Removal and Growth of Microorganisms across Treatment and Simulated Distribution at a Pilot-Scale Direct Potable Reuse Facility

Scott E. Miller[1,2], Roberto A. Rodriguez[3], and Kara L. Nelson[1,2,*]

[1]Department of Civil and Environmental Engineering, College of Engineering, University of California, Berkeley, CA, United States;
[2]National Science Foundation Engineering Research Center for Re-inventing the Nation's Urban Water Infrastructure, Berkeley, CA, United States;
[3]School of Public Health, University of Texas Health Sciences Center at Houston, TX, United States

Corresponding author email: karanelson@berkeley.edu

Key words: advanced water treatment, log removal values, flow cytometry, ATP, assimilable organic carbon, chlorine residual

Table of Contents

1. SUPPLEMENTARY METHODS 2
1.1. Conventional wastewater treatment plant 2
1.2. Overview of major treatment processes of the pilot treatment facility 2
1.3. Ozone operation 2
1.4. Microfiltration and ultrafiltration operation 3
1.5. Reverse osmosis and nanofiltration operation 3
1.6. UV / advanced oxidation process operation 3
1.7. Granular activated carbon filter operation 3
2. SUPPLEMENTARY TABLES 5
3. SUPPLEMENTARY FIGURES 10
4. REFERENCES 13

1. Supplementary Methods

1.1. Conventional wastewater treatment plant

The Roberto R. Bustamante Wastewater Treatment Plant supplied unchlorinated secondary wastewater effluent to the pilot advanced treatment train. The Bustamante plant is a conventional activated sludge facility with primary clarifiers, aeration basins designed for full nitrification of ammonia, and secondary clarifiers. It should be noted that despite its design, the facility often fails to fully nitrify ammonia during high loading periods.

1.2. Overview of major treatment processes of the pilot treatment facility

Unchlorinated secondary wastewater effluent was treated sequentially by the following processes (detailed information on each process is provided in later sections):

(1) **ozonation**, with a fixed target ozone concentration of 3.5 mg/L, ~5 min of storage time, and no detectable effluent residual. Ozonation was operated from October 26, 2015 – January 9, 2016 to evaluate potential benefits to MF/UF operation (e.g., lower biofouling and longer run-times between membrane backwashing);
(2) **chloramination**, with a target concentration of 2 – 4 mg/L as Cl_2, added to reduce biofouling of downstream membranes;
(3) **parallel microfiltration (MF)** and **ultrafiltration (UF)** membranes, with nominal pore sizes of 0.1 μm and 0.04 μm, respectively;
(4) **parallel** membrane desalination units: one tightly-bound **nanofiltration** (**NF**; Dow, model NF90-400/34i) and one **reverse osmosis** (**RO**; Hydranautics, model ESPA-LD), both operated at an overall permeate recovery of ~80% and each composed of 12 first-stage elements feeding 6 second-stage elements;
(5) an **UV-advanced oxidation process (AOP)** consisting of low-pressure UV light (Trojan UV Swift SC B08^{TM}) supplied at a dose of 840 mJ/cm^2 and H_2O_2 dosed to a target concentration of 4 mg/L. The AOP operational setpoints were chosen to target a 1.2-$\log_{10}$ reduction of N-nitrosodimethylamine by photolysis and a 0.5-$\log_{10}$ reduction of 1,4-dioxane with advanced oxidation;
(6) three **parallel granular activated carbon (GAC)** filters for quenching residual H_2O_2, consisting of a different type of filter media and operated at empty bed contact times of five minutes (GAC 1 and GAC 2) or 15 minutes (GAC 3).

1.3. Ozone operation

Ozone was operated during the latter half of the pilot test study to investigate possible benefits of ozonation on operation of downstream membranes (e.g., lower biofouling and longer run times). The ozonation unit was operated from October 26, 2015 to January 9, 2016, except for the dates of November 24 – December 2, 2015. The ozone dose was set as 0.5 mg/L of O_3 per 1 mg/L of total organic carbon in the secondary wastewater feed to the pilot, which was measured as ~6 – 8 mg/L. Therefore, the ozone setpoint was set to 3.5 mg/L and was fed at a constant rate. Ozone treatment occurred in a reaction tank (~1 min hydraulic retention time), and effluent water was sent to an ozone decay tank (~4.5 min hydraulic retention time) to ensure no ozone reached the microfiltration or ultrafiltration membranes.

