted into a 5 mL volumetric flask. Subsequently, another 1 mL hexane was added in the solution, and went through the same vortex step. The top hexane phase was collected and aggregated with the previous extract. The final volume was adjusted with hexane to 5 mL and stored in the GC vials. Since TCT was unaffected by the derivatization step, any variations in TCT area counts can be attributed to the extraction efficiency or instrument measurements.

The extracted samples were analyzed by HP 6890 series GC with ECD (Agilent, Palo Alto, USA). The column was an Agilent 19091H-433 GC–ECD capillary column (30 m × 0.25 mm i.d, 0.25-µm film thickness). The injector and detector temperatures were all set at 250°C. Nitrogen was used as the carrier gas at a flowrate of 0.9 mL/min. The GC oven temperature was started from initial temperature of 40°C, which was held for 30 s, followed by an increase at a rate of 10°C /min to 240°C, which was held for 2.5 min, and then increased at 10°C /min to 250°C, which was held for 3.5 min. The total run time was 27.5 min. A volume of 2.0 µL sample was injected into the GC.

The detection limit of pentachlorophenol was 10 ng/L. The detection limits of dichlorophenol, trichlorophenol, and tetrachlorophenol are 25 ng/L. During the operation of the PoU manifold system, no chlorophenols were detected in the influent control water samples.

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8. Total mass of DNA extracted

Table S5. Total mass of DNA extracted[1]

Sample type	PCP treatment (ng/L)	Operation days	DNA concentrations (ng/L water or ng/cm^2 fabric area)
Influent	0	58	14
		65	16
	50	42	5
		58	9
		66	10
	100	42	7
		51	4
		58	15
		66	14
Effluent	0	42	637
		58	737
		65	53
	50	42	359
		58	661
		66	46
	100	42	775
		51	382
		58	524
		66	36
Fabric[3]	0	67 (end)	1034 (389)
	50	67 (end)	216 (83)
	100	67 (end)	399 (145)

[1] Standard deviations are shown in parenthesis.
[2] The averages are taken from the total sample number of two (duplicate PoU filter replicates of each treatment).
[3] Fabric DNA was extracted from four fabric pieces of each PoU filter replicate. The averages and standard deviations are taken from the total sample number of eight for each treatment.

9. The number of quality-filtered sequences per sample

Table S6 Number of quality-filtered sequences per sample

Sample name (Sample code)	Number of quality filtered sequences
Influent at day 58 (12Infl1)	16969
Influent at day 65 (15Infl1)	18123
Influent spiked with 100 ng/L PCP at day 42 (11Infl3)	16730
Influent spiked with 100 ng/L PCP at day 51 (30Infl3)	21080
Influent spiked with 100 ng/L PCP at day 58 (12Infl3)	8906
Influent spiked with 100 ng/L PCP at day 66 (15Infl3)	33370
Influent spiked with 50 ng/L PCP at day 42 (11Infl2)	11826
Influent spiked with 50 ng/L PCP at day 58 (12Infl2)	15114
Influent spiked with 50 ng/L PCP at day 66 (15Infl2)	11140
Effluent from PoU filter replicate 1 at day 58 (12Effl1A)	31902
Effluent from PoU filter replicate 1 at day 65 (15Effl1A)	28817
Effluent from PoU filter replicate 2 at day 65 (15Effl1B)	21222
Effluent from PoU filter replicate 2 at day 42 (11Effl1B)	34660
Effluent from PoU filter replicate 2 at day 58 (12Effl1B)	32940
Effluent from PoU filter replicate 1 spiked with 50 ng/L PCP at day 42 (11Effl2A)	29800
Effluent from PoU filter replicate 1 spiked with 50 ng/L PCP at day 58 (12Effl2A)	24947
Effluent from PoU filter replicate 1 spiked with 50 ng/L PCP at day 66 (15Effl2A)	28433
Effluent from PoU filter replicate 2 spiked with 50 ng/L PCP at day 42 (11Effl2B)	35147
Effluent from PoU filter replicate 2 spiked with 50 ng/L PCP at day 58 (12Effl2B)	51757
Effluent from PoU filter replicate 2 spiked with 50 ng/L PCP at day 66 (15Effl2B)	34253
Effluent from PoU filter replicate 1 spiked with 100 ng/L PCP at day 51 (30Effl3A)	29097
Effluent from PoU filter replicate 1 spiked with 100 ng/L PCP at day 58 (12Effl3A)	39211
Effluent from PoU filter replicate 1 spiked with 100 ng/L PCP at day 66 (15Effl3A)	35273
Effluent from PoU filter replicate 2 spiked with 100 ng/L PCP at day 42 (11Effl3B)	29200
Effluent from PoU filter replicate 2 spiked with 100 ng/L PCP at day 51 (30Effl3B)	27007
Effluent from PoU filter replicate 2 spiked with 100 ng/L PCP at day 58 (12Effl3B)	45935
Effluent from PoU filter replicate 2 spiked with 100 ng/L PCP at day 66 (15Effl3B)	27316
Fabric from PoU filter replicate 1 (F1A1)	30653
Fabric from PoU filter replicate 1 (F1A2)	34762
Fabric from PoU filter replicate 1 (F1A3)	34063
Fabric from PoU filter replicate 1 (F1A4)	33036
Fabric from PoU filter replicate 2 (F1B1)	23241
Fabric from PoU filter replicate 2 (F1B2)	19724
Fabric from PoU filter replicate 2 (F1B3)	21551
Fabric from PoU filter replicate 2 (F1B4)	25802
Fabric from PoU filter replicate 1 with 50 ng/L PCP (F2A1)	30989
Fabric from PoU filter replicate 1 with 50 ng/L PCP (F2A2)	35717
Fabric from PoU filter replicate 1 with 50 ng/L PCP (F2A3)	39039

