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Data Set: *Escherichia coli* Persistence on Unlined Iron in Chlorinated and Chloraminated Drinking Water

Office of Research and Development
National Homeland Security Research Center

Data Set: *Escherichia coli* Persistence on Unlined Iron in Chlorinated and Chloraminated Drinking Water

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List of Acronyms

ATCC	American Type Culture Collection
BAR	Biofilm Annular Reactor
hr	Hour
MPN	Most Probable Number
NHSRC	National Homeland Security Research Center
rpm	Revolutions per minute
SD	Standard deviation
%RSD	Relative Standard Deviation

Executive Summary

Data on *Escherichia coli* persistence are presented. Unlined iron coupons were conditioned in chlorinated and chloraminated water for two months in biofilm annular reactors (dynamic ring-shaped growth chambers for culturing aggregates of microbes on solid surfaces) under controlled conditions. *E. coli* suspensions were pulse injected into the reactors with the water dechlorinated, with the chlorine residual present, and with dechlorination during injection but a disinfectant residual added thereafter. Colilert® reagent and Quanti-Trays® were used to enumerate the numbers of viable *E. coli* persisting on the coupons (excised surface samples) and in the bulk water phase. *E. coli* persistence on the coupons was not observed for more than 3 days under the most favorable conditions (no disinfectant residual). When disinfectant residual was present, either after injection of *E. coli* or during the entire experiment, persistence of less than one day was observed.

1.0 Introduction

Concern over the risk for deliberate contamination of drinking water systems, and research aimed at mitigating this risk, has intensified with the spate of global terrorist activity. The data in this report describes the persistence of *Escherichia coli* on corroded iron surfaces in chlorinated and chloraminated drinking water. The data were generated for a research project specifically aimed at developing a model for pathogen attachment and detachment in drinking water distribution system infrastructure. The project was a collaborative effort between EPA/NHSRC and Pegasus Technical Services (Contract EC-C-05-056). Validation of attachment/detachment models requires experimental data. Some of this data has been acquired from previous studies reported in the literature (Szabo et al. 2006; Szabo et al, 2007). However, it was found that the sampling intervals were too long to draw conclusions about the model's representation of the data. Therefore, it was determined that controlled experiments would be performed under conditions similar to Szabo et al. (2006, 2007), but with shorter sampling intervals.

The goal of this data report is two-fold: 1) summarize *E. coli* persistence data for the aforementioned project and 2) provide the data and data collection methods to the broader research community. This document, containing both the data collection methods and the data, is available online through the NHSRC Web site (www.epa.gov/nhsrc/). Therefore, any publications generated from the aforementioned project can reference this data and the data is easily accessed. In addition, the data provided in this open format is accessible to and available for use by other researchers in the fields of microbiology, data modeling or water quality.

2.0
Materials and Methods

2.1 Experimental System

Modified methods in Szabo et al. (2006, 2007) were utilized in this study. Drinking water distribution system conditions were simulated using Biofilm Annular Reactors (BAR) (BioSurface Technologies Corporation, Bozeman, Montana) (ring-shaped growth chambers for culturing aggregates of microbes on solid surfaces) under controlled shear conditions. The BAR contains 20 flush-mounted rectangular polycarbonate slides attached to a rotating polycarbonate cylinder, which is inside of a stationary glass outer cylinder. The rotational speed of the inner drum was set to 100 rpm for all experiments, which generates a shear on the inner drum equivalent 30.5 cm/s flow in a 10.2 cm diameter pipe. However, this calculation is only valid for a smooth inner drum. The biofilm and corrosion layers protruded from the polycarbonate slide surface as they formed, thus the exact shear was not able to be determined at the rough biofilm/corrosion surface. So, the value reported for shear is an estimate of the flow conditions.

Components of the BARs were cleaned with soap and water and rinsed with tap water before assembly. The assembled reactors were filled with a 100 mg/L free chlorine solution, which was circulated by rotating the drum at 100 rpm for 2 hr. The reactor was then drained and flushed with tap water.

A mixture of corrosion and biofilm was developed on iron coupons before contamination experiments. Coupons were made of 99.5% pure iron, and each had a surface area of 1 cm^2 and thickness of 0.05 cm. Coupons were attached to the polycarbonate slides with acrylic cement (TAP Plastics, Oakland, California). The slides/coupons were installed after reactor disinfection, and then the flow to the reactor commenced. Flow rate was maintained at 200-300 mL/min during experimentation, so mean residence time in the reactor was approximately 3.3-5.0 min. This range keeps the reactor well flushed, which minimizes the potential for re-adherence of spiked microorganisms after contamination. Coupons were conditioned in this manner for two months.

