1 **Sediment and Biofilm Affect Disinfectant Decay Rates During Long-**

2 **term Operation of Simulated Reclaimed Water Distribution Systems**
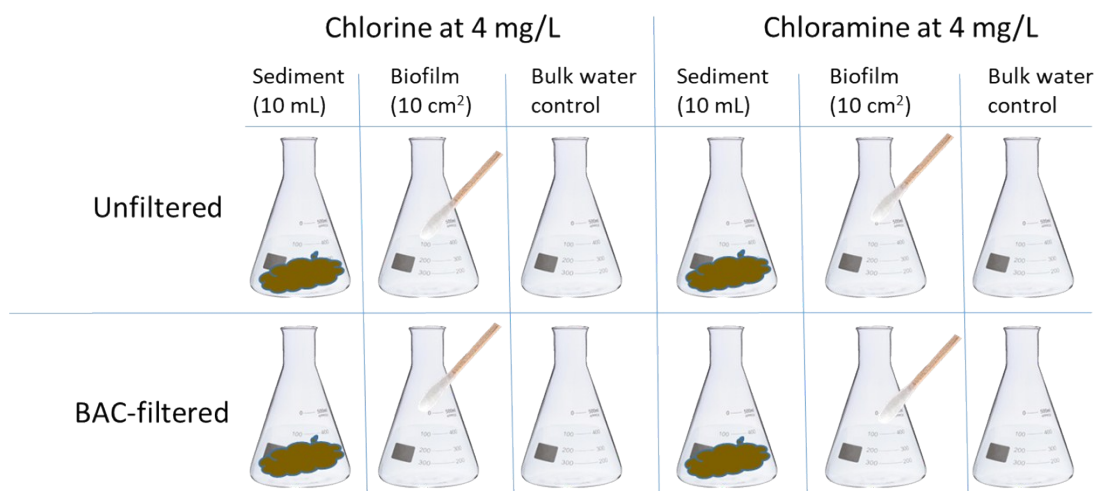
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4 Ni Zhu[1], Kris Mapili[2], Haniyyah Majeed[1], Amy Pruden[1], Marc A. Edwards[1*]

5 [1] Via Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA 24061,
6 USA

7 [2] Stantec, Fairfax, V 22030-6001

8 * Corresponding author. Tel.: +1 540 231 7236. Email address: edwardsm@vt.edu (M.A.
9 Edwards).