Table S6 Number of quality-filtered sequences per sample (continue)

Sample name (Sample code)	Number of quality filtered sequences
Fabric from PoU filter replicate 1 with 50 ng/L PCP (F2A4)	35982
Fabric from PoU filter replicate 2 with 50 ng/L PCP (F2B1)	24329
Fabric from PoU filter replicate 2 with 50 ng/L PCP (F2B2)	24866
Fabric from PoU filter replicate 2 with 50 ng/L PCP (F2B3)	25130
Fabric from PoU filter replicate 2 with 50 ng/L PCP (F2B4)	34280
Fabric from PoU filter replicate 1 with 100 ng/L PCP (F3A1)	31916
Fabric from PoU filter replicate 1 with 100 ng/L PCP (F3A2)	34024
Fabric from PoU filter replicate 1 with 100 ng/L PCP (F3A3)	35265
Fabric from PoU filter replicate 1 with 100 ng/L PCP (F3A4)	35306
Fabric from PoU filter replicate 2 with 100 ng/L PCP (F3B1)	26131
Fabric from PoU filter replicate 2 with 100 ng/L PCP (F3B2)	25446
Fabric from PoU filter replicate 2 with 100 ng/L PCP (F3B3)	33834
Fabric from PoU filter replicate 2 with 100 ng/L PCP (F3B4)	32299

10. Relative abundance of dominant OTUs (>1%) in influent, effluent, and fabric samples. Data are available in a separate file: Table S7_Relative abundance of OTUs.XLSX.

Influent community members were similar between PCP treatments (p>0.05, data not shown), despite the variation of the relative abundance among samples collected on different dates. Two OTUs (*Mycobacterium* and unclassified Bacteria OTU23) became "highly dominant" in the influent reservoirs that contained 50 and 100 ng/L PCP (Table 1). However, the relative abundances of these two OTUs were not significantly different between PCP treatments (p>0.05, Kruskal-Wallis test), not resulting in a significant influent community shift relative to the control.

11. Statistical analysis of bacterial community structure

Table S8. Bacterial composition dissimilarity across PoU filter and PCP treatments.

Mean ± standard deviation	Taxa-based dissimilarity index		Phylogeny-based dissimilarity index	
	Jaccard	Abundance-based Jaccard	Unweighted UniFrac	Weighted UniFrac
I. Within paired samples that received the same treatment				
Influent	0.57 ± 0.03^{a}	0.08 ± 0.02^{a}	0.54 ± 0.03^{a}	0.22 ± 0.03^{a}
Fabric	0.62 ± 0.05^{b}	0.03 ± 0.01^{b}	0.61 ± 0.05^{b}	0.06 ± 0.02^{b}
Effluent	0.64 ± 0.08^{b}	0.02 ± 0.01^{c}	0.65 ± 0.07^{b}	0.11 ± 0.03^{c}
Between influent and effluent	0.76 ± 0.07^{c}	0.30 ± 0.14^{d}	0.75 ± 0.07^{c}	0.46 ± 0.05^{d}
Between fabric and effluent	0.75 ± 0.06^{c}	0.12 ± 0.05^{e}	0.75 ± 0.05^{c}	0.49 ± 0.05^{e}
Between influent and fabric	0.66 ± 0.06^{b}	0.12 ± 0.05^{e}	0.64 ± 0.06^{b}	0.42 ± 0.05^{f}
II. Between PCP and control samples				
Influent	0.56 ± 0.04^{a}	0.07 ± 0.03^{a}	0.53 ± 0.04^{a}	0.20 ± 0.06^{a}
Fabric	0.64 ± 0.05^{b}	0.09 ± 0.02^{f}	0.63 ± 0.06^{b}	0.14 ± 0.02^{g}
Effluent	0.66 ± 0.08^{b}	0.02 ± 0.01^{c}	0.67 ± 0.07^{b}	0.14 ± 0.03^{g}

Letters indicate significant ($P < 0.001$) differences based on Kruskal-Wallis test for each distance index. Groups with the same letters indicate they are not significantly different from each other.