1.4. Microfiltration and ultrafiltration operation

Table S1: Microfiltration and ultrafiltration operating and cleaning parameters

Parameter	**Microfiltration**	**Ultrafiltration**
Nominal Pore Size	0.01 um	0.04 um
Recovery	95.40%	~94%
Backwash Interval	~22 min	25 min
Maintenance Clean Interval	48 hours	36 hours
Integrity Test Interval	24 hours	24 hours
Maintenance Clean		
Solution	1,250 mg/L NaOCl	200 mg/L NaOCl
Target Residual	600 mg/L NaOCl	NA
Recovery Clean		
Step 1 Solution	1% NaOH + 1,000 ppm NaOCl	~1% citric acid + pH ~2 by HCl
Step 1 Duration	~2 hours	~2 hours
Step 2 Solution	2% citric acid	500 ppm NaOCl
Step 2 Duration	~1 hour	~2 hours

1.5. Reverse osmosis and nanofiltration operation

The nanofiltration (NF; Dow, model NF90-400/34i) and reverse osmosis (RO; Hydranautics, model ESPA-LD) membranes were each configured with 12 first-stage elements that fed six second-stage elements. They were operated at a flux of ~12 gallons per square foot per day and an overall permeate recovery of ~80%.

To limit mineral scaling, sulfuric acid was added to the RO and NF feed waters to a pH setpoint of 6.5 before November 12 ("Phase 1") and a pH of 6.1 after November 12 ("Phase 2"). Over the course of Phase 1 the salt passage in the RO increased, indicating damage to the membranes attributable to formation of excessive scale. The second-stage RO elements were replaced on November 11, 2015, which restored salt rejection efficacy before the start of Phase 2.

1.6. UV / advanced oxidation process operation

The H_2O_2-based UV-advanced oxidation process (AOP) was designed to reduce 1.2 $\log_{10}$ of NDMA by photolysis and 0.5 $\log_{10}$ of dioxane by advanced oxidation. As part of the pilot testing, the AOP was fed RO permeate between 9/25/15 – 11/18/15 and NF permeate between 11/19/15 – 1/22/16. Arcadis engineers observed no noticeable impact on AOP performance between using RO or NF permeates as feed.

1.7. Granular activated carbon filter operation

Three granular activated carbon (GAC) filters were established one week before full operation of the advanced treatment facility. The filter media was catalytic re-agglomerated bituminous GAC (column 1; Calgon Centaur(R) HSL 8x30), catalytic coconut-shell derived GAC (column 2; Evoqua AquaCarb(R) 830), and re-agglomerated bituminous GAC (column 3; Calgon Filtrasorb(R) 300), respectively. Columns 1 and 2 were manufactured with proprietary "catalytic" properties that purportedly increase rates of reaction with oxidants like hydrogen peroxide by

increasing the number of sites available for catalysis. GAC filters were constructed identically (except for media type) at the pilot site in unsterile conditions. GAC media was installed as six-foot deep media medis loaded into 4-inch diameter clear PVC pipes that were exposed to ambient light.

Columns 1 and 2 were operated in catalytic mode at 0.8 gpm, corresponding to a surface loading rate of 9.2 gpm per square foot and an empty bed contact time of 5 minutes. Column 3 was operated in adsorption mode at a flow rate of 0.25 gpm, corresponding to a surface loading rate of 2.9 gpm per square foot and an empty bed contact time of 15 minutes. Column 3 was operated in adsorption mode to assess potential benefits of additional total organic carbon removal. The filter columns were backwashed with stored GAC filtrate approximately every 2 – 4 weeks after appreciable increases in head loss.

2. Supplementary Tables

Table S2: Summary statistics for the standard error of the mean (i.e., precision of) for primary measurements for cell counts, ATP, and assimilable organic carbon assays. Sample count (n) is the number of triplicate measurements taken for each assay across the entire study period.

