ENGINEERING & RESEARCH SERVICES

FINAL REPORT

RESEARCH STUDY:

Regrowth Potential of Water Pathogens in Sediment

and Sewage Treatment Plant Effluent

and

Affinity of Water Pathogens to Attach to Soil Fractions.

by
NSF International
Engineering & Research Services

Work Completed under Project Number PA#248286

Test Facility:
NSF International
789 Dixboro Road
Ann Arbor, MI 48105

Sponsor:
Harris County Storm Water Quality Section
9800 Northwest Freeway, Ste 305
Houston, TX 77092

January 17, 2007

Contents:

Study 1: J-00031440

Introduction

The purpose of this study was to evaluate the potential for regrowth in sewage treatment plant effluent of *Escherichia coli (E. coli)* and other known waterborne pathogens.

Materials

Effluent:

Collected by NSF
Sterilize
De-chlorinate
BOD measured at 2.5 mg/L, not adjusted due to lower than expected level

Organisms at a concentration of ~ 20,000 CFU/100mL for each organism:

E. coli (ATCC 11229)
E. coli 0157 (ATCC 43890)
Shigella dysenteriae (ATCC 12037)
Vibrio parahaemolyiticus (ATCC 17802)

Methods

Sample points**:**

Experimental Groups: Every 12 hours for 108 hours
Controls: Every 24 hours for 96 hours

Control flasks:

Negative controls: Sterilized effluent, 3 replicates, added carbon source (0.5% glucose)
Positive controls: Sterilized effluent, single replicate, spiked with organism, added carbon source (0.5% glucose)
Baseline control: Sterile Buffered Deionized Water (SBDW), single replicate, spiked with organism

Test flasks:

Experimental: Sterilized effluent, 3 replicates, spiked with organism

The samples were set up and enumerated separately per organism as follows:

- Add organisms as 40mL of 100,000mpn/100ml organism to 160mL of effluent or buffered water
- Place on rotary shaker at 25 rpm to provide slight continuous movement at 20-25^{o}C (68-77^{o}F)
- Pull aliquot for serial dilution and spread plate in duplicate. Analyses for *E. coli* ATCC 43890 utilized Sorbitol Mackonkey Agar and were incubated for 24 hours at 35ºC. Analyses for *E. coli* ATCC 11229 utilized LES mEndo Agar and were incubated for 24 hours at 35ºC. Analyses for *S. dysenteriae* ATCC 12037 utilized Hektoen Enteric Agar and were incubated for 24-48 hours at 35ºC. Analyses for *V. parahaemolyticus* ATCC 17802 utilized TCBS Agar and were incubated for 48 hours at 35ºC.

Results

Microbiological results and summary for all samples are available in Tables 1-1 through 1-8. All statistical analyses are attached in Appendix A. Results for the negative controls were acceptable, indicating that the sterilization by autoclave was effective.

E. coli 43890: Throughout the test the positive control demonstrated higher populations than the baseline control or the experimental flasks. The baseline control was consistently lower than the positive control and experimental flasks throughout the test. Statistical analyses indicated that there were significant differences between the three groups at all time points 24 hours and later. They also indicated that there were significant differences in the comparison between only the experimental flasks and the baseline control at all time points 24 hours and later.

E. coli 11229: After the initial time point, the positive control demonstrated higher populations than the baseline control or the experimental flasks for the remainder of the test. The baseline control was consistently lower than the positive control and experimental flasks throughout the test. Statistical analyses indicated that there were significant differences between the three groups at all time points 24 hours and later. They also indicated that there were significant differences in the comparison between only the experimental flasks and the baseline control at 48 hours and 72 hours.

S. dysenteriae 12037: The positive control and baseline control remained fairly steady before dropping to near non-detect levels at 96 hours, while the experimental flasks remained approximately the same throughout the test. Statistical analyses indicated that there were significant differences between the three groups at 48 hours and 72 hours. They also indicated that there were significant differences in the comparison between only the experimental flasks and the baseline control at 48 hours and 72 hours.

V. parahaemolyticus 17802: This organism did not survive or multiply the 0 hour time point. No statistical analyses were performed due to non-detect results.

Table 1-1. Cell densities of *E. coli* 43890 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Baseline	Positive Control	Experimental
0	1.03E+03	1.22E+03	8.58E+02
24	7.10E+02	3.70E+03	2.70E+03
48	7.60E+01	1.21E+05	3.98E+04
72	6.50E+01	4.95E+05	4.53E+05
96	4.00E+01	2.89E+05	2.37E+05

Figure 1-1. Cell densities of *E. coli* 43890 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

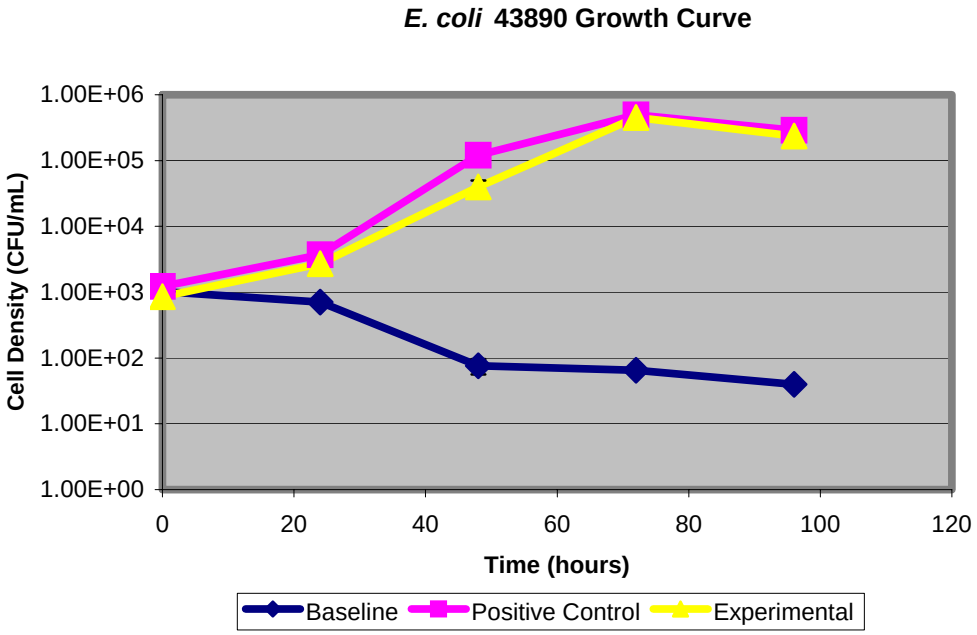


Table 1-2. Cell densities of *E. coli* 43890 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Experimental
0	8.58E+02
12	7.48E+02
24	2.70E+03
36	1.68E+04
48	3.98E+04
60	3.79E+05
72	4.53E+05
84	2.20E+05
96	2.37E+05
108	3.95E+05

Figure 1-2. Cell densities of *E. coli* 43890 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

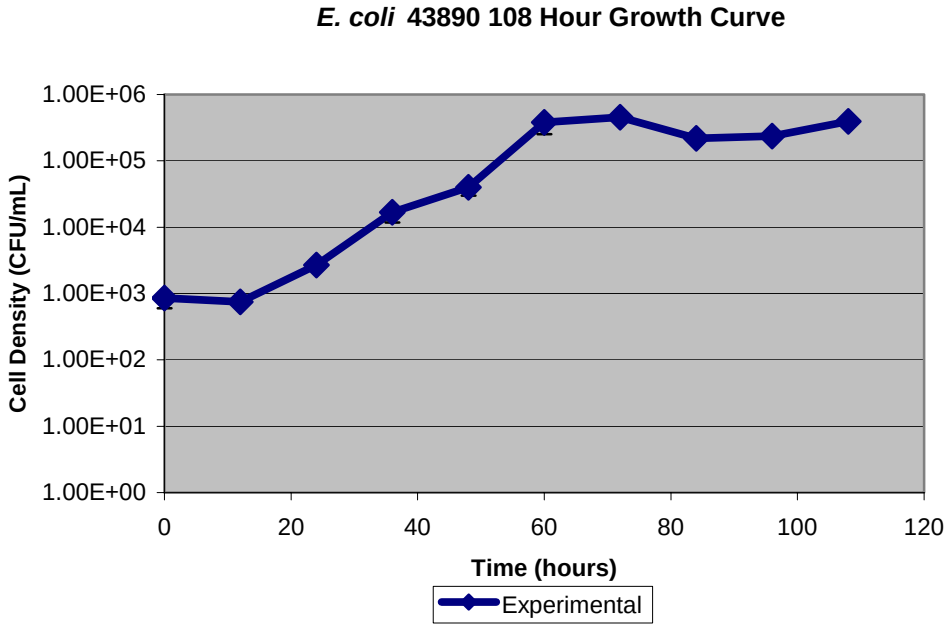


Table 1-3. Cell densities of *E. coli* 11229 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Baseline	Positive Control	Experimental
0	6.40E+02	3.20E+02	4.77E+02
24	9.20E+02	3.80E+03	1.13E+03
48	3.80E+02	3.00E+05	1.20E+05
72	6.50E+01	4.95E+05	4.53E+05
96	2.25E+02	5.05E+05	6.01E+05