Table S9. Multivariate permutational analysis (PERMANOVA) results comparing the bacterial composition between PoU filter location and PCP treatment.

	Degrees of freedom	Sum of squares	Mean square	F.model	R^2	Pr(>F)
Unweighted UniFrac distance						
PoU filter location	2	2.69	1.35	6.97	0.22	0.005
PCP treatment	2	0.56	0.28	1.46	0.046	0.045
Residuals	46	8.89	0.19		0.73	
Total	50	12.14			1.00	
Weighted UniFrac distance						
PoU filter location	2	3.34	1.67	221.49	0.88	0.005
PCP treatment	2	0.09	0.04	5.89	0.024	0.005
Residuals	46	0.35	0.008		0.092	
Total	50	3.77			1	

12. OTUs correlated with Weighted Unifrac based PCoA plots of fabric and effluent samples.

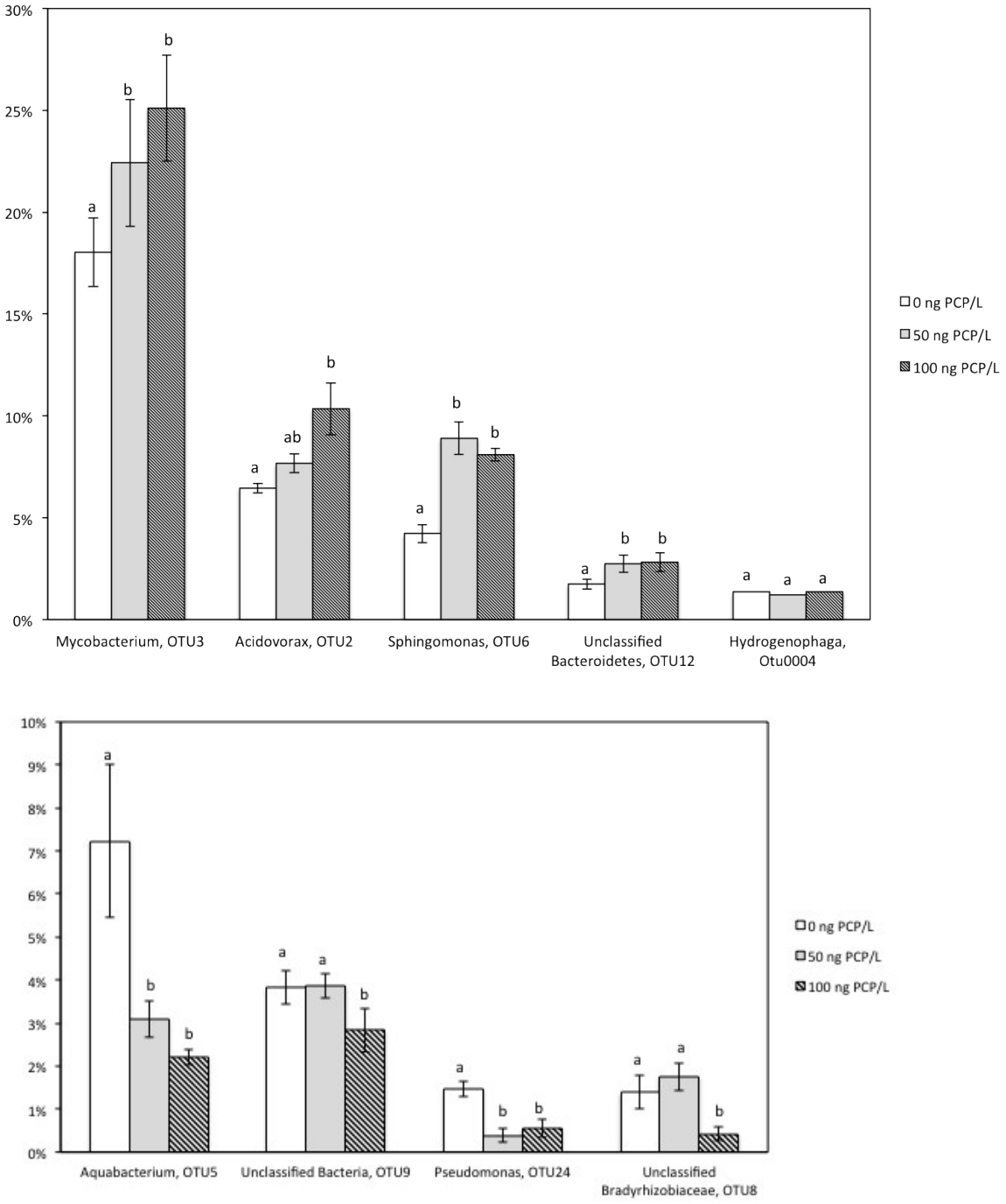


Fig. S5. Relative abundance (y-axis) versus specified taxa (x-axis) for dominant OTUs (>1% relative abundance) of fabric samples that were correlated with Weighted Unifrac based PCoA plot (PCoA 1, 54%; PCoA 2, 16%). Letter Groups with the same letters indicate significant differences (p<0.05) between PCP treatments based on Tukey's pairwise analysis. They are not significantly different from each other.