Coupon conditioning and contamination experiments were conducted in both chlorinated and chloraminated water. Chlorinated Cincinnati tap water was supplied from a tap in the laboratory. Chloraminated water was made by adding ammonia to Cincinnati tap water in a Cl_2:N ratio of 4:1. Water was fed to the reactors from a 15.2 cm diameter pipe loop that was constantly supplied with chloraminated water. Key water quality parameters measured for the duration of the study are summarized in Table 1 as the mean ± standard deviation. Free chlorine data was acquired from online free chlorine sensors that recorded data continuously. Sensors were polled every two minutes, which resulted in 43,200 data points being used to calculate the mean and standard deviation over the two month conditioning period. Water quality data from the chloraminated pipe loop was acquired through daily grab samples resulting in 60 data points.

Table 1. Conditioning Period Water Quality

Parameter	Free Chlorine Mean ± SD	Chloramine Mean ± SD
Free/Total Chlorine (mg/L)	0.90±0.05	1.95±0.36
pH	8.64±0.06	8.89±0.31
Temperature (ºF)	65.3±3.2	72.4±2.0

2.2 Contamination

Experiments were performed in BAR with chlorinated water and with chloraminated water. Reactor contamination was performed three ways (Experiments 1, 2 and 3 described below) in the chlorinated reactors and two ways (Experiments 2 and 3) in the chloraminated reactors. In all contamination experiments, *E. coli* was pulse injected at a target initial density of 10^7 MPN/mL in the bulk water phase. BAR rotational speed was 100 rpm. Immediately before *E. coli* pulse injection, flow to the reactor was stopped and it was run in batch mode for 1 hr after injection (with the inner drum rotating), after which flow was reintroduced. This 1 hr period allowed the *E. coli* a chance to adhere to the coupons in the presence of shear without being washed out of the reactor. Sampling intervals varied during the first day after *E. coli* injection, but coupon samples were always removed 1 day after injection and further samples were withdrawn at 1 day intervals, if necessary. Samples consisted of 10 mL of bulk phase water and one iron coupon.

Experiment 1: Full Dechlorination

Tap water in the biofilm annular reactors was dechlorinated by co-injecting 1 mL of a 10% sodium thiosulfate solution with *E. coli* before the 1 hr batch contact period. After flow was reestablished, a 10% sodium thiosulfate solution was continuously injected with a syringe pump at 2 mL/hr for the rest of the experiment. Dechlorination occurred when flow was present and during the 1 hr period of batch operation. Coupons and bulk phase samples were extracted at 30 sec, 15 min, 30 min, 45 min, 1 hr, 2 hr, 4 hr, 8 hr and 24 hr after injection, and thereafter as necessary.

Experiment 2: Dechlorination During Injection

Biofilm annular reactors were dechlorinated or dechloraminated during injection by co-injecting 1 mL of a 10% sodium thiosulfate solution with *E. coli*. Dechlorination or de chloramination only occurred during the 1 hr period of batch operation. After the 1 hr batch operation, flow was re-established; disinfectant residual then returned within a few minutes. Coupons and bulk phase samples were extracted at 30 sec, 1 hr, 2 hr, 4 hr and 8 hr after injection, and thereafter as necessary.

Experiment 3: Full Chlorination/Chloramination

Tap water flowing through the biofilm annular reactors retained its chlorine residual for the entire experiment. This includes the 1 hr batch operation period. Coupons and bulk phase samples were extracted at 30 sec, 1 hr and 2 hr, and thereafter as necessary.

2.3 *E. coli* Culturing and Analysis

E. coli K-12 (ATCC 25204) was used in all experiments. *E. coli* was subcultured in Terrific broth by inoculating the broth and incubating at 37º C for 24 hr. *E. coli* was enumerated using Colilert® reagent for detection of *E. coli* and Quanti-Tray/2000® (Idexx Corporation, Westbrook, Maine), which produces the data needed for the most probable number (MPN) enumeration method. Homogenized corrosion material suspended in 100 mL of sterile 0.05M KH_2PO_4 buffer (pH 7.2) and 10 mL of bulk water from the BAR were used for enumeration of the biofilm and bulk water phases, respectively. Ten fold serial dilutions were made using sterile buffer. Positive tray wells were counted after incubation. Positive wells for the Colilert reagent are defined by a change in color from clear (water white) to yellow. The most probable number (MPN) was determined using the Quanti-Tray/2000 MPN Table (http://www.idexx.com/pubwebresources/pdf/en_us/water/qt97mpntable.pdf).