Figure 1-3. Cell densities of *E. coli* 11229 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

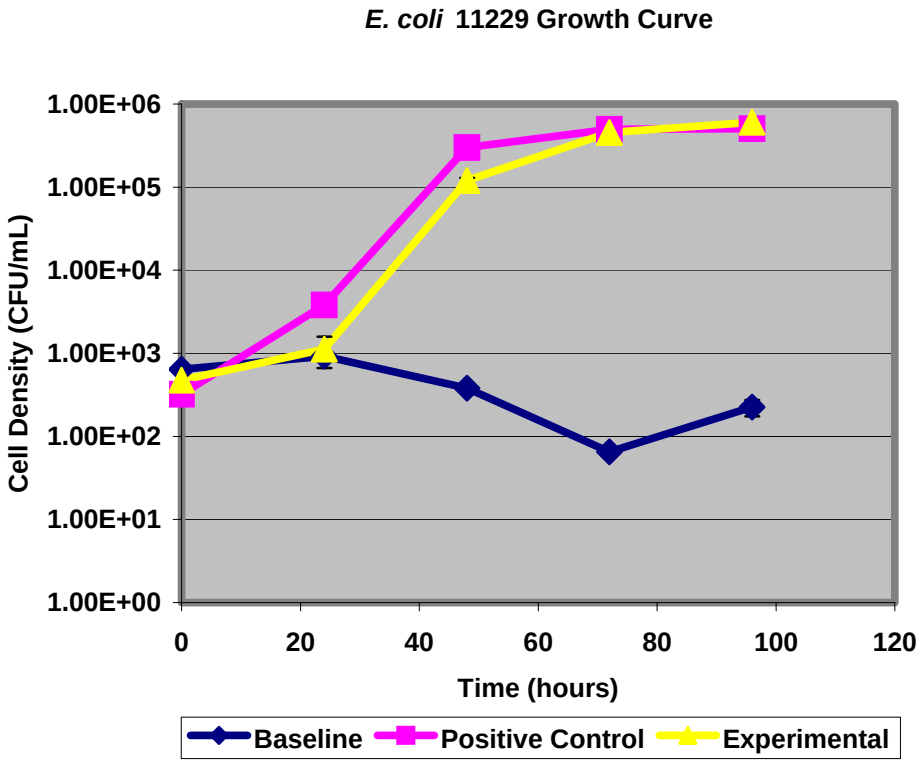


Table 1-4. Cell densities of *E. coli* 11229 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Experimental
0	4.77E+02
12	8.57E+02
24	1.13E+03
36	5.84E+04
48	1.20E+05
60	2.86E+05
72	4.53E+05
84	3.27E+05
96	6.01E+05
108	1.50E+06

Figure 1-4. Cell densities of *E. coli* 11229 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

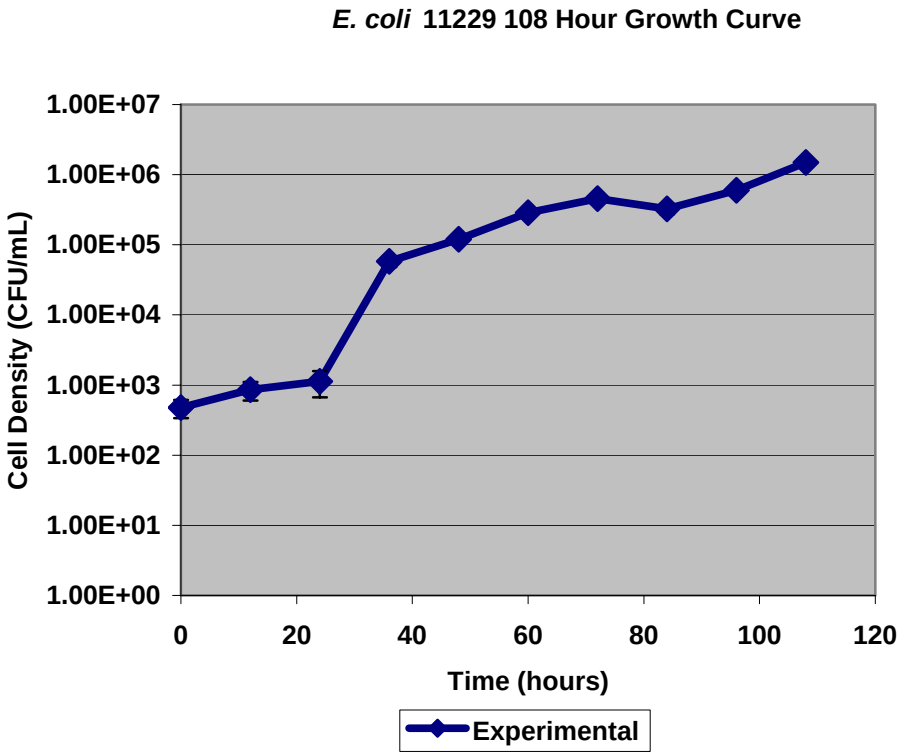


Table 1-5. Cell densities of *S. dysenteriae* 12037 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Baseline	Positive Control	Experimental
0	3.75E+03	3.85E+03	3.65E+03
24	3.25E+03	3.00E+03	3.77E+03
48	1.00E+02	3.20E+04	5.30E+03
72	1.55E+02	2.00E+04	6.63E+03
96	1.00E+00	2.90E+01	3.70E+03

Figure 1-5. Cell densities of *S. dysenteriae* 12037 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

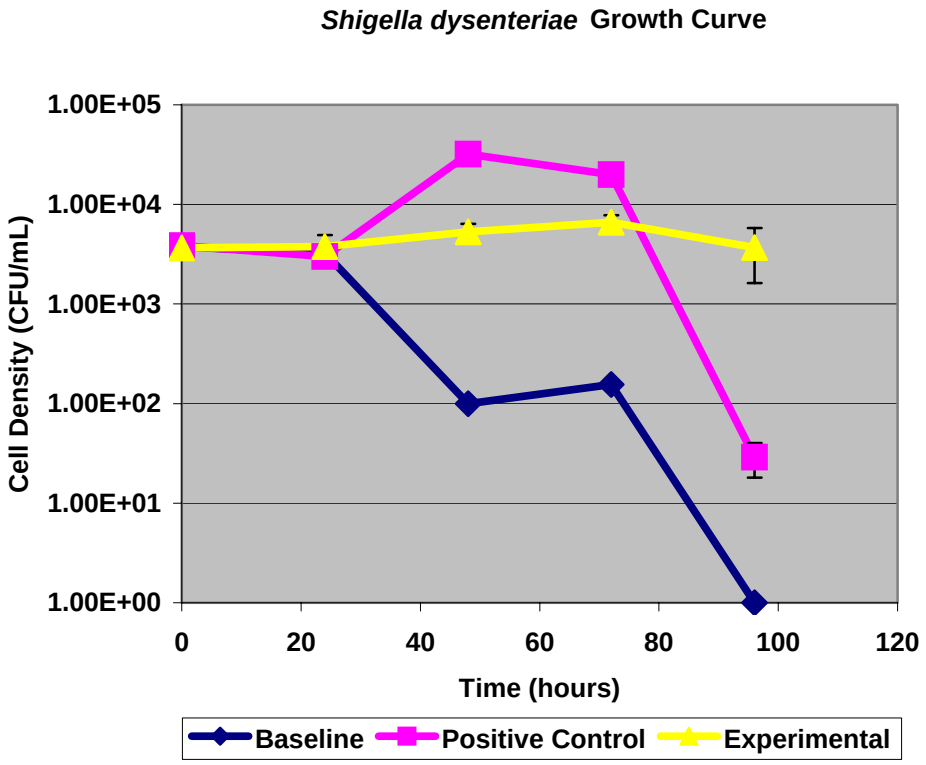


Table 1-6. Cell densities of *S. dysenteriae* 12037 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Experimental
0	3.65E+03
12	7.57E+03
24	3.77E+03
36	5.73E+03
48	5.30E+03
60	5.70E+03
72	6.63E+03
84	4.93E+03
96	3.70E+03
108	3.59E+03