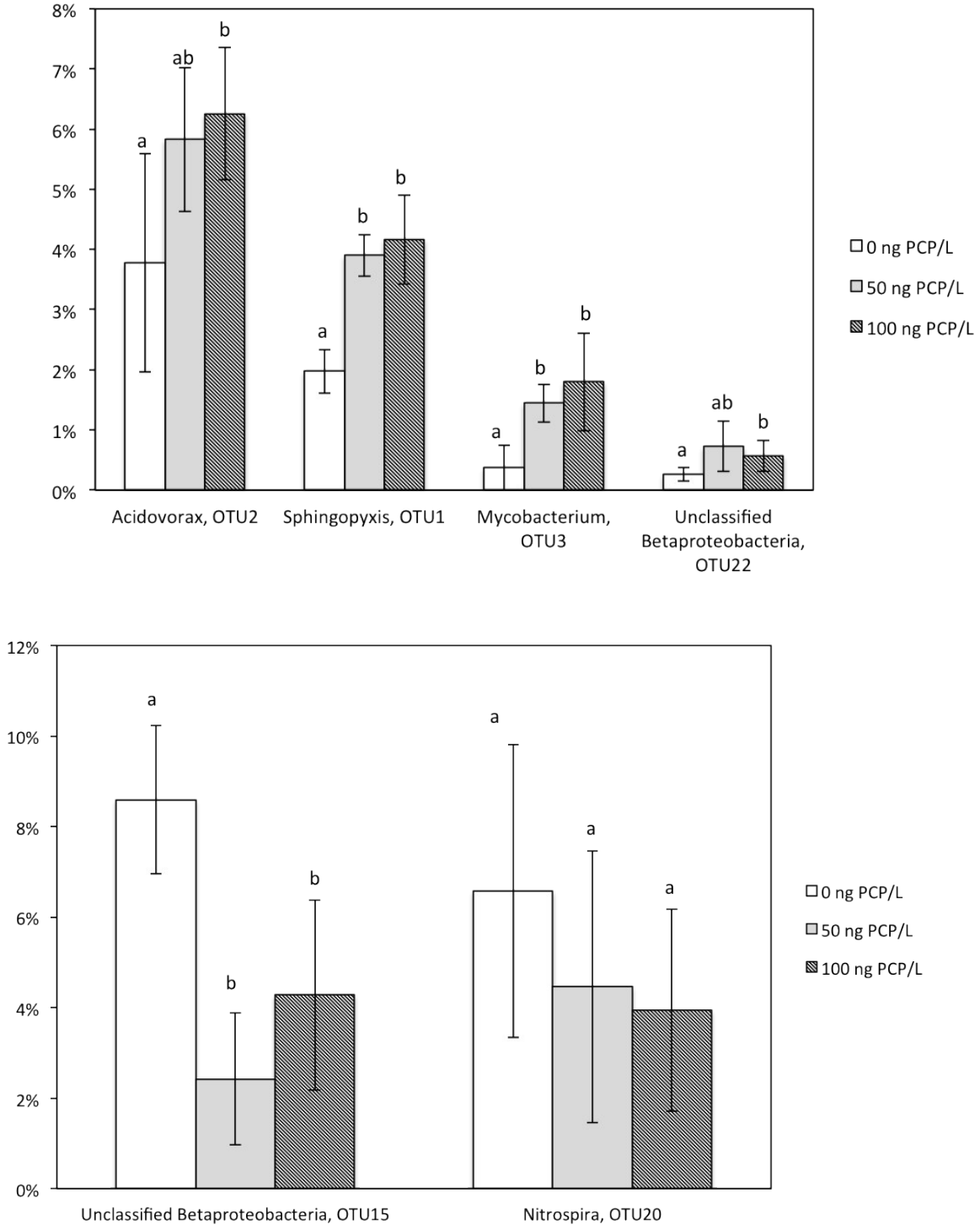


Fig. S6. Dominant OTUs (>1%) of effluent samples that were correlated with Weighted Unifrac based PCoA plot (PCoA 1, 30%; PCoA 2, 14%). Letters indicate whether significant differences (p<0.05) occur between PCP treatments based on Tukey's pairwise analysis. Bars with the same letter are not significantly different from each other.