	WW 2ndary	Ozone	Chlora-mine	MF/UF	MF/UF Storage Tank	NF/RO	NF/RO Storage	AOP	GAC	Reservoir	SDS	Full Scale DWDS
ASSIMILABLE ORGANIC CARBON – Standard Error of the Mean of Primary Measurements (%)												
Average	2	4	4	4	2	17	44	28	14	NA	NA	NA
St. Dev.	1	3	3	3	2	18	33	30	16	NA	NA	NA
Median	2	4	4	3	2	13	43	28	5	NA	NA	NA
Sample Count (n)	10	5	6	18	7	18	7	5	33	2	2	0
TOTAL CELL COUNT – Standard Error of the Mean of Primary Measurements (%)												
Average	2	3	4	23	5	16	7	11	3	40	7	28
St. Dev.	1	3	4	26	4	14	3	9	2	22	7	20
Median	1	2	3	11	4	12	8	8	2	37	5	27
Count	12	12	12	24	12	24	12	12	36	90	90	11
INTACT CELL COUNT – Standard Error of the Mean of Primary Measurements (%)												
Average	2	4	3	28	4	19	15	13	3	66	13	38
St. Dev.	2	4	3	28	3	13	18	7	2	36	16	40
Median	1	2	2	25	4	17	6	14	3	77	7	18
Count	12	12	12	24	12	24	12	12	36	90	90	11
(repeated header for reference)	**WW 2ndary**	**Ozone**	**Chlora-mine**	**MF/UF**	**MF/UF Storage Tank**	**NF/RO**	**NF/RO Storage**	**AOP**	**GAC**	**Reservoir**	**SDS**	**Full Scale DWDS**
TOTAL ATP – Standard Error of the Mean of Primary Measurements (%)												
Average	6	4	3	2	1	2	1	5	3	5	2	NA
St. Dev.	4	3	2	1	1	2	1	3	4	3	2	NA
Median	5	3	3	2	1	1	1	4	2	4	1	NA
Count	8	8	8	16	8	16	8	8	24	15	48	0
INTRACELLULAR ATP – Standard Error of the Mean of Primary Measurements (%)												
Average	16	10	3	3	1	2	2	5	3	4	2	NA
St. Dev.	9	15	4	3	1	2	2	4	2	2	1	NA
Median	13	3	2	2	1	1	1	5	2	4	1	NA
Count	8	8	8	16	8	16	8	8	24	15	48	0

Table S3: Nutrient and mineral buffers added to samples in the AOC assay to create carbon-limiting conditions, per protocol in Drinking Water Microbiology Group at Eawag, Switzerland (Hammes, 2015).

(1) Phosphate-Nitrogen Buffer	Concentration (g/L)
Sodium phosphate dibasic	1.28
Potassium phosphate monobasic	0.3
Ammonium sulfate	1.77
(2) Iron-Chloride Solution (10 mM)	
Iron (III) chloride hexahydrate	2.7
(3) Trace Element Solution	
Calcium carbonate	8
Magnesium chloride	1.15
Copper sulfate	0.15
Cobalt chloride	0.13
Zinc oxide	0.4
Boric acid	0.12
Magnesium chloride	13.42
Sodium molybdenum	1.04

Table S4: Determination of the lower limit of quantification of flow cytometric total and intact cell counts. All samples were 0.1 μm-filtered bottled mineral water (Evian, France) run with 1,000 uL of volume on a BD Accuri™ C6 flow cytometer.

Sample Blank w/o Fluorescent Dye		**Total Cell Counts Assay**		**Intact Cell Counts Assay**	
Replicate #	Count [cells/mL]	Replicate #	cells/mL	Replicate #	cells/mL
1	0	1	5	1	6
2	0	2	2	2	13
3	0	3	3	3	6
4	0	4	3	4	5
5	0	5	5	5	10
6	0	6	2	6	3
7		7	9	7	6
8		8	1	8	18
9		9	3	9	3
10		10	8	10	8
11		11	1	11	4
Average	0	Average	3.8	Average	7.5
Standard Deviation	0.0	Standard Deviation	2.6	Standard Deviation	4.7
		3x Standard Deviation	7.9	3x Standard Deviation	14.1
		Limit of Quantification	**11.7**	**Limit of Quantification**	**21.6**

Table S5: Summary statistics for total and intact cell count measurements across the pilot treatment train. For calculation of average and standard deviation, all samples with cell counts below the limit of quantification ("LoQ") were set to the LoQ.