Figure 1-6. Cell densities of *S. dysenteriae* 12037 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

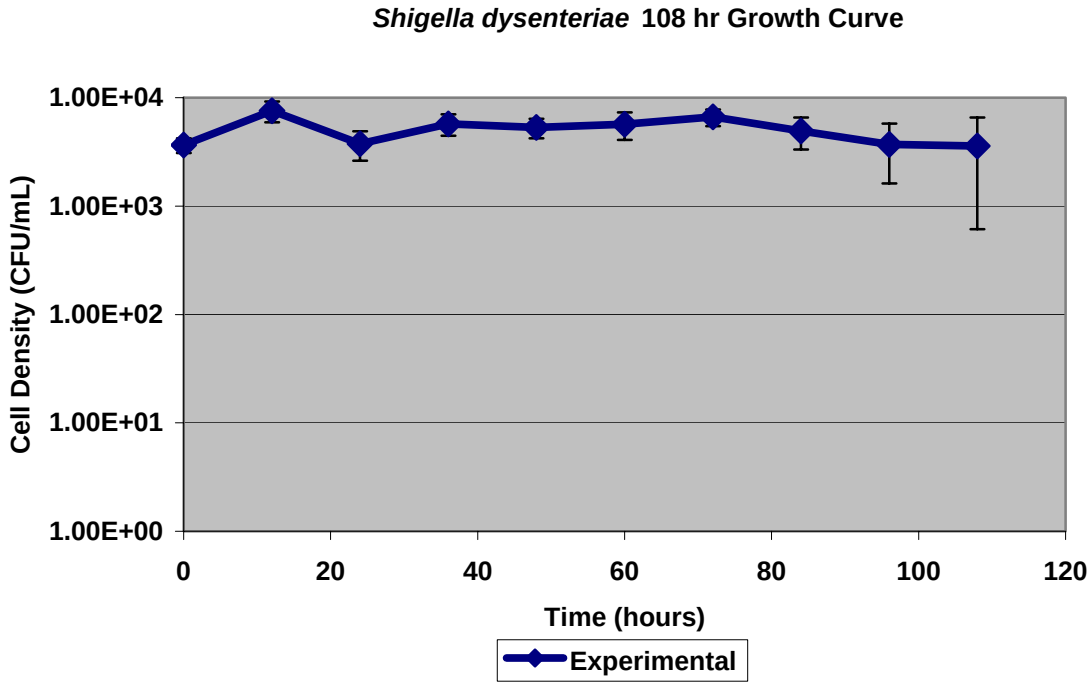


Table 1-7. Cell densities of *V. parahaemolyticus* 17802 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Baseline	Positive Control	Experimental
0	9.10E+01	1.45E+02	7.20E+01
24	1.00E+00	1.00E+00	1.00E+00
48	1.00E+00	1.00E+00	1.00E+00
72	1.00E+00	1.00E+00	1.00E+00
96	1.00E+00	1.00E+00	1.00E+00

Figure 1-7. Cell densities of *V. parahaemolyticus* 17802 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

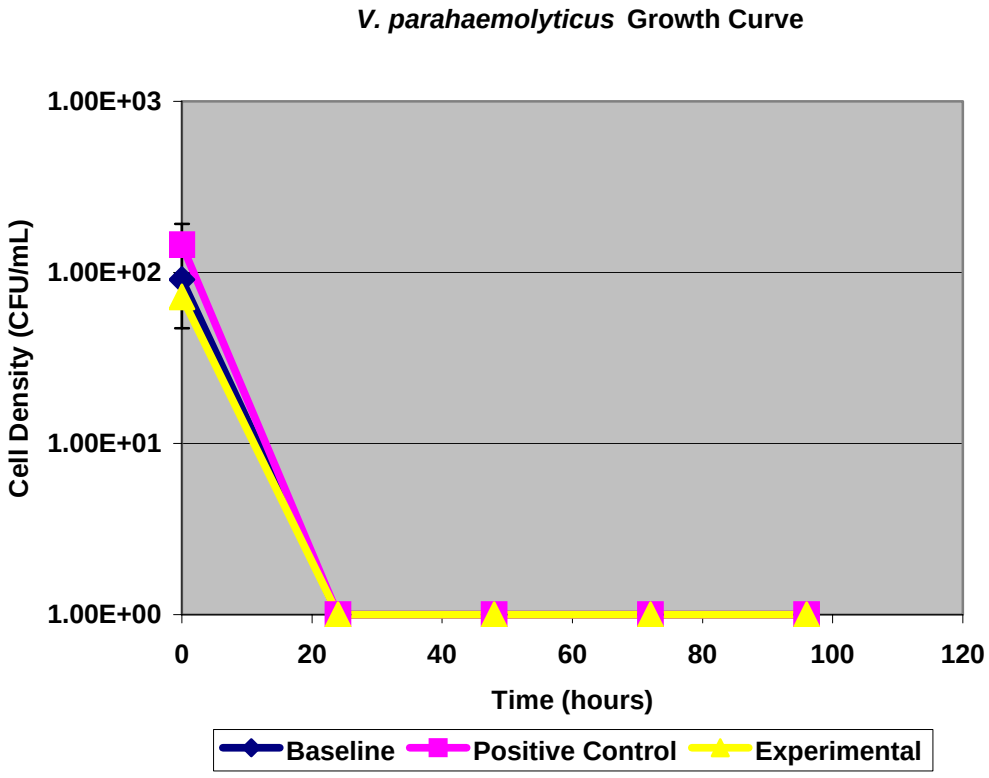
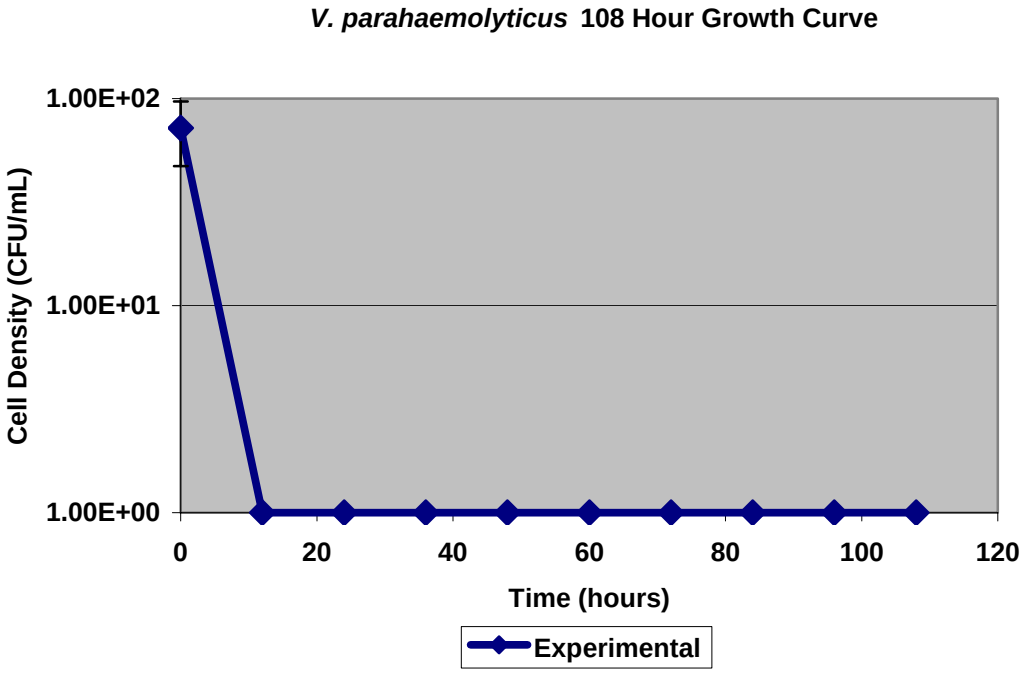


Table 1-8. Cell densities of *V. parahaemolyticus* 17802 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Experimental
0	7.20E+01
12	1.00E+00
24	1.00E+00
36	1.00E+00
48	1.00E+00
60	1.00E+00
72	1.00E+00
84	1.00E+00
96	1.00E+00
108	1.00E+00

Figure 1-8. Cell densities of *V. parahaemolyticus* 17802 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.



Study 2: J-00026494

Introduction

The purpose of this study was to evaluate the potential for regrowth in buffered water with sterilized sediment substrate of *Escherichia coli (E. coli)* and other known waterborne pathogens.