13. Water Chemistry of PoU filter

13.1 Influent and effluent pH measurements

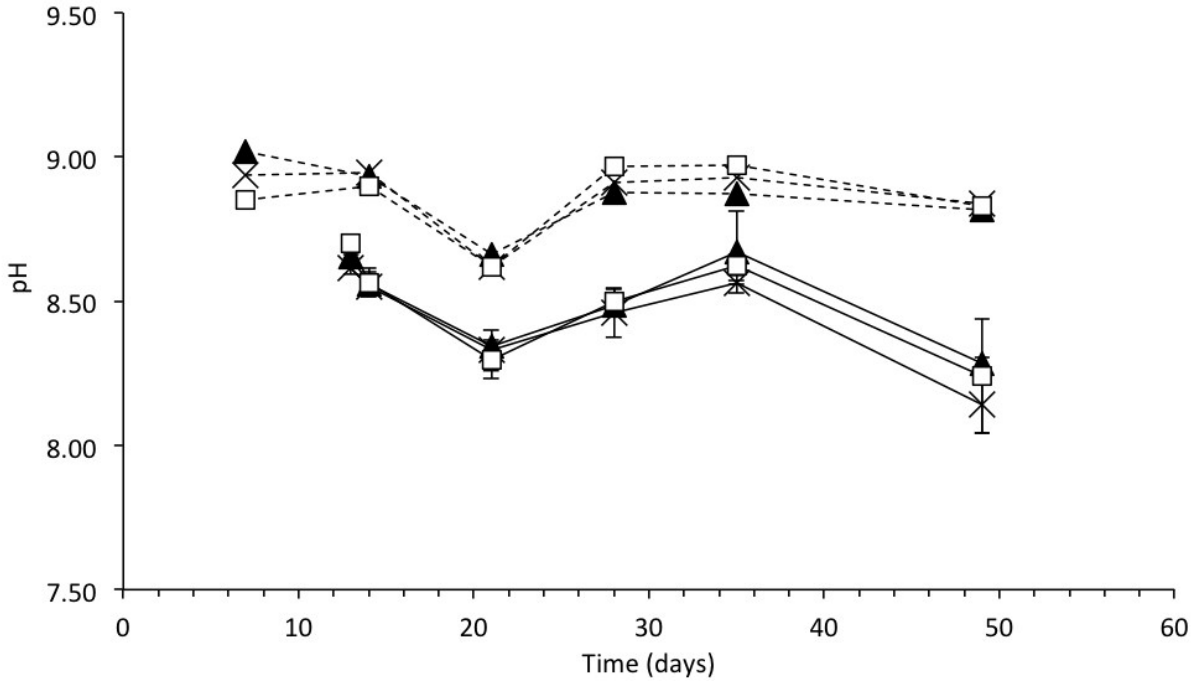
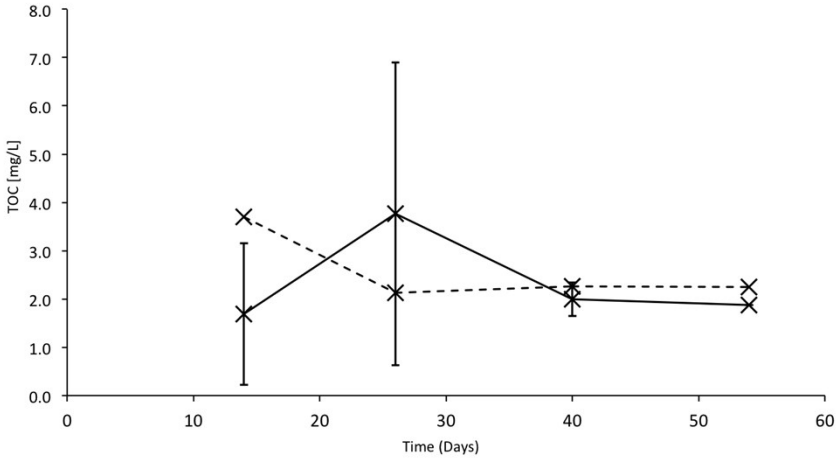


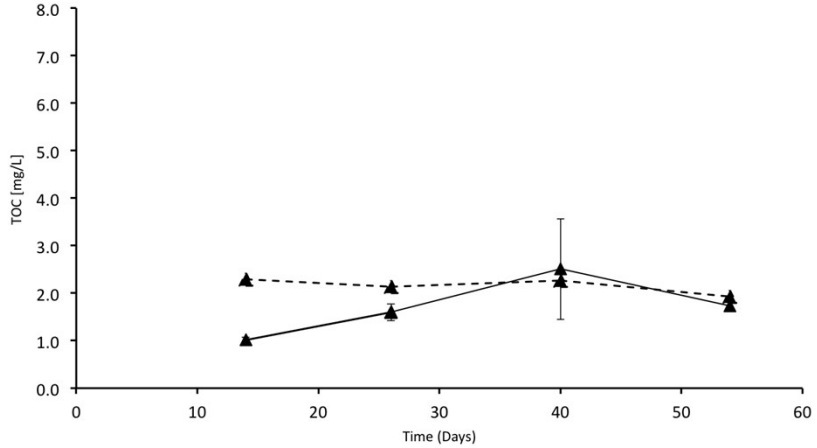
Fig. S7. pH measurements in influent (dashed line) and effluent samples (solid line) of PoU filters during operation. The PoU filters were fed with 0 ng PCP/L (cross), 50 ng PCP/L (triangle), or 100 ng PCP/L (square). Standard deviations of effluent samples are indicated by the error bars.