Grouped Sampling Location	Flow Cytometry Assay	Avg. (cells/mL)	St. Dev. (cells/mL)	Count (n)	Count (n) less than LoQ	% Less than LoQ
WW 2ndary	Total Cell Count	1.68E+07	1.93E+07	10	0	0
WW 2ndary	Intact Cell Count	1.30E+07	1.56E+07	10	0	0
Ozone	Total Cell Count	1.44E+07	1.92E+07	8	0	0
Ozone	Intact Cell Count	6.62E+06	1.24E+07	8	0	0
Chloramine	Total Cell Count	1.23E+07	1.62E+07	10	0	0
Chloramine	Intact Cell Count	5.11E+06	8.15E+06	10	0	0
MF/UF	Total Cell Count	6.91E+02	1.21E+03	17	0	0
MF/UF	Intact Cell Count	4.49E+02	9.61E+02	17	4	24
MF/UF Tank	Total Cell Count	3.15E+03	1.82E+03	8	0	0
MF/UF Tank	Intact Cell Count	2.31E+03	1.42E+03	8	0	0
NF/RO	Total Cell Count	7.40E+01	8.70E+01	24	0	0
NF/RO	Intact Cell Count	3.34E+01	2.69E+01	24	17	71
NF/RO Tank	Total Cell Count	6.50E+02	9.57E+02	9	0	0
NF/RO Tank	Intact Cell Count	3.74E+02	6.36E+02	9	4	44
AOP	Total Cell Count	4.66E+02	6.47E+02	7	0	0
AOP	Intact Cell Count	1.65E+02	1.96E+02	7	0	0
GAC	Total Cell Count	1.08E+04	4.62E+03	33	0	0
GAC	Intact Cell Count	7.88E+03	3.28E+03	33	0	0
Reservoir	Total Cell Count	5.35E+01	4.69E+01	30	1	3
Reservoir	Intact Cell Count	2.50E+01	8.73E+00	30	24	80
SDS	Total Cell Count	1.27E+04	2.07E+04	88	0	0
SDS	Intact Cell Count	3.70E+03	9.76E+03	88	2	2

Table S6: Summary statistics for total and intracellular ATP measurements across the pilot treatment train. For calculation of average and standard deviation, all samples with cell counts below the limit of quantification ("LoQ") were set to the LoQ.

Grouped Sampling Location	ATP Assay	Avg. (cells/mL)	St. Dev. (cells/mL)	Count (n)	Count (n) less than LoQ	% Less than LoQ
WW 2ndary	Total ATP	5.88E+00	2.69E+00	8	0	0
WW 2ndary	Intracellular ATP	5.14E+00	2.51E+00	8	0	0
Ozone	Total ATP	3.83E+00	2.02E+00	8	0	0
Ozone	Intracellular ATP	1.26E+00	8.71E-01	8	0	0
Chloramine	Total ATP	4.19E+00	2.52E+00	8	0	0
Chloramine	Intracellular ATP	8.16E-01	6.94E-01	8	0	0
MF/UF	Total ATP	2.63E+00	1.35E+00	16	0	0
MF/UF	Intracellular ATP	2.22E-01	3.69E-01	16	0	0
MF/UF Tank	Total ATP	2.80E+00	1.38E+00	8	0	0
MF/UF Tank	Intracellular ATP	1.50E-01	1.02E-01	8	0	0
NF/RO	Total ATP	5.44E-03	5.17E-03	16	0	0
NF/RO	Intracellular ATP	2.12E-04	2.55E-04	16	1	6
NF/RO Tank	Total ATP	4.72E-03	2.29E-03	8	0	0
NF/RO Tank	Intracellular ATP	1.95E-04	1.59E-04	8	1	13
AOP	Total ATP	2.08E-04	8.20E-05	8	0	0
AOP	Intracellular ATP	5.37E-05	6.75E-05	8	2	25
GAC	Total ATP	2.48E-03	1.18E-03	21	0	0
GAC	Intracellular ATP	1.08E-03	9.22E-04	21	0	0
Reservoir	Total ATP	4.95E-04	1.91E-04	15	0	0
Reservoir	Intracellular ATP	-7.08E-05	2.25E-04	15	6	40
SDS	Total ATP	1.06E-02	1.45E-02	48	0	0
SDS	Intracellular ATP	1.13E-03	1.51E-03	48	2	4

Table S7: AOC concentrations after major treatment processes in the pilot facility. AOC concentrations (in μg/L) averaged from all sampling dates are shown followed by standard deviations from technical triplicate measurements in parentheses. Sample count (n) is shown to the right of each data subset. Data for individual treatment processes and combined parallel processes are shown on separate rows (e.g., "MF/UF"). Data on the right half of columns show MF and UF data collected at 9 hours or more after a maintenance clean or recovery clean.