Materials

Sediment substrate:

Collected by Harris County personnel
Sterilized by autoclaving

Organisms at a concentration of ~ 20,000 CFU/100mL for each organism:

E. coli (ATCC 11229)
E. coli 0157 (ATCC 43890)
Shigella dysenteriae (ATCC 12037)
Vibrio parahaemolyiticus (ATCC 17802)

Methods

Sample points**:**

Experimental Groups: Every 12 hours for 108 hours
Controls: Every 24 hours for 108 hours

Control flasks:

Negative controls: Sterilized sediment, 3 replicates, added carbon source (0.5% glucose)
Positive controls: Sterilized sediment, single replicate, spiked with organism, added carbon source (0.5% glucose)
Baseline control: Sterile Buffered Deionized Water (SBDW), single replicate, spiked with organism

Test flasks:

Experimental: Sterilized sediment, 3 replicates, spiked with organism

The samples were set up and enumerated separately per organism as follows:

- Add organisms as 40mL of 100,000mpn/100ml organism to 160mL of buffered water with ~1 tablespoon sediment (triplicate beakers)
- Place on rotary shaker at 25 rpm to provide slight continuous movement at 20-25^{o}C (68-77^{o}F)
- Pull aliquot for serial dilution and spread plate in duplicate. Analyses for *E. coli* ATCC 43890 utilized Sorbitol Mackonkey Agar and were incubated for 24 hours at 35ºC. Analyses for *E. coli* ATCC 11229 utilized LES mEndo Agar and were incubated for 24 hours at 35ºC. Analyses for *S. dysenteriae* ATCC 12037 utilized Hektoen Enteric Agar and were incubated for 24-48 hours at 35ºC. Analyses for *V. parahaemolyticus* ATCC 17802 utilized TCBS Agar and were incubated for 48 hours at 35ºC.

Results

Microbiological results and summary for all samples are available in Tables 2-1 through 2-8. All statistical analyses are attached in Appendix B. Results for the negative controls were acceptable, indicating that the sterilization by autoclave was effective.

E. coli 43890: Throughout the test the positive control demonstrated higher populations than the baseline control or the experimental flasks. The baseline control and experimental flask were approximately the same throughout the test. Statistical analyses indicated that there were significant differences between the three groups at all time points 24 hours and later. They also indicated that there were significant differences in the comparison between only the experimental flasks and the baseline control at all time points 48 hours and later.

E. coli 11229: After the initial time point, the positive control demonstrated higher populations than the baseline control or the experimental flasks for the remainder of the test. The baseline control and experimental flask were approximately the same throughout the test. Statistical analyses indicated that there were significant differences between the three groups at all time points 48 hours and later. They also indicated that there were significant differences in the comparison between only the experimental flasks and the baseline control at 48 hours and 72 hours.

S. dysenteriae 12037: After the initial time point, the positive control and baseline control dropped to non-detect levels, while the experimental flasks continued to multiply for the remainder of the test. Statistical analyses indicated that there were significant differences between the three groups at all time points. They also indicated that there were significant differences in the comparison between only the experimental flasks and the baseline control at all time points.

V. parahaemolyticus 17802: This organism did not survive or multiply at any of the time points. No statistical analyses were performed due to non-detect results.

Table 2-1. Cell densities of *E. coli* 43890 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Baseline	Positive Control	Experimental
0	1.70E+03	5.86E+03	5.04E+03
24	1.59E+03	3.11E+05	2.32E+03
48	1.40E+03	6.10E+05	2.50E+03
72	1.28E+03	4.95E+05	1.82E+03
96	1.03E+03	5.10E+05	2.72E+03

Figure 2-1. Cell densities of *E. coli* 43890 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

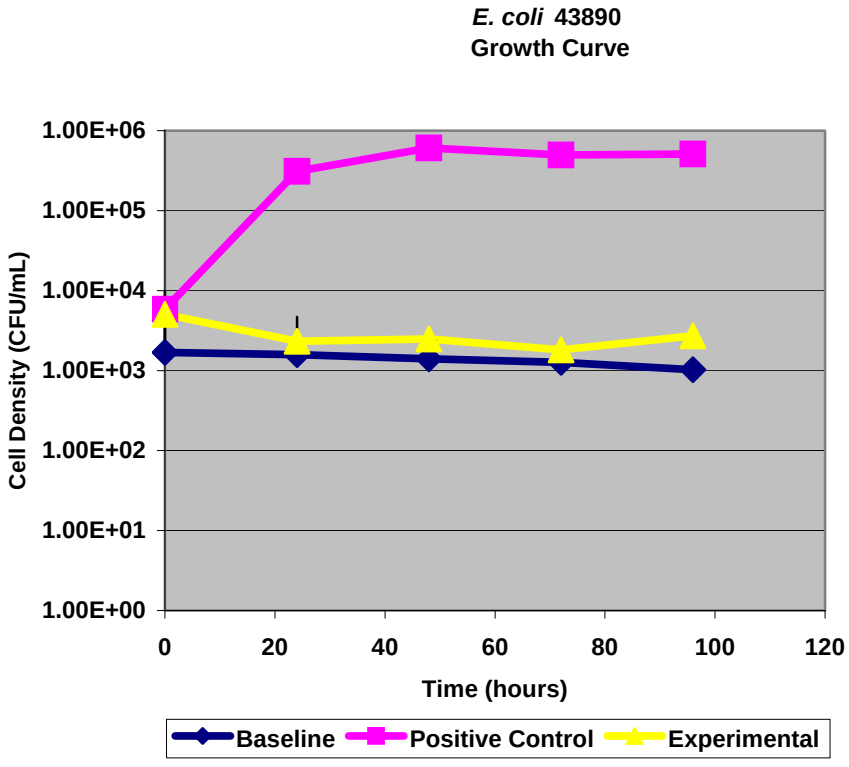


Table 2-2. Cell densities of *E. coli* 43890 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Experimental
0	5.04E+03
12	3.13E+03
24	2.32E+03
36	1.76E+03
48	2.50E+03
60	2.93E+03
72	1.82E+03
84	5.86E+03
96	2.72E+03
108	2.09E+03

Figure 2-2. Cell densities of *E. coli* 43890 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

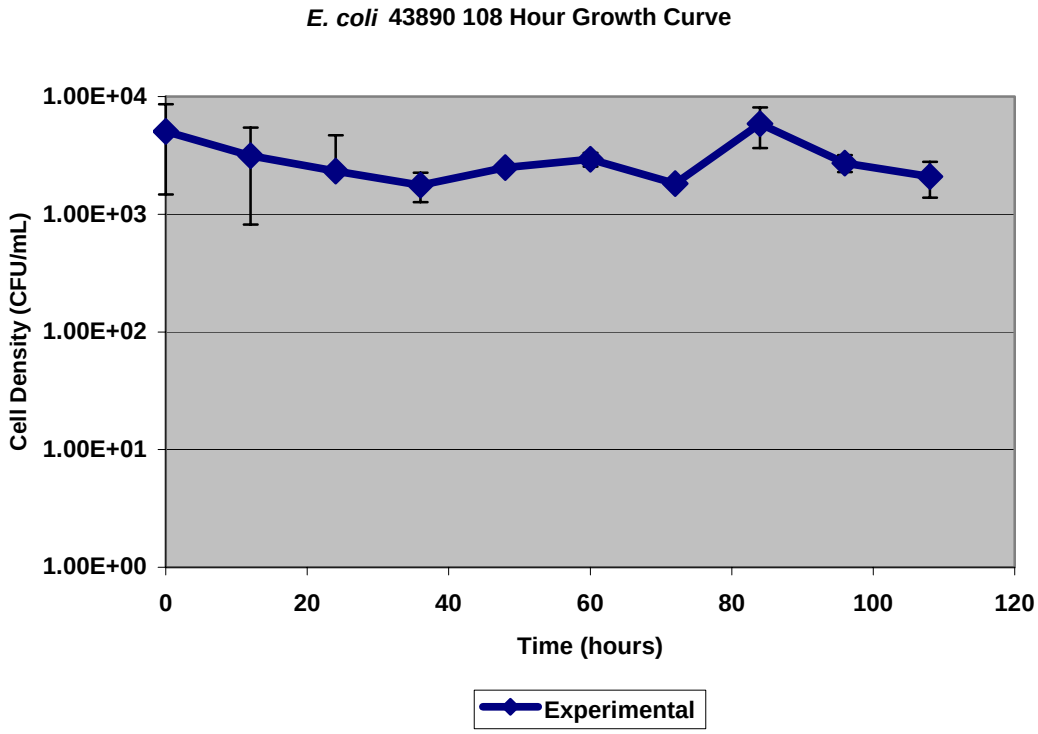


Table 2-3. Cell densities of *E. coli* 11229 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Baseline	Positive Control	Experimental
0	1.13E+03	1.03E+03	1.15E+03
24	9.95E+02	2.82E+05	1.07E+04
48	1.63E+03	7.14E+05	2.14E+03
72	5.60E+03	1.33E+06	2.34E+03
96	9.15E+03	6.90E+05	6.49E+03