10 **Supplementary Information**

12 **Supplementary Figure S1** Experimental design of flask test aimed at quantifying disinfectant
13 demand associated with bulk water, biofilm, and sediment collected from Pipe RWDS. Unfiltered
14 and BAC-filtered influent water corresponding to that from the Pipe rigs were used in the test.
15 Initial chlorine or chloramine levels for the flask tests were targeted at 4 mg/L to simulate the rig
16 reservoir conditions. 10 cm^2 of swabbed biofilm and 10 mL of sediment samples collected from
17 the corresponding Pipe rig conditions were added to each flask representing those conditions. All
18 flasks were incubated at room temperature (25 °C) during the decay tests.
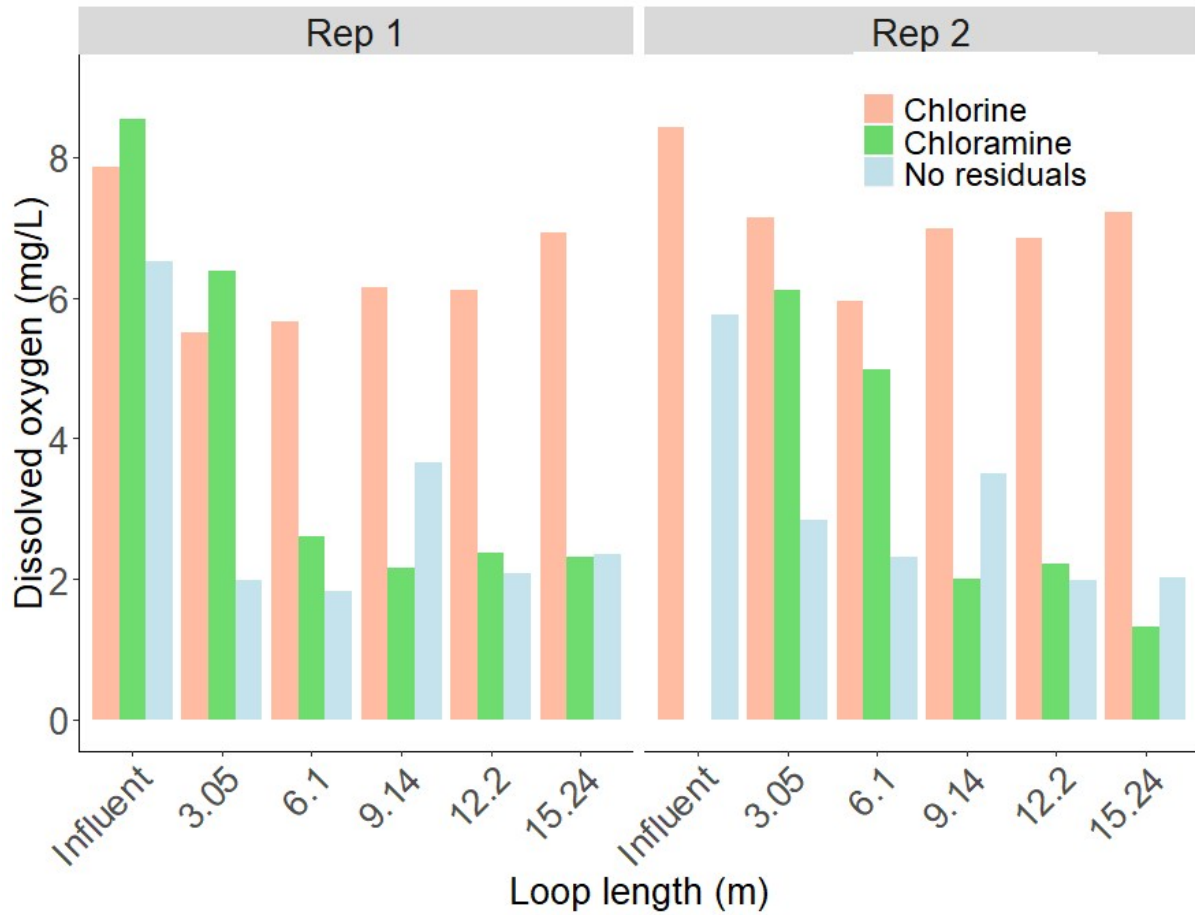
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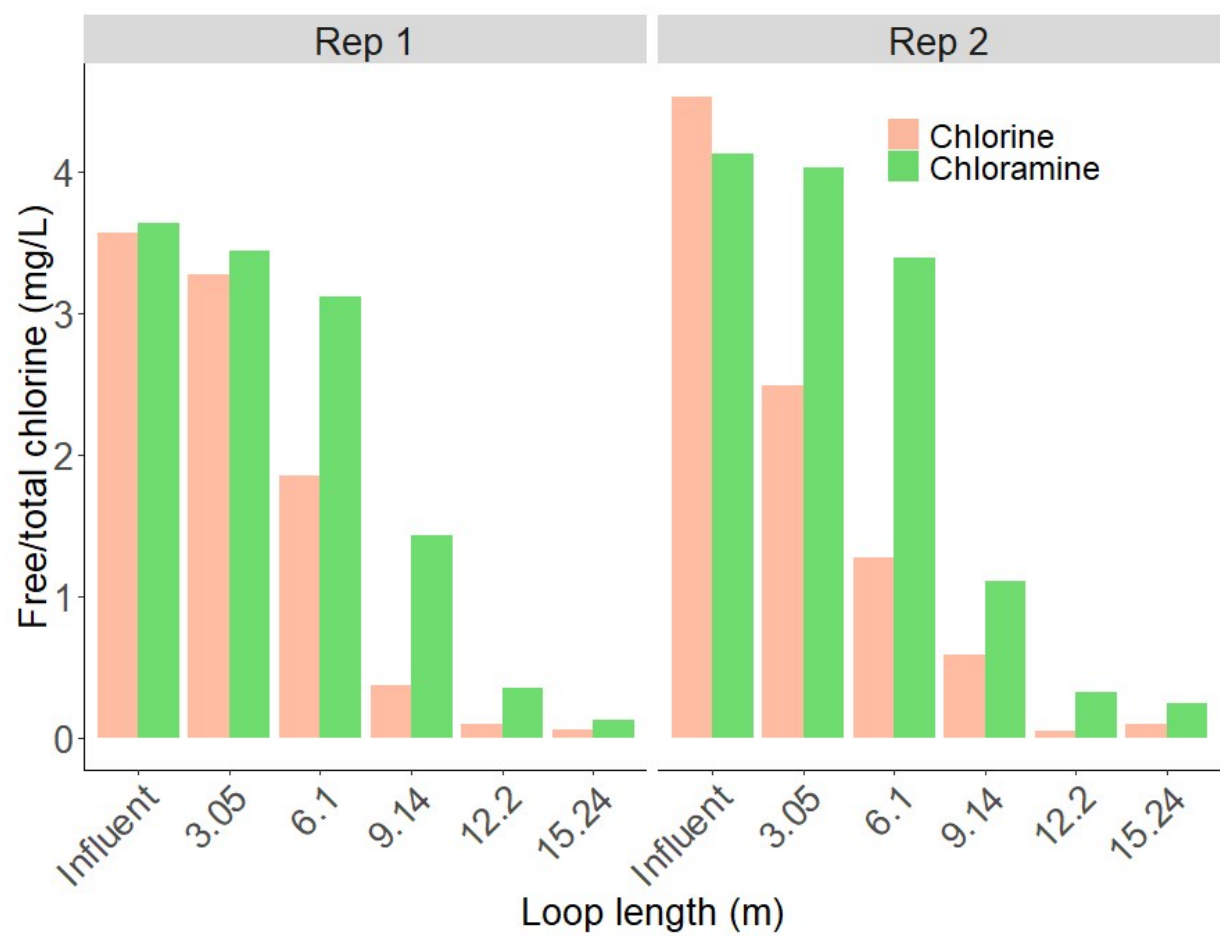
Supplementary Figure S2 Comparison of chlorine and chloramine measurements in Tube RWDS
duplicates (Rep 1, Rep2) operated in parallel displayed comparable trends of disinfectant
depletion.