	All Samples						**MF and UF Samples Collected 9+ hours After Cleaning**					
Sample Location	All Days	(n)	Ozone "On"	(n)	Ozone "Off"	(n)	All Days	(n)	Ozone "On"	(n)	Ozone "Off"	(n)
Secondary Wastewater Feed	335 (103)	10	333 (79)	6	338 (146)	4						
Ozone	790 (500)	6	790 (500)	6	NA	NA						
Chloramine	465 (299)	6	943 (NA)	1	440 (213)	5						
MF	398 (207)	8	414 (164)	4	380 (269)	4	303 (195)	6	443 (188)	3	162 (28)	3
UF	260 (157)	10	258 (105)	5	260 (197)	5	203 (95)	6	232 (101)	4	146 (64)	2
MF + UF ("MF/UF")	320 (185)	18	327 (150)	9	312 (225)	9	253 (155)	12	323 (172)	7	156 (39)	5
MF/UF Storage Tank	345 (170)	6	373 (0.35)	2	330 (218)	4						
RO	0 (0)	8	0 (0)	3	0 (0)	5						
NF	0 (0)	8	0 (0)	3	0 (0)	5						
NF + RO ("NF/RO")	0 (0)	16	0 (0)	7	0 (0)	9						
NF/RO Storage Tank	0 (0)	6	0 (0)	2	0 (0)	4						
AOP	0 (0)	6	0 (0)	2	0 (0)	4						
GAC 1	67 (55)	6	154 (NA)	1	50 (39)	5						
GAC 2	66 (35)	6	64 (NA)	1	67 (39)	5						
GAC 3	47 (26)	6	21 (NA)	1	52 (27)	5						