Figure 2-3. Cell densities of *E. coli* 11229 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

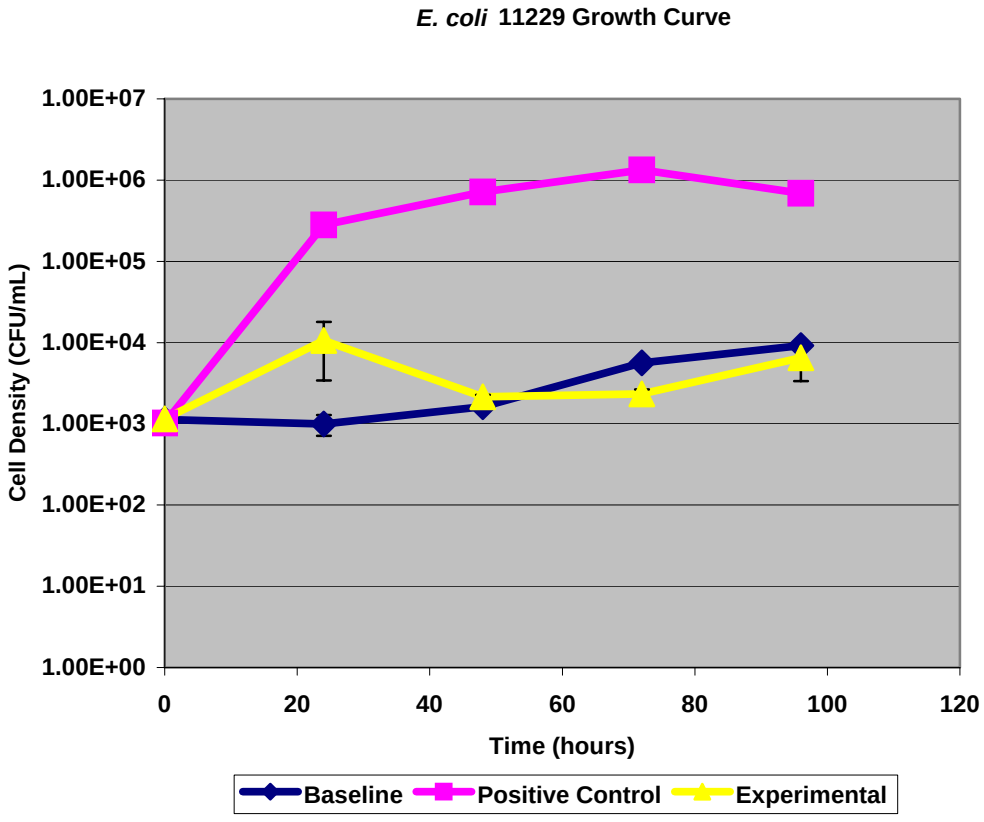


Table 2-4. Cell densities of *E. coli* 11229 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Experimental
0	1.15E+03
12	4.26E+03
24	1.07E+04
36	9.25E+03
48	2.14E+03
60	5.13E+03
72	2.34E+03
84	5.43E+03
96	6.49E+03
108	1.10E+04

Figure 2-4. Cell densities of *E. coli* 11229 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

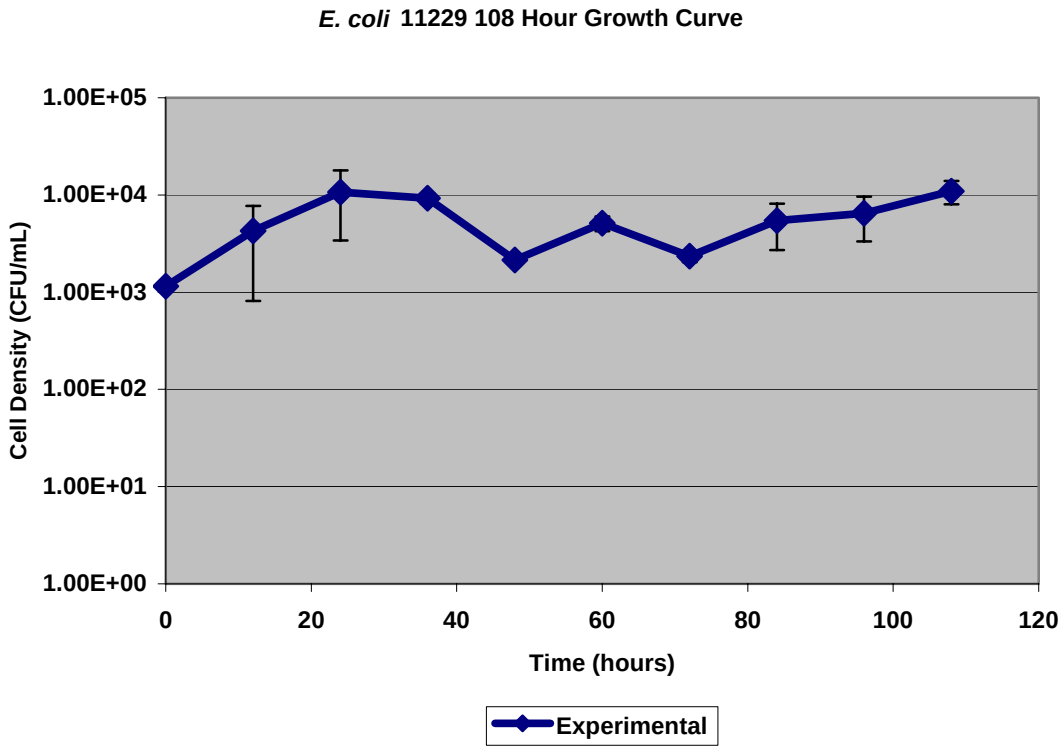


Table 2-5. Cell densities of *S. dysenteriae* 12037 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Baseline	Positive Control	Experimental
0	6.30E+02	6.80E+02	4.00E+02
24	5.40E+01	1.00E+00	1.86E+02
48	1.00E+00	1.00E+00	1.31E+05
72	1.00E+00	1.00E+00	5.10E+04
96	1.00E+00	1.00E+00	2.93E+05
120	1.00E+00	1.00E+00	3.02E+05

Figure 2-5. Cell densities of *S. dysenteriae* 12037 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

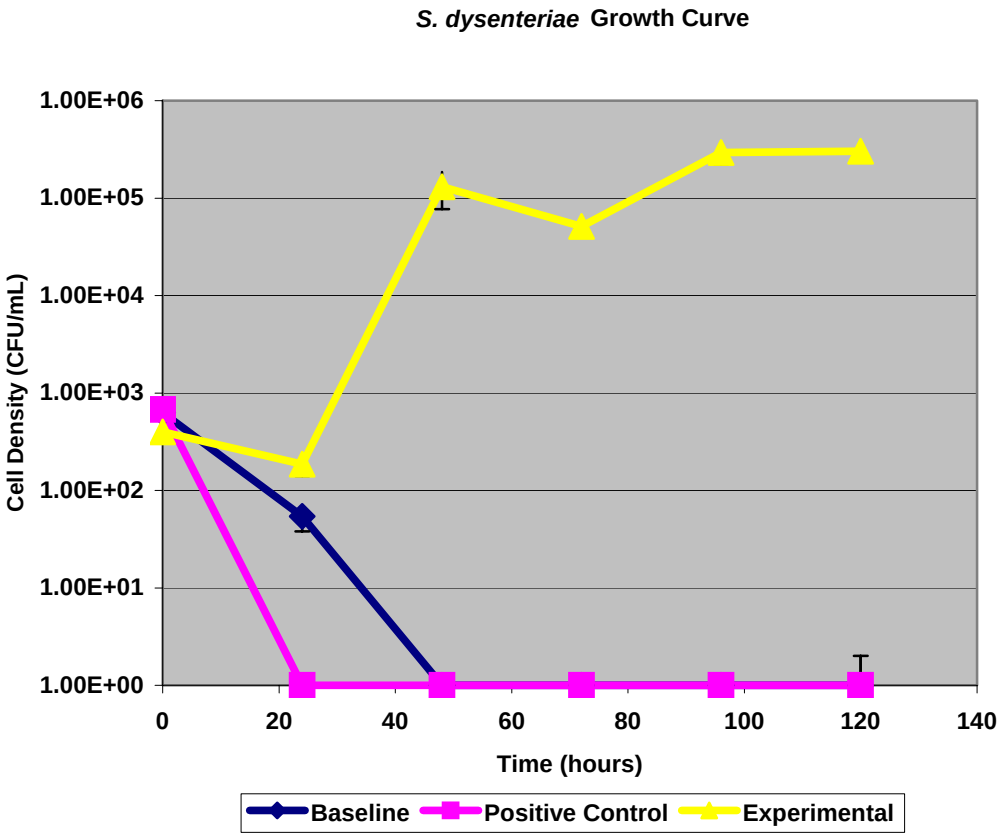


Table 2-6. Cell densities of *S. dysenteriae* 12037 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Experimental
0	4.00E+02
12	2.59E+02
24	1.86E+02
36	9.02E+04
48	1.31E+05
60	3.17E+05
72	5.10E+04
84	3.14E+05
96	2.93E+05
108	2.65E+05
120	3.02E+05