13.2 TOC

(a)



(b)



(c)

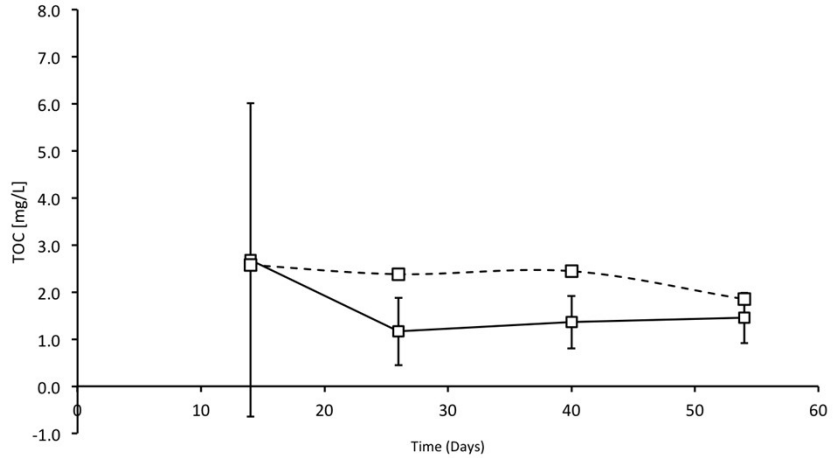


Fig. S8. TOC concentrations in influent (dashed line) and effluent samples (solid line) during operation. (a) 0 ng PCP/L(cross); (b) 50 ng PCP/L(triangle); and (c) 100 ng PCP /L (square). Standard deviations of effluent samples are indicated by the error bars.

13.3 Average water quality of all treatments in influent and effluent

Table S10. Average influent water quality of all treatments*.

Parameter	Avg. Conc.
pH	8.86 (0.12)
Total alkalinity (mg/L as CaCO3)	49.7 (9.5)
Total Cl_2 residual (mg/L as Cl2)	2.5 (0.5)
TOC (mg/L as C)	2.3 (0.4)
DOC (mg/L as C)	2.2 (0.2)
SUVA (L mg-1 cm-1)	0.016 (0.003)
HPC (CFU/mL)	160 (250)
PCP (ng/L)	Control: <10 ng/L 50 ng /L PCP treatment: 62 (10) 100 ng /L PCP treatment: 121 (13)

*Standard deviations are shown in parentheses

Table S11. Average effluent water quality of PoU Filters*.

Parameter	**Treatment**		
	0 ng/L PCP	50 ng/L PCP	100 ng/L PCP
pH	8.45 (0.17)	8.51 (0.16)	8.41 (0.39)
PCP (ng/L)	< 10 ng/L	< 10 ng/L	< 10 ng/L
Total Cl_2 residual (mg/L as Cl2)	< 0.2 mg/L	< 0.2 mg/L	< 0.2 mg/L
TOC (mg/L as C)	2. (1.66)	1.7 (0.7)	1.77 (1.55)

HPC (CFU/mL)	1.1×10^5 (7.4×10^4)	4.5×10^4 (3.1×10^5)	1.6×10^4 (3.2×10^4)

*Standard deviations are shown in the parenthesis

13.4 Estimation of the depth of penetration of PCP into activated carbon block

To determine the approximate penetration depth of PCP in the activated carbon block, the following assumptions were made. Biodegradation of PCP was neglected. Equilibrium adsorption of PCP by the activated carbon block was assumed to be modeled by a Freundlich isotherm. This is represented symbolically as follows:

where $q_s(mg/g\ GAC) = K_F\, C_s^{1/n}$ (eq. S1)

q_s is the PCP mass adsorbed on the solid phase, C_s is PCP concentration in aqueous phase, K_f and n are constants of the Freundlich isotherm model. Literature values of the constants K_f and n were reported by Dobb et al's[2] for Filtrasorb 300 granular activated carbon, which typically has a surface area of 1000 m^2/g GAC.[3] At the pH conditions of Ann Arbor tap water, pH 9, K_f = 100 $mg^{1+n}g^{-1}L^{-n}$ and n=0.41, were chosen in this approximation.