3. Supplementary Figures

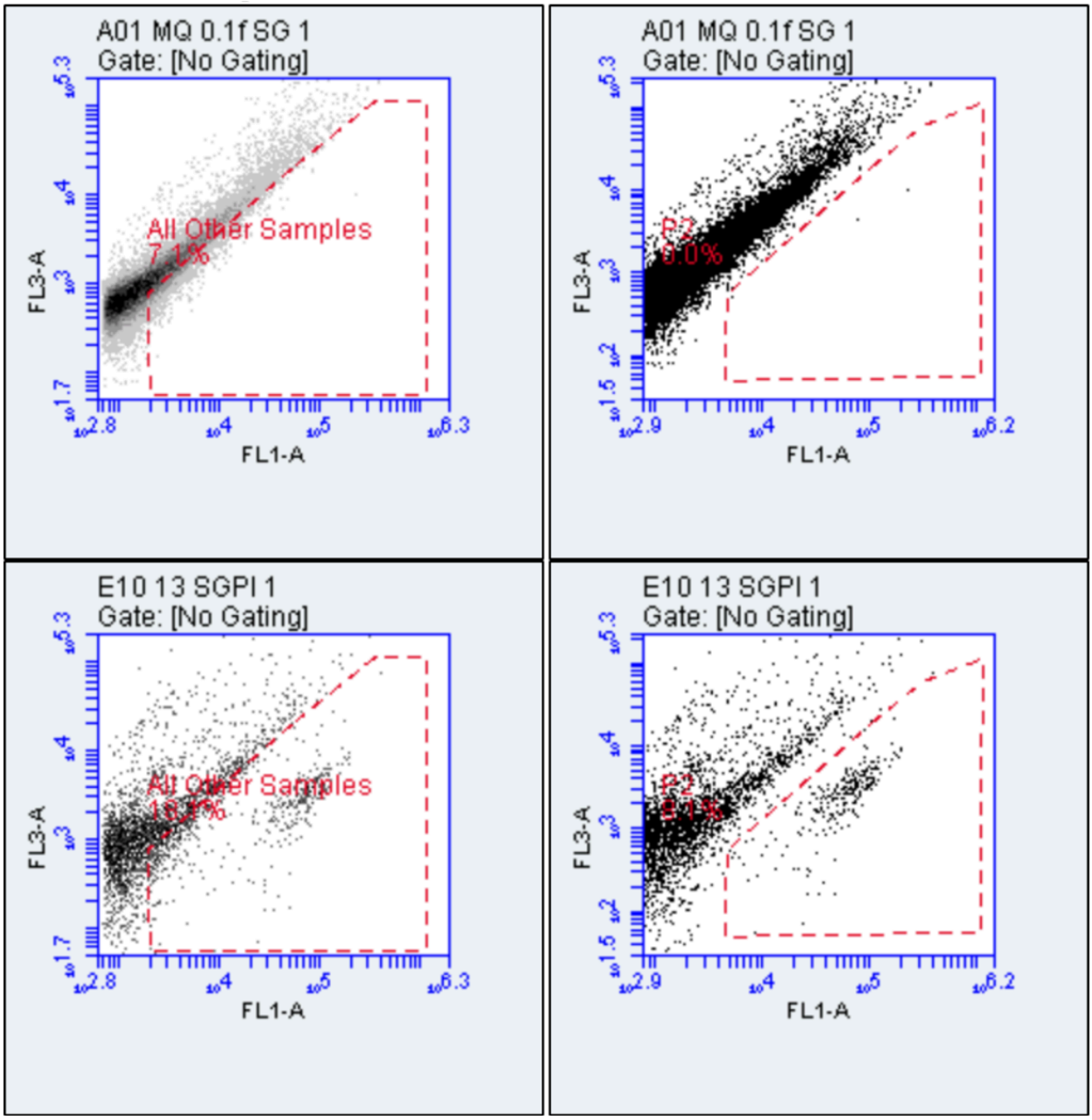

Figure S1: Adjusted gates for flow cytometry on the BD Accuri C6 for microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and UV-advanced oxidation. The top two and bottom two pictures show cell counts for 0.1 µm-filtered nanopure water ("MQ 0.1f SG 1") and reverse osmosis permeate ("13 SGPI 1"), respectively. The gate applied in the left-hand column is a publicly available gate developed by researchers at the Swiss Federal Institute of Aquatic Science and Technology ("Eawag, Switzerland) for aquatic samples analyzed on the BD Accuri™ C6 flow cytometer (Gatza, Hammes, & Prest, 2013), whereas the gate applied in the right-hand column is modified from the Eawag template to avoid excessive noise in low-cell count samples.

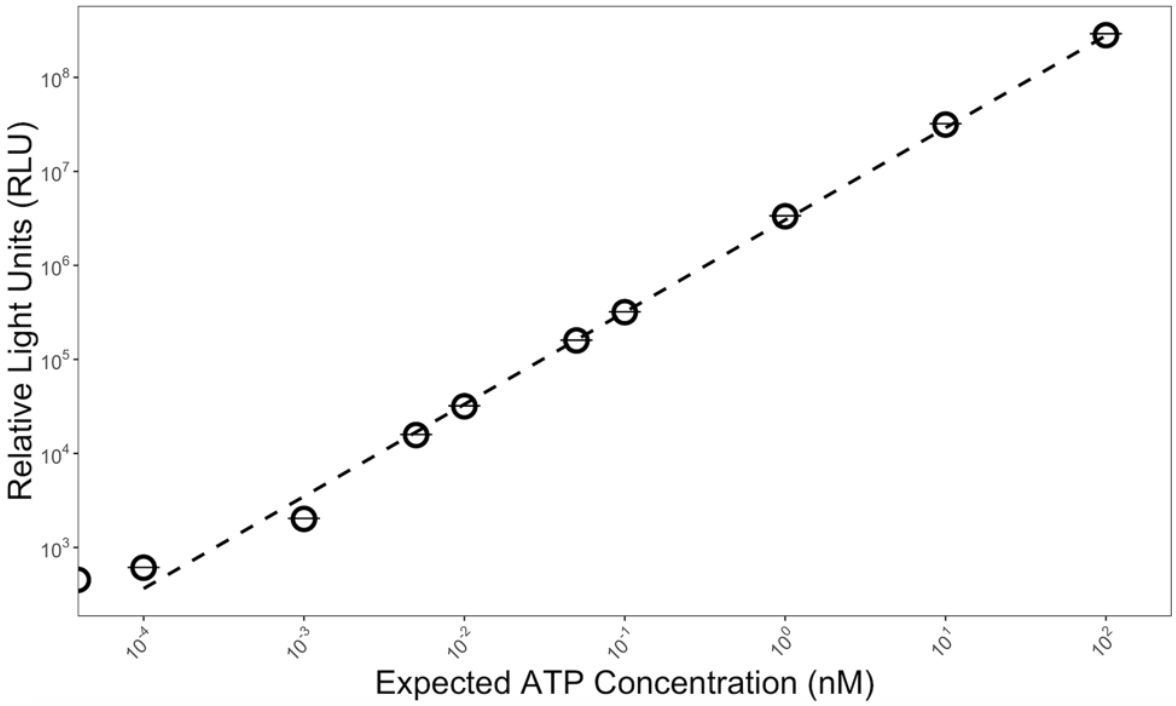

Figure S2: Calibration curve for determining ATP concentrations. This calibration curve was created by diluting pure ATP standard diluted in 0.1-µm filtered and autoclaved ultrapure water. The curve was experimentally determined to be y = 2,808,903*x + 410267, where "y" is luminescence in RLU and "x" is ATP concentration in nM. Error bars show the standard deviation of triplicate measurements. The curve was found to be linear between ATP concentrations of 10^{-4} and 10^{2} nM with an R^2 of 0.9998.