Figure 2-6. Cell densities of *S. dysenteriae* 12037 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

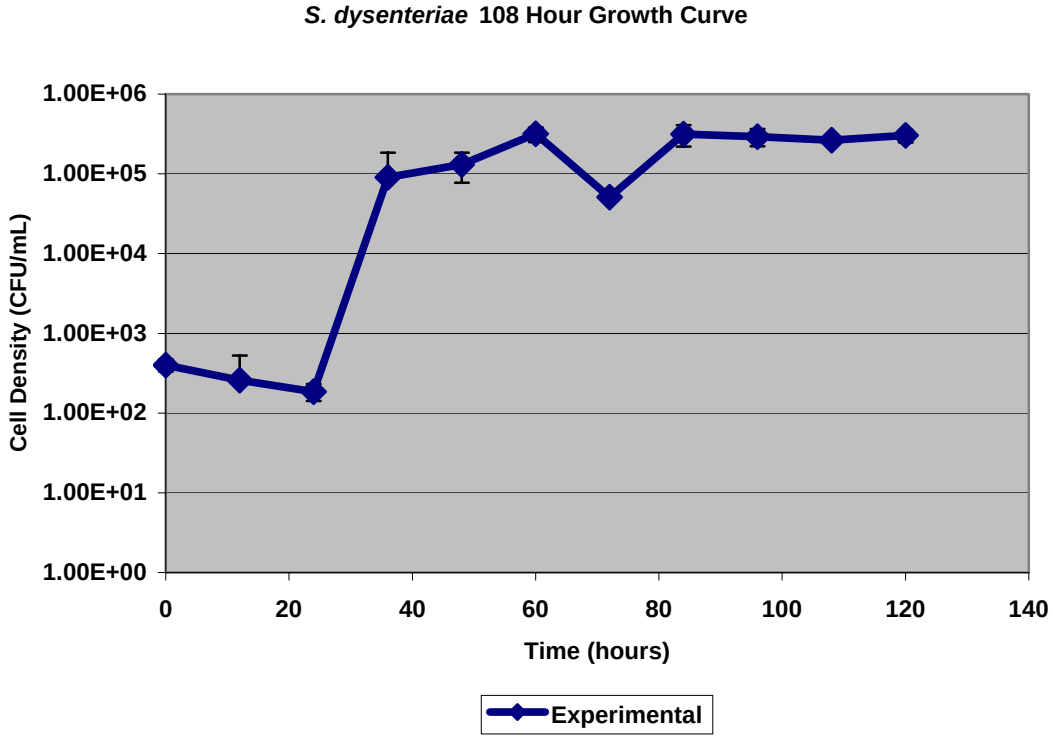


Table 2-7. Cell densities of *V. parahaemolyticus* 17802 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Baseline	Positive Control	Experimental
0	1.00E+00	1.00E+00	9.00E+00
24	1.00E+00	1.00E+00	1.00E+00
48	1.00E+00	1.00E+00	1.00E+00

Figure 2-7. Cell densities of *V. parahaemolyticus* 17802 for the Baseline, Positive Control, and Experimental flasks at various time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

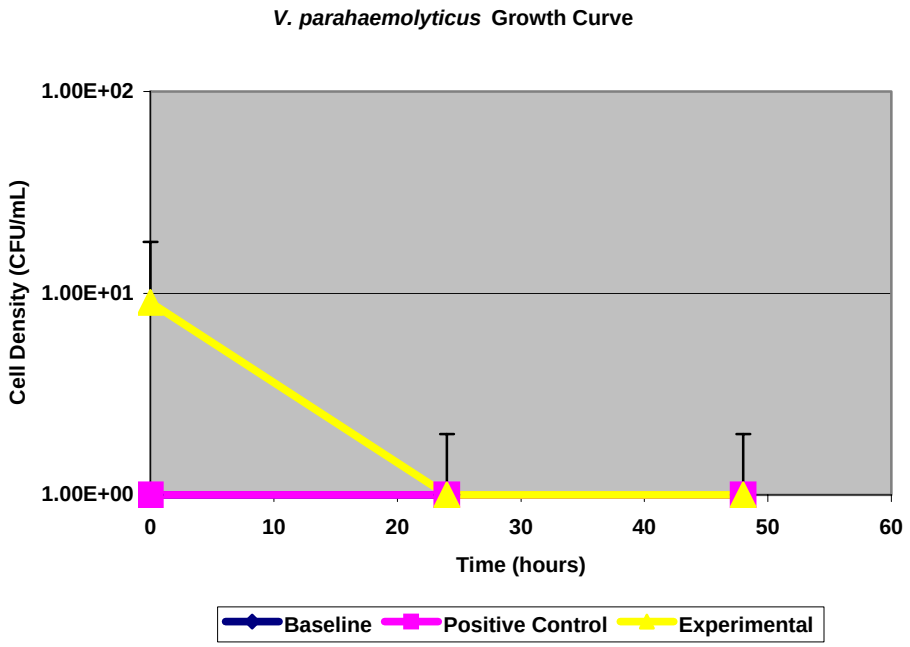


Table 2-8. Cell densities of *V. parahaemolyticus* 17802 for the Experimental flasks at all time points. Results are given in Colony Forming Units per mL (CFU/mL) and are the mean of three replicates.

Time	Experimental
0	9.00E+00
12	1.00E+00
24	1.00E+00
36	1.00E+00
48	1.00E+00

Study 3: J-00031444

Introduction
The purpose of this test was to evaluate the affinity of *Escherichia coli (E. coli)* and a known waterborne pathogen to attach to the sand and silt of a specific Harris County detention basin using sterilized sewage treatment plant effluent.

Samples
NW Sand and NW Silt representing samples from the Northwest side of the detention basin.
SE Sand and SE Silt representing samples from the Southeast side of the detention basin.

Materials
No. 10 sieve
No. 230 sieve
E. coli solution at a concentration of ~100,000mpn/100ml
Shigella dysenteriae solution at a concentration of ~100,000mpn/100ml
Buffered water
Rotary shaker
Autoclave
Laboratory oven
Sterilized effluent- BOD measured at 2.5 mg/L, not adjusted due to lower than expected level

Methods

- Harris County collected the sediment samples and sent to NSF
- NSF collected sterilized sewage treatment plant effluent
- Fractionated samples sterilized by autoclaving then dried
- *E. coli* (ATCC 11229) addition at a concentration of ~10^5 CFU/100 mL
- *Shigella dysenteriae* (ATCC 12037) addition at a concentration of ~10^5 CFU/100 mL
- The following samples were set up on a rotary shaker at 25 rpm and 20-25^{o}C (68-77^{o}F) and enumerated separately per organism at 0 and 1 hour of exposure
    - Experimental (organism spiked) groups:
        - Northwest basin sand and effluent– 3 replicates
        - Northwest basin silt and effluent – 3 replicates
        - Southeast basin sand and effluent – 3 replicates
        - Southeast basin silt and effluent – 3 replicate
    - Negative controls
        - Sterilized sand and effluent – 3 replicates
        - Sterilized silt and effluent – 3 replicates
    - Background Sample –
        - *E. coli* (ATCC 11229) and sterilized effluent– 3 replicates

        - *Shigella dysenteriae* (ATCC 12037) and sterilized effluent– 3 replicates
- Analyses for *E. coli* ATCC 11229 utilized LES mEndo Agar and were incubated for 24 hours at 35ºC. Analyses for *S. dysenteriae* ATCC 12037 utilized Hektoen Enteric Agar and were incubated for 24-48 hours at 35ºC.