In all experiments, coupons and bulk samples were removed and assayed before injection to assure that no *E. coli* were detected. Sterile buffer used for dilution was also assayed during each experiment to ensure that it was sterile. All control samples had zero positive wells and are not included in the tables in the following section.

2.4 Comparison of the Colilert Assay with and without Corrosion Present

The question arose during the study whether the Colilert analysis was influenced by particulate matter from the coupon samples. Ten 100 mL bottles of sterile buffer were spiked with the *E. coli* to achieve a density of around 15 MPN/mL. Five of these bottles were analyzed after spiking using the Colilert method described in section 2.3. The other five bottles had 0.511-0.517 g of ground coupon corrosion material from a chlorinated BAR experiment added. This corrosion material was not exposed to *E. coli*. These samples were also enumerated using Colilert. Two negative controls were performed. One was the sterile buffer (no *E. coli* added) and the other was the buffer and corrosion material (no *E. coli* added). Both showed no positive wells, which was the expected result.

The enumeration results of five bottles with and without corrosion were compared using a two tailed paired t-test (α=0.05). The null hypothesis was that the mean values of the bottles with and without corrosion were equal.

3.0 Results

3.1 Chlorinated Reactor Results

E. coli persistence data in the chlorinated BAR is presented in Table 2. *E. coli* was detected up to three days (72 hr) after injection on the coupons in Experiment 1 (full dechlorination). In Experiment 2 (dechlorinated during the one hr batch operation after injection), most *E. coli* persisted for 8 hr and disappeared by one day after injection. However, one positive well was detected on a coupon sampled at 24 and 48 hr after injection. In Experiment 3 (full chlorination), no *E. coli* was observed on the coupon after 1 hr past injection.

The discrepancy between the expected initial *E. coli* bulk phase density and actual measured density may be due to three factors. First, the actual liquid volume of the BAR is difficult to estimate. The water level in the BAR can vary depending on the amount of flow, which changes the overall volume, and flow can vary over time as pressure in the distribution system changes. The calculated initial density was based on a 1000 mL volume, but the actual volume is likely between 950 to 1000 mL. Second, *E. coli* adsorbed to the iron coupons in the reactor, but the amount of adsorbed *E. coli* on the iron coupons does not account for all of the missing mass in solution. Adsorption also likely occurred to other reactor surfaces (largely polycarbonate and glass) but the amount was not quantified. Finally, some *E. coli* was inactivated by the disinfectant residual when it was present.

3.2 Chloraminated Reactor Results

E. coli persistence data in the chloraminated BAR is presented in Table 3. A fully dechlorinated experiment was not performed since it was not expected that the results would differ from the same experiment described in section 3.1. The 1 hr dechlorination experiment shows a 3-log *E. coli* reduction from the coupon surfaces 2 hr after injection. One positive well was observed in one experiment at 24 hr, but no *E. coli* were detected at 48 hr. When chloramine was present during the entire experiment, most *E. coli* was gone from the coupon surfaces by 2 hr, and none was detected at 24 hr after injection.

The same analysis of bulk phase concentration discussed for the chlorinated reactors in section 3.1 is also true for the chloraminated reactors.

3.3 Colilert Comparison with and without Corrosion

Results are presented in Table 4. The t-test results showed no difference between the means of the two data sets (P=0.91), so the null hypothesis was accepted.

Table 2. *E. coli* Persistence Data (Chlorinated Water)