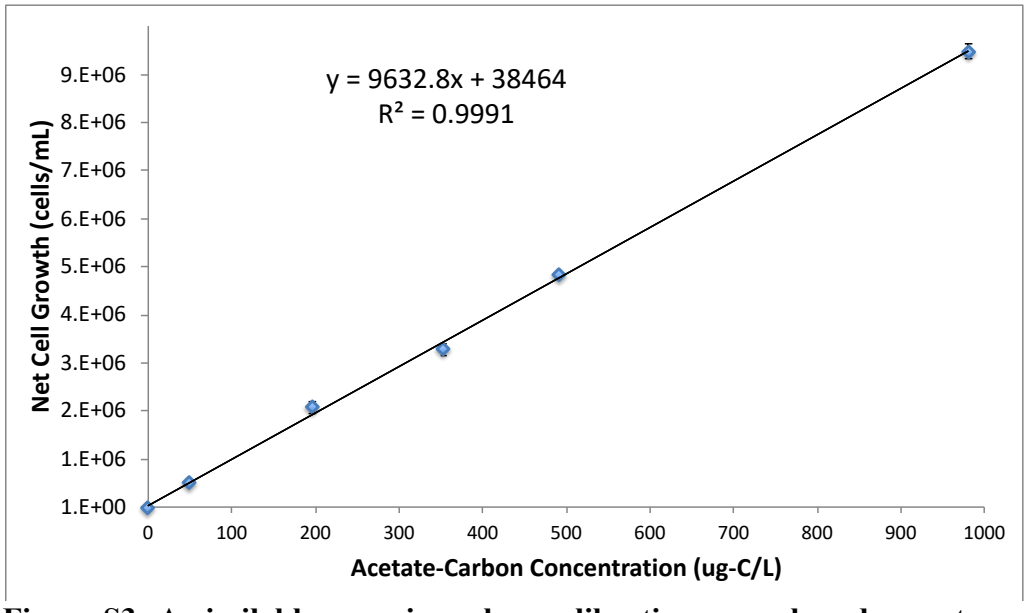

Figure S3: Assimilable organic carbon calibration curve based on net growth of total flow cytometric cell counts on known concentrations of acetate-carbon. Samples were performed in triplicate. Standard deviation of each sample is shown by vertical bars.