Results

Microbiological results and summary for all samples are available in Tables 3-1 through 3-6. Results for the negative controls were acceptable, indicating that the sterilization by autoclave was effective. All test samples for sand and silt demonstrated less than a 90% bacterial reduction for *E. coli* and *S. dysenteriae*. This was true for samples from both locations. There were no statistically significant differences between the groups for either organism.

Table 3-1. Cell densities of *E. coli* in Sand and Silt samples at two exposure points. Results are given in CFU/100 mL and are the means of three replicates.

	NW Sand	NW Silt	SE Sand	SE Silt	Control
Hour 0	7.30E+02	7.60E+02	7.53E+02	8.65E+02	7.45E+02
Hour 1	7.35E+02	8.13E+02	8.33E+02	7.77E+02	8.95E+02

Table 3-2. Per cent reductions in cell densities of *E. coli*. Reductions are calculated in comparison to the baseline control.

	NW Sand	NW Silt	SE Sand	SE Silt
Hour 0	2.0%	-2.0%	-1.1%	-16.1%
Hour 1	17.9%	9.1%	6.9%	13.2%

Figure 3-1. Cell density of *E. coli* for various soil fractions at times 0 Hour and 1 Hour. Results are given in CFU/mL and are the mean of three replicates.

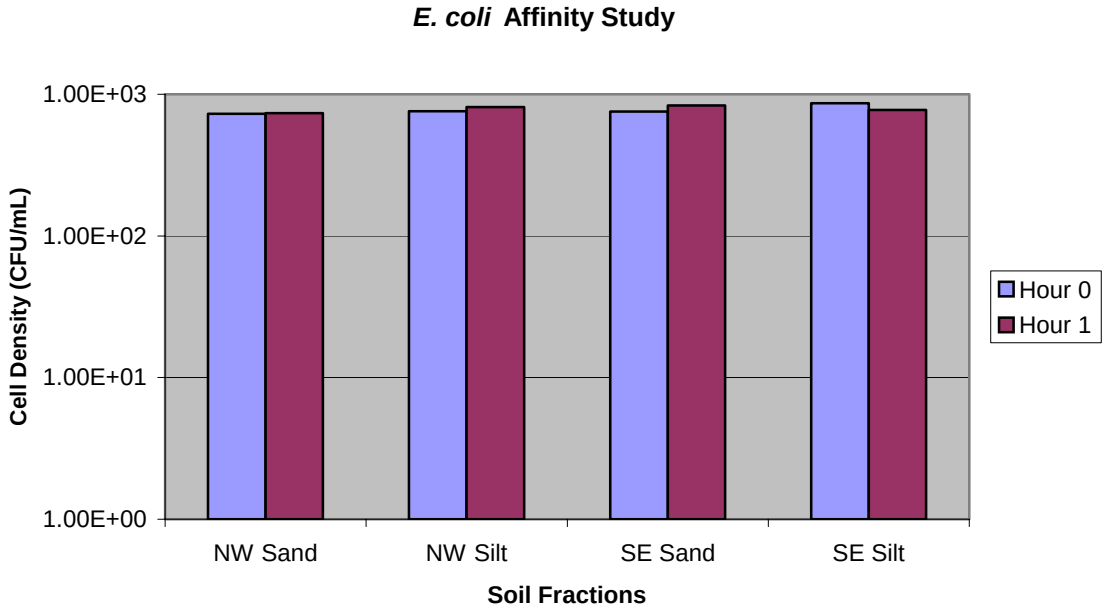


Table 3-3. Cell densities of *S. dysenteriae* in Sand/Silt/Clay samples at two exposure points. Results are given in CFU/100 mL and are the means of three replicates.

	NW Sand	NW Silt	SE Sand	SE Silt	Control
Hour 0	6.55E+02	6.42E+02	6.50E+02	5.93E+02	7.35E+02
Hour 1	5.62E+02	6.15E+02	5.12E+02	6.18E+02	7.65E+02

Table 3-4. Per cent reductions in cell densities of *S. dysenteriae*. Reductions are calculated in comparison to the baseline control.

	NW Sand	NW Silt	SE Sand	SE Silt
Hour 0	10.9%	12.7%	11.6%	19.3%
Hour 1	26.6%	19.6%	33.1%	19.2%

Figure 3-2. Cell density of *S. dysenteriae* for various soil fractions at times 0 Hour and 1 Hour. Results are given in CFU/mL and are the mean of three replicates.

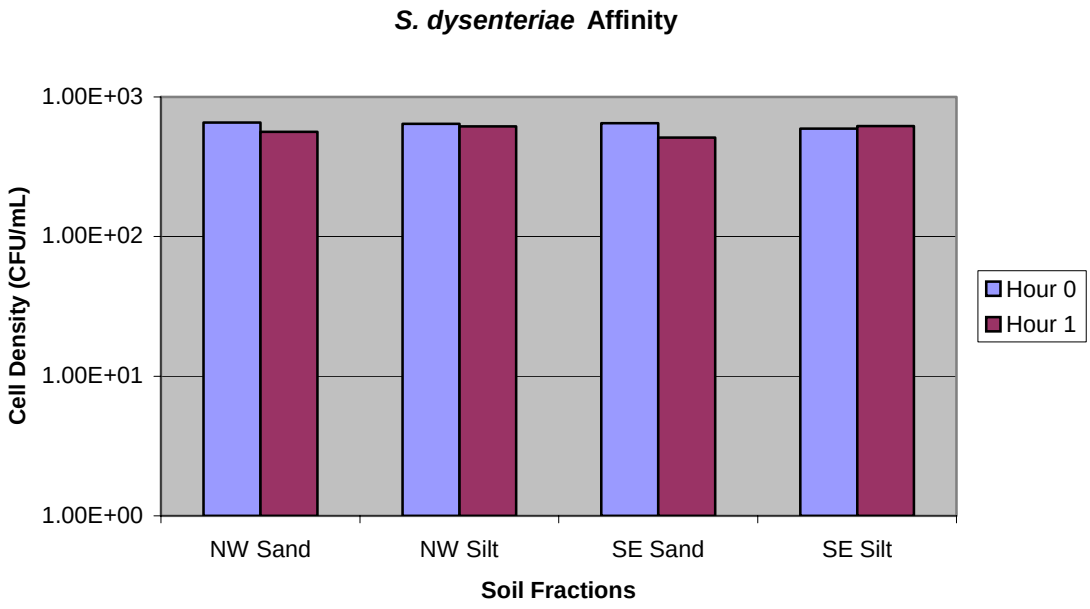


Table 3-5. Single factor ANOVA test- Summary of *E. coli* results from 0 Hour exposure data. Results were determined to be statistically significant between the groups.

Groups	*Count*	*Sum*	*Average*	*Variance*		
NW Sand	6	4380	730	7080		
NW Silt	6	4560	760	13160		
SE Sand	6	4520	753.3333	12066.67		
SE Silt	6	5190	865	32350		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	64812.5	3	21604.17	1.336547	0.290636	3.098393
Within Groups	323283.3	20	16164.17			
Total	388095.8	23				

Table 3-6. Single factor ANOVA test- Summary of *E. coli* results from 1 Hour exposure data. Results were determined to be statistically significant between the groups.

Groups	*Count*	*Sum*	*Average*	*Variance*		
NW Sand	6	4410	735	23150		
NW Silt	6	4880	813.3333	36266.67		
SE Sand	6	5000	833.3333	5026.667		
SE Silt	6	4660	776.6667	21426.67		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	33745.83	3	11248.61	0.523983	0.670767	3.098393
Within Groups	429350	20	21467.5			
Total	463095.8	23				

Study 4: J-00031445

Introduction
The purpose of this test was to evaluate the affinity of *Escherichia coli (E. coli)* to attach to soil fractions collected from a detention basin in Harris County, TX.

Samples
NW Sand/Silt, NW Sand/Silt/Clay, and NW Clay representing samples from the Northwest side of the detention basin.
SE Sand/Silt, SE Sand/Silt/Clay, and SE Clay representing samples from the Southeast side of the detention basin.

Materials
No. 10 sieve
No. 230 sieve
E. coli solution at a concentration of ~100,000mpn/100ml
Buffered water
Rotary shaker
Autoclave
Laboratory oven

Methods
Due to the limited number of vessels that the shaker table can accommodate, the testing of the three sand, silt, and/or clay scenarios was performed in three separate tests.