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Supplementary Figure S3 Comparison of dissolved oxygen profiles in duplicate Tube rigs (Rep 1, Rep2). Dissolved oxygen was consumed in the chloramine and no disinfectant residual Tube rigs, but remained high in the chlorine Tube rigs.

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Supplementary Figure S4 BART Nitrifier results indicated the presence of high levels of
nitrifiers in the bulk water and sediment of the chloramine conditions. Development of pink color
indicates the presence of nitrifiers. The intensity of the pink color is proportional to the potential
nitrifiers population and is compared to a colorimetric chart to estimate the nitrifier abundance.
The highest pink intensity approximates to 100,000 cfu/mL.

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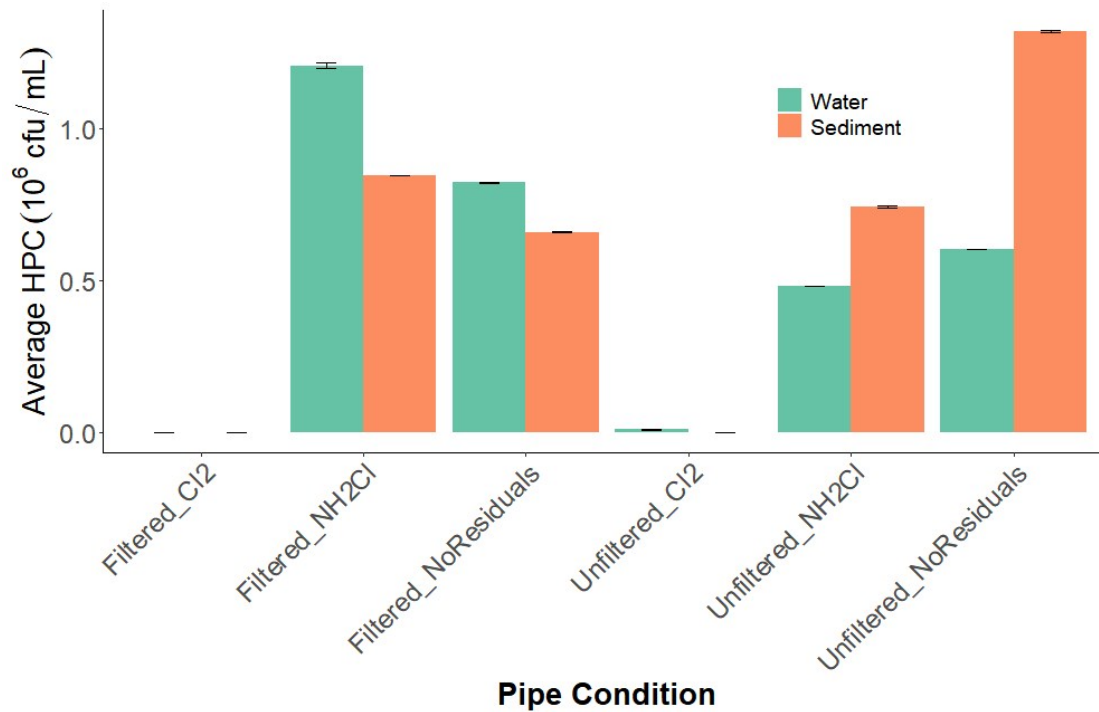
Supplementary Figure S5 HPCs in the bulk water and sediment of the Pipe Rigs enumerated
after 5 days of incubation according to Standard Methods 9215. Error bars indicate standard
deviation of triplicate plates.

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52 **Table S1** Nitrifiers BART results comparing the BAC-filtered and unfiltered waters and three
53 disinfectant conditions in the first pipe segment

Approximate Nitrifiers Population ($\log_{10}$ cfu/mL)	Unfiltered		BAC-Filtered	
	Water	Sediment	Water	Sediment
Chlorine	*bd*	*bd*	*bd*	*bd*
Chloramine	5	5	5	5
No Residual	3	3	*bd*	*bd*

54 Note1. *bd* – below detection if there was no color change

55 Note 2. 7.5mL of water/sediment sample as per the product manual was used in each vial to semi-
56 quantitatively evaluate the nitrifiers population. The upper detection limit is 10^5 cfu/mL.

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58 **Table S2** First-order decay constants derived from the flask tests containing 10 mL sediment or
59 10 cm² biofilm swabs of corresponding disinfectant conditions from the Pipe RWDS. The color
60 from red to green corresponds to decreasing decay constants and slower decay rate.

First order decay constant (hr^{-1})	Chlorine			Chloramine		
	Sediment	Biofilm	Control	Sediment	Biofilm	Control
Unfiltered	-0.032 ($R^2 = 0.96$)	-0.054 ($R^2 = 0.92$)	-0.037 ($R^2 = 0.97$)	-0.024 ($R^2 = 0.92$)	-0.014 ($R^2 = 0.95$)	-0.007 ($R^2 = 0.82$)
Filtered	-0.014 ($R^2 = 0.96$)	-0.053 ($R^2 = 0.79$)	-0.012 ($R^2 = 0.98$)	-0.045 ($R^2 = 0.97$)	-0.027 ($R^2 = 0.98$)	-0.004 ($R^2 = 0.89$)

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