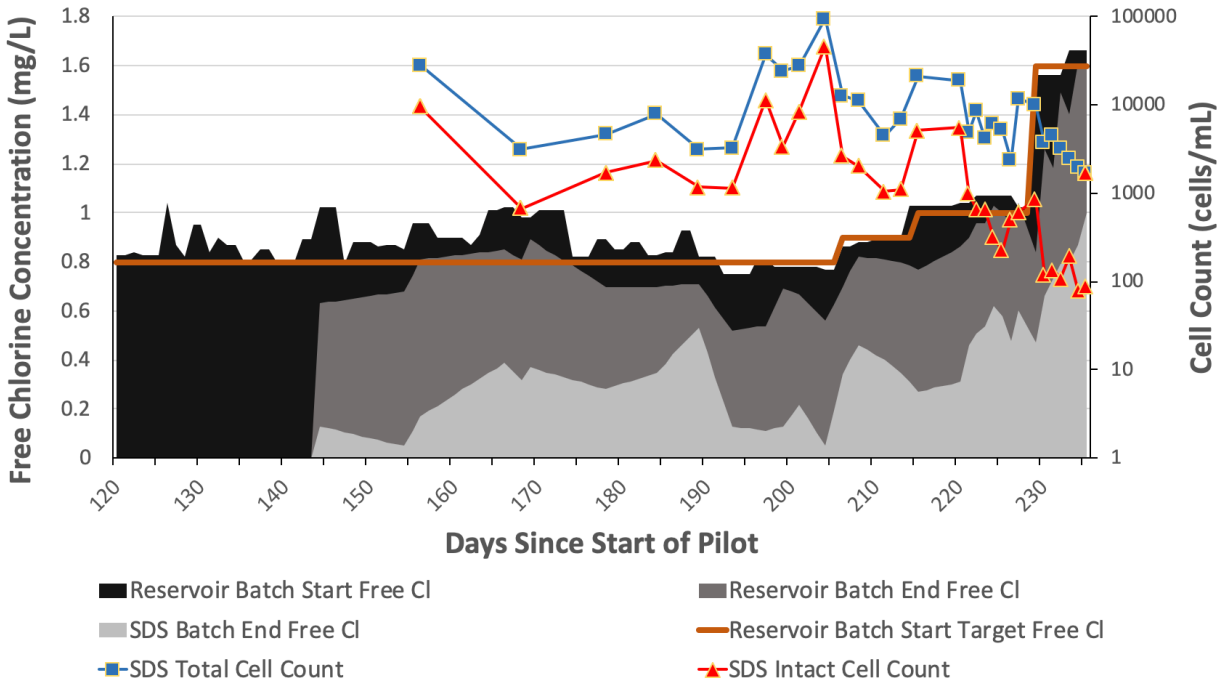

Figure S4: Time series of the reservoir and SDS free chlorine concentrations, and SDS total and intact cell counts. All free chlorine and cell count data represent averages of the three simulated distribution systems for that day. Cell count measurements were conducted in technical triplicate.

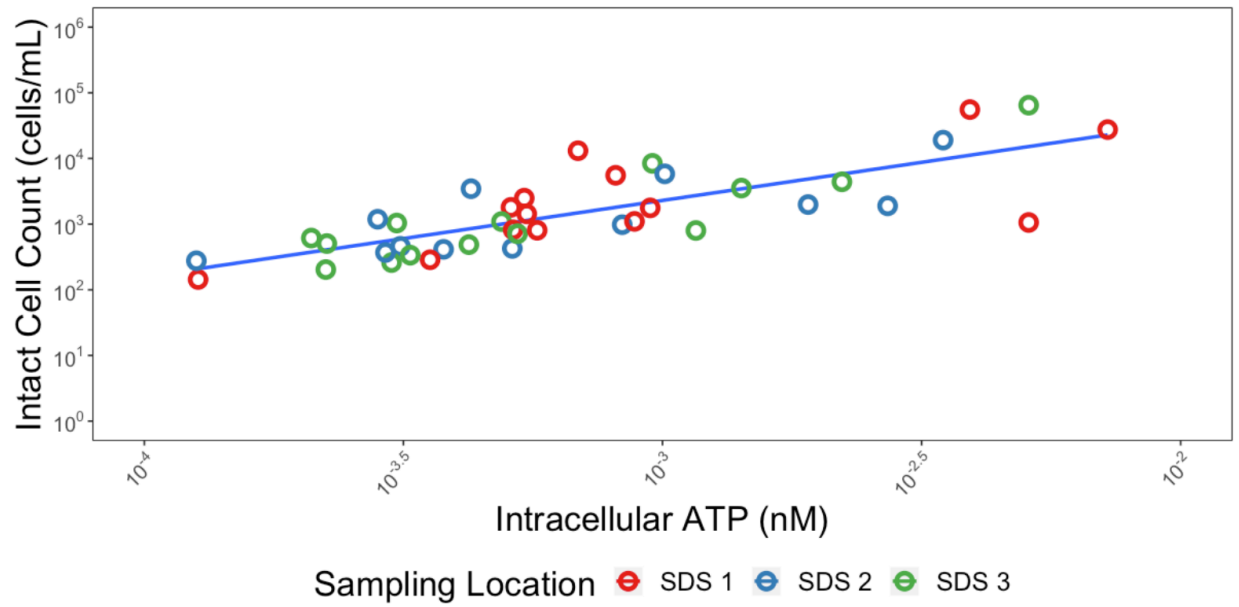

Figure S5: Relationship of intact cell counts and intracellular ATP in the SDS. A linear correlation was found ($R^2 = 0.47$; $p < 0.001$; $n = 43$), and an average ATP-per-cell value of $6.87 \pm 7.72 \times 10^{-10}$ nmol/cell was calculated from the dataset. All measurements were conducted in technical triplicate.

4. References

Gatza, E., Hammes, F., & Prest, E. (2013). *Assessing Water Quality with the BD Accuri™ C6 Flow Cytometer* (pp. 1–12).

Hammes, F. (2015, February 18). Assimilable Organic Carbon (AOC): Filtration Method. Zurich, Switzerland.