Perfect mass transfer was assumed, i.e., all of the sorbed PCP was contained within a layer that was equilibrated with influent concentrations of PCP. Therefore, C_s was the PCP influent concentration, 50 or 100 ng/L. With these values and eq. S1, the adsorbed concentrations on the solid phase (q_s) were calculated as 1.72 and 2.29 mg PCP/g Filtrasorb carbon, or 1.72×10^{-7} and 2.29×10^{-7} mg/cm^2 carbon, exposed to 50 and 100 ng/L PCP treatments. Since the specific area of activated carbon block was measured as 600 m^2/g, the masses of PCP adsorbed per mass of activated carbon block were estimated to be 1.03 and 1.37 mg PCP/g ACB carbon for the 50 and 100 ng/L PCP treatments.

The depth of the PCP saturated zone was then estimated for the ACB at the end of filter operation by assuming all of the PCP fed to the filter was held in the saturated zone (and the PCP mass in the solution phase (void volume) was negligible). The following equations were used:

$$Mass\ of\ PCP - saturated\ carbon\ zone\ (g\ carbon) = \frac{The\ total\ PCP\ mass\ adsorbed\ \ (mg\ PCP)}{The\ mass\ PCP\ adsorbed\ on\ the\ activated\ carbon\ block\ (mg\ PCP/g\ carbon)}$$

(e.q. S2)

$$Volume\ of\ PCP-saturated\ carbon\ zone\ (cm^3) = \frac{Mass\ of\ saturated\ carbon\ zone\ (g\ carbon)}{The\ density\ of\ PCP\ of\ activated\ carbon\ block\ (g/cm^3)}$$

(e.q. S3)

The depth of PCP penetration into the ACB, is the difference of the outer radius of the ACB, R_0 and the radius, R_1, to the inner edge of the PCP-saturated ACB volume (see Fig. 1). The latter dimension can be estimated by calculating the annulus area of the saturated zone, A as follows:

$$Annulus\ area\ of\ saturated\ zone,\ A\ (cm^2) = \frac{Volume\ of\ PCP-saturated\ carbon\ zone\ (cm^3)}{The\ hHeight\ of\ activated\ carbon\ block\ (cm)}$$

(e.q. S4)

A is related to R_0 and R_1 by,

$$A = \pi R_0^2 - \pi R_1^2 \qquad \text{(e.q. S5)}$$

Therefore, the approximate PCP penetration depth = R_0-R_1 can be determined from eqs. S4 and S5.

At the end of filter operation, the total mass of PCP fed was 0.027 and 0.054 mg at 50 and 100 ng/L PCP treatment. The ACB density was measured as 0.19 g/cm^3. The height and the outer radius of the activated carbon block (R_0) were 5.9 and 2.5 cm, respectively. With these values, the area of the PCP-saturated zone was estimated to be 0.023 cm^2 in 50 ng/L PCP treatment, and 0.035 cm^2 in 100 ng/L PCP treatment. The depth of penetration of PCP into activated carbon block was estimated to be about 15 μm for 50 ng/L PCP treatment, and 22 μm with 100 ng/L PCP treatment.

14. Estimation of cell protein obtained from the cell load in influents

If no cell growth occurred on the fabric, the bacterial cell mass that accumulated on the fabric would be equal to or less than (due to decay) the cell load from the influent of PoU filters over the period of operation. It was assumed that only 1% of cells are culturable using the HPC method.[4]

$$Cells\ applied\ to\ fabric = \frac{average\ influent\ conc\ (CFU/mL)\ \times\ volume\ of\ water\ processed\ (}{1\%\ (culturability\ of\ HPC\ test)}$$

(e.q. S6)

Based on measured data, the average concentration of influent HPC was 160 CFU/mL (Table S10). The total volume of water processed was 519 L. Using these values and e.q. S6, the total number of cells applied to the fabric was estimated to be 8.7×10^9 cells.

To convert the number of cells to protein from applied cells, it was assumed that all cells have the same dry weight as actively growing cell of *E.coli*, 2.8×10^{-13} g, where protein mass comprised 55% of total cell mass.[5] In addition, 25% of cells that grew over the course of the study were assumed to remain at the end (i.e., an observed yield of 0.25 g cell theoretical oxygen demand per g of electron donor as theoretical oxygen demand); the rest would have been lysed and cytoplasmic matter would have been recycled as food to other growing cells. Therefore, the protein mass expected on the fabric at the end of the study is estimated to be 3.3×10^{-4} g. Since the fabric area was 94.2 cm^2, the protein from applied cells per fabric area would be 3.6 $\mu g/cm^2$. This compares to average measured cell protein on the fabric of 23 $\mu g/cm^2$. The difference implies that substantial cell growth occurred on the fabric.