Study 4a: Sand and Silt Sand, Silt and Clay

- Harris County to collect the sediment samples and send to NSF
- Fractionated samples will be sterilized by autoclaving then dried
- Buffered water, not DI, will be used to cause less shock to cells
- *E. coli* (ATCC11229) addition will be at a concentration of ~10^5 CFU/100 mL
- The following samples were set up on a rotary shaker at 25 rpm and 20-25^{o}C (68-77^{o}F) and enumerated at 0 and 1 hours.
    - Experimental (organism spiked) groups:
        - Northwest basin Sand and Silt – 3 replicates
        - Northwest basin Sand, Silt and Clay – 3 replicates
        - Southeast basin Sand and Silt – 3 replicates
        - Southeast basin Sand, Silt and Clay – 3 replicate
    - Negative controls
        - Buffered Water and Sand and Silt – 3 replicates
        - Buffered Water and Sand, Silt and Clay – 3 replicates
    - Background Sample – E. *coli* (ATCC11229 and buffered water – 3 replicates

Study 4b: Clay

- Harris County to collect the sediment samples and send to NSF
- Fractionated samples will be sterilized by autoclaving then dried
- Buffered water, not DI, will be used to cause less shock to cells
- E. *coli* (ATCC11229) addition will be at a concentration of 10^5
- The following samples were set up on a rotary shaker at 25 rpm and 20-25^{o}C (68-77^{o}F) and enumerated at 0 and 1 hours.
    - Experimental (organism spiked) groups:
        - Northwest basin clay only – 3 replicates
        - Southeast basin clay only – 3 replicates
    - Negative controls
        - Buffered Water and clay – 3 replicates
        - Buffered Water and clay – 3 replicates
    - Background Sample – E. *coli* (ATCC11229) and buffered water – 3 replicates

Study 4c: Sand, Silt, and Clay

- Harris County to collect the sediment samples and send to NSF
- Fractionated samples will be sterilized by autoclaving then dried
- Buffered water, not DI, will be used to cause less shock to cells
- *E*. *coli* (ATCC 11229) addition will be at a concentration of 10^5
- The following samples were set up on a rotary shaker at 25 rpm and 20-25^{o}C (68-77^{o}F) and enumerated at 0 and 1 hours.
    - Experimental (organism spiked) groups:
        - Northwest basin Sand, Silt and Clay – 3 replicates
        - Southeast basin Sand, Silt and Clay – 3 replicate
    - Negative controls
        - Buffered Water and Sand, Silt and Clay – 3 replicates
    - Background Sample – *E*. *coli* (ATCC 11229 and buffered water – 3 replicates

Analyses for *E. coli* ATCC 11229 utilized LES mEndo Agar and were incubated for 24 hours at 35ºC.

Results

Microbiological results and summary for all samples are available in Tables 4-1 through 4-6. Results for the negative controls were acceptable, indicating that the sterilization by autoclave was effective. All test samples for sand/silt, sand/silt/clay, and clay demonstrated less than 90% bacterial reduction. This was true for samples from both locations. There was a statistically significant difference between groups at the 0 Hour time point and the 1 Hour time point.

Table 4-1. Cell densities of *E. coli* in Sand/Silt samples at two exposure points. Results are given in CFU/100 mL and are the means of three replicates.

	NW Sand/Silt	SE Sand/Silt	Control
Hour 0	5.40E+04	4.58E+04	4.61E+04
Hour 1	4.26E+04	3.15E+04	3.48E+04

Table 4-2. Cell densities of *E. coli* in Sand/Silt/Clay samples at two exposure points. Results are given in CFU/100 mL and are the means of three replicates.

	NW Sand/Silt/Clay	SE Sand/Silt/Clay	Control
Hour 0	2.56E+03	1.67E+03	2.56E+03
Hour 1	1.64E+03	1.08E+03	2.17E+03

Table 4-3. Cell densities of *E. coli* in Clay samples at two exposure points. Results are given in CFU/100 mL and are the means of three replicates.

	NW Clay	SE Clay	Control
Hour 0	9.42E+02	9.68E+02	9.90E+02
Hour 1	1.03E+03	7.67E+02	9.13E+02

Figure 4-1. Cell density of *E. coli* for various soil fractions at times 0 Hour and 1 Hour. Results are given in CFU/mL and are the mean of three replicates.

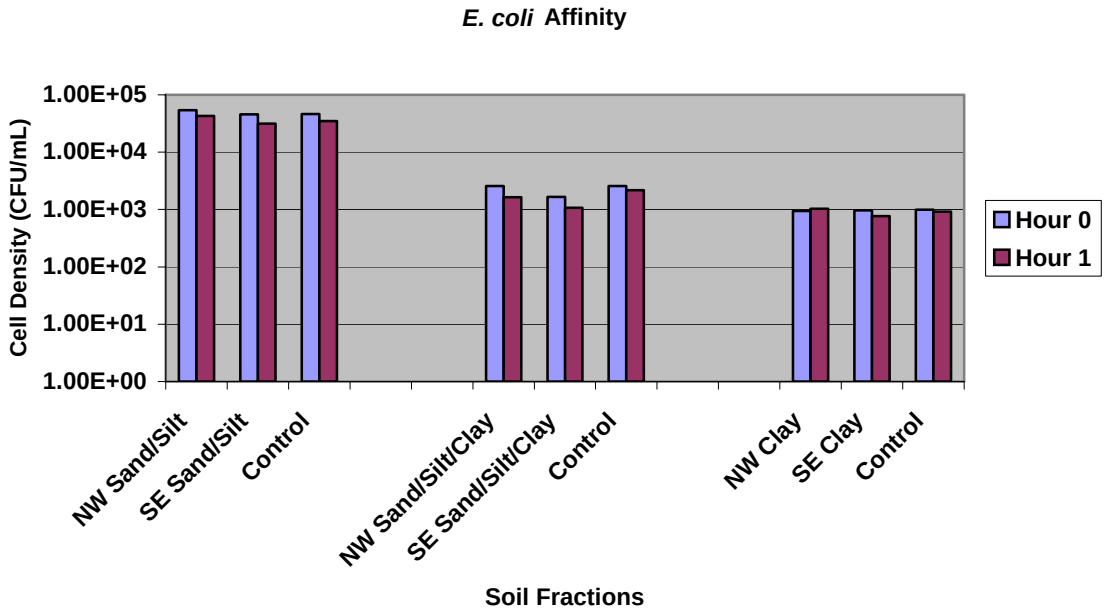


Table 4-4. Per cent reductions in cell densities of *E. coli*. Reductions are calculated in comparison to the baseline control.

	NW Sand/Silt	SE Sand/Silt	NW Sand/Silt/Clay	SE Sand/Silt/Clay	NW Clay	SE Clay
Hour 0	-17.2%	0.7%	0.1%	34.9%	4.9%	2.2%
Hour 1	-22.4%	9.5%	24.3%	50.3%	-12.8%	16.1%

Table 4-5. Single factor ANOVA test- Summary of results from 0 Hour exposure data. Results were determined to be statistically significant between the groups.

Groups	*Count*	*Sum*	*Average*	*Variance*		
NW Sand/Silt	6	324200	54033.33	3.15E+08		
SE Sand/Silt	6	274600	45766.67	1.11E+08		
Background 1	6	276600	46100	1.91E+08		
NW Sand/Silt/Clay	6	15340	2556.667	81026.67		
SE Sand/Silt/Clay	6	10000	1666.667	86306.67		
Background 2	6	15350	2558.333	34336.67		
NW Clay	6	5650	941.6667	28416.67		
SE Clay	6	5810	968.3333	30856.67		
Background 3	6	5940	990	10560		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	2.68E+10	8	3.35E+09	48.82899	1.18E-19	2.152134
Within Groups	3.09E+09	45	68635649			
Total	2.99E+10	53				

Table 4-6. Single factor ANOVA test- Summary of results from 1 Hour exposure data. Results were determined to be statistically significant between the groups.

Groups	*Count*	*Sum*	*Average*	*Variance*		
NW Sand/Silt	6	255800	42633.33	2.18E+08		
SE Sand/Silt	6	189200	31533.33	1.01E+08		
Background 1	6	209000	34833.33	57222667		
NW Sand/Silt/Clay	6	9850	1641.667	23456.67		
SE Sand/Silt/Clay	6	6460	1076.667	90506.67		
Background 2	6	13010	2168.333	224696.7		
NW Clay	6	6180	1030	19680		
SE Clay	6	4600	766.6667	9986.667		
Background 3	6	5480	913.3333	95706.67		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1.52E+10	8	1.89E+09	45.31396	5.15E-19	2.152134
Within Groups	1.88E+09	45	41805337			
Total	1.7E+10	53				