Experiment 1-Full Dechlorination *Initial Bulk Phase (Calculated) = 7.4E+07 (MPN/mL)*				
Time (h)	**Coupon Density (MPN/cm²)**		**Bulk Phase Density (MPN/mL)**	
	Reactor 1	**Reactor 2**	**Reactor 1**	**Reactor 2**
0.01	1.14E+04	3.46E+04	1.95E+07	2.23E+07
0.25	4.35E+04	9.73E+04	1.59E+07	1.46E+07
0.50	3.10E+04	2.72E+05	1.26E+07	1.18E+07
0.75	3.10E+04	1.35E+05	1.59E+07	1.26E+07
1.0	3.66E+04	9.17E+04	1.03E+07	6.51E+06
2.0	1.60E+04	1.79E+04	1.20E+02	2.42E+02
4.0	1.57E+04	4.46E+04	7.94E+00	1.12E+02
8.0	1.48E+04	1.35E+05	4.79E+00	6.13E+01
25.50	1.09E+03	3.83E+02	2.79E+00	1.00E-01
48.00	5.75E+02	5.75E+02	7.50E-01	0.00E+00
72.00	0.00E+00	2.00E+00	0.00E+00	0.00E+00
96.00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
120.00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Experiment 2-Partial Dechlorination ***Initial Bulk Phase (Calculated) = 6.4E+07 (MPN/mL)***				
Time (h)	**Coupon Density (MPN/cm²)**		**Bulk Phase Density (MPN/mL)**	
	Reactor 1	**Reactor 2**	**Reactor 1**	**Reactor 2**
0.01	3.46E+04	8.65E+04	1.74E+07	2.72E+07
0.50	7.71E+04	6.15E+04	2.25E+04	7.45E+05
1.00	2.65E+04	1.95E+05	2.80E+05	7.24E+05
2.00	1.47E+02	6.07E+02	0.00E+00	0.00E+00
4.00	4.56E+01	3.50E+02	0.00E+00	0.00E+00
8.00	4.56E+01	1.22E+02	0.00E+00	0.00E+00
24.00	0.00E+00	1.00E+00		
48.00	0.00E+00	1.00E+00		
72.00	0.00E+00	0.00E+00		
Experiment 3-Full Chlorination ***Initial Bulk Phase (Calculated) = 6.4E+07 (MPN/mL)***				
Time (h)	**Coupon Density (MPN/cm²)**		**Bulk Phase Density (MPN/mL)**	
	Reactor 1	**Reactor 2**	**Reactor 1**	**Reactor 2**
0.01	6.13E+02	3.45E+02	2.42E+02	2.42E+02
1.00	3.36E+01	4.79E+01	4.35E+01	3.87E+01
2.00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
24.00	0.00E+00	8.60E+00	0.00E+00	0.00E+00
48.00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

MPN, most probable number

Table 3. *E. coli* Persistence Data (Chloraminated Water)

Time (h)	Coupon Density (MPN/cm²)		Bulk Phase Density (MPN/mL)	
	Reactor 1	Reactor 2	Reactor 1	Reactor 2
Experiment 2-Partial Dechloramination (During 1 hr injection period) *Initial Bulk Phase Density (Calculated) = 1.35E+06 (MPN/mL)*				
0.01	2.42E+05	2.42E+05	2.42E+02	2.42E+02
1.00	1.02E+06	2.61E+06	2.42E+02	2.42E+02
2.00	1.99E+03	9.70E+02	4.10E-01	1.19E+01
4.00	3.65E+02	3.65E+02	2.00E-01	0.00E+00
6.00	6.38E+01	2.61E+02	0.00E+00	0.00E+00
25.99	0.00E+00	1.00E+00	0.00E+00	0.00E+00
48.00	0.00E+00	0.00E+00		
Experiment 3-Full Chloramination *Initial Bulk Phase Density (Calculated) = 1.35E+06 (MPN/mL)*				
0.01	6.87E+04	2.30E+04	2.42E+04	2.42E+04
1.00	3.10E+00	0.00E+00	0.00E+00	0.00E+00
2.00	2.00E+00	4.10E+00	6.30E-01	6.30E-01
24.00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Table 4. Impact of Corrosion Material on *E. coli* Count with Colilert® Reagent

Sample	Large Wells	Small Wells	Colilert Count (MPN/ 100 mL)	Titer (MPN) *(Sample Volume = 100 mL)*			%RSD	t-test *P* (α=0.05)	Corrosion Mass Added(g)
				Sample	Sample Mean	Standard Deviation			
Dilution Blank (Buffer)	0	0	0	0					
Coupon Blank (Buffer + coupon)	0	0	0	0					0.5167
1 (Buffer + *E. coli*	49	43	1413.6	141360					
2 (Buffer + *E. coli*)	49	42	1299.7	129970					
3 (Buffer + *E. coli*)	49	42	1299.7	129970	1.41E+05	1.88E+04	13.3%		
4 (Buffer + *E. coli*)	49	42	1299.7	129970					
5 (Buffer + *E. coli*)	49	45	1732.9	173290					
6 (Buffer + *E. coli* + coupon)	49	40	1119.9	111990				0.91	0.5112
7 (Buffer + *E. coli* + coupon)	49	42	1299.7	129970					0.5135
8 (Buffer + *E. coli* + coupon)	49	43	1413.6	141360	1.42E+05	2.35E+04	16.5%		0.5116
9 (Buffer + *E. coli* + coupon)	49	45	1732.9	173290					0.5113
10 (Buffer + *E. coli* + coupon)	49	44	1553.1	155310					0.5131

4.0
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