15. Estimation of cell protein formed from PCP utilization

15.1 Theoretical yield of PCP

The theoretical yield of PCP is calculated based on the stoichiometry of the redox reaction. It was assumed that the starting material was completely consumed and no side or reverse reactions occur. The balanced organic half-reaction for PCP is given in equation S7.

$$0.33\ CO_2 + 1.28\ H^+ + e^- + 0.28\ Cl^- = 0.06\ C_6HCl_5O + 0.61\ H_2O \quad \text{(eq. S7)}$$

The amount of energy that can be acquired from the redox reaction is the difference in the Gibb's formation potential for the reactants and products. Based on the free energies of formation (G_f^0) shown in Table S12, the standard free energy change of the reaction (ΔG^0) at 1 atm pressure and 1M concentration is:

$$\Delta G^0 = \sum G_f^0(products) - \sum G_f^0(reactants) = 13.58\ kJ/mole = 13.58\ kJ/e-eq$$

The standard free energy change of the reaction at pH 7 ($\Delta G^{0'}$) is calculated to be $\Delta G^{0'} = \Delta G^0 + m\Delta G_f^0(H^+) = -35.91\ kJ/e-eq$. The theoretical yield (f_s°) of PCP is estimated thermodynamically by the fraction of electron donor, A (eq. S8 and S9). It was assumed that ammonia is the nitrogen source and is available for cell synthesis. The efficiency of energy transfer, ε, was assumed to be 0.6. n is +1 if ΔG_p is positive, and vice versa.

$$A = -\frac{\frac{\Delta G_p}{\epsilon^n} + \frac{\Delta G_{pyruvate}}{\epsilon}}{\epsilon(\Delta G_a^{\circ'} - \Delta G_d^{\circ'})} \quad \text{(eq. S8)}$$

$$f_s^0 = \frac{1}{1+A} \quad \text{(eq. S9)}$$

ΔG_p is the energy required to convert the cell carbon source to an intermediate stage. It is calculated by subtracting the amount of energy that can be formed from the PCP redox reaction ($\Delta G^{0'}$) from the free energy of formation of pyruvate (Table S12) as follows:

$$\Delta G_p = 35.09 - \Delta G^{0'} = 72.49\ kJ/e-eq$$

ΔG_a and ΔG_d are the free energies per electron equivalent for oxidation half reactions for the electron acceptor and electron donor, respectively. Oxygen is the electron acceptor because we assumed the water is saturated throughout the PoU filter and the biofilm thickness during operating periods is not sufficient to cause anaerobic zones. As a minimum, this is a conservative estimate and will overestimate cell yield from PCP growth. ΔG_d is equal to $\Delta G^{0'}$ since PCP is assumed to be the electron donor in this calculation. With the calculated $\Delta G^{0'}$, the values shown in Table S12, eq. S8 and S9, the theoretical yield of PCP (f_s°) is calculated at 0.14.

15.2 Theoretical oxygen demand of PCP on PCP-fed fabric membranes

In order to determine the biomass that can be formed on the fabric due to growth on PCP, it was assumed that all of the PCP applied to the filter system adsorbed on the activated carbon block (2.8×10^{-4} and 5.7×10^{-4} mg PCP/cm^2 in 50 and 100 ng/L PCP-fed filters, respectively) and was all utilized by the biomass (to obtain the most conservative estimate). Based on the theoretical oxygen demand (ThOD) of PCP, which is 0.54 mg ThOD/mg PCP, the PCP adsorbed on the activated carbon block contained 1.5×10^{-4} and 3.1×10^{-4} mg ThOD/cm^2 for 50 and 100 ng/L PCP fed-fabrics, respectively.

15.3 Theoretical cell biomass and cell protein yielded from PCP utilization

The biomass that can be yielded from the PCP is the product of the theoretical yield (f_s°) and the theoretical oxygen demand (ThOD) provided by the substrate. Thus, the amount of biomass that could grow on PCP was calculated to be 2.2×10^{-5} and 4.3×10^{-5} mg biomass as ThOD/cm^2 for 50 and 100 ng/L PCP fed-fabrics, respectively. Since cell protein comprises 55% of a prokaryotic cell by weight,[5] the cell protein yields are thus 1.2×10^{-2} and 2.5×10^{-2} μg cell protein/cm^2 for 50 and 100 ng/L PCP fed-fabrics, respectively. This is three orders of magnitude lower than the actual measured amount of biomass on the fabric, and indicates that under the conservative assumptions used in this estimate, PCP was not a major contributor to cell growth.

Table S12. The free energies of formation (G_f^0) for the compounds at 25 °C, 1 atm and 1M concentration.

Compound	G_f^0
C_6HCl_5O (at pH 0)	-144.8 kJ/mol[6]
H_2O (at pH 0)	-237.17 kJ/mol[7]
CO_2 (at pH 0)	-394.4 kJ/mol[7]
H^+ (at pH 0)	0 kJ/mol[7]
Cl^- (at pH 0)	-131.4 kJ/mol[8]
H^+ (at pH 7)	-39.83 kJ/mol[7]
Pyruvate (at pH 7)	35.09 kJ/e-eq[9]
O_2 (at pH 7)	−78.72 kJ/e-eq[9]

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