WWTP Effluent-borne Pathogen Regrowth Potential and Sediment Attachment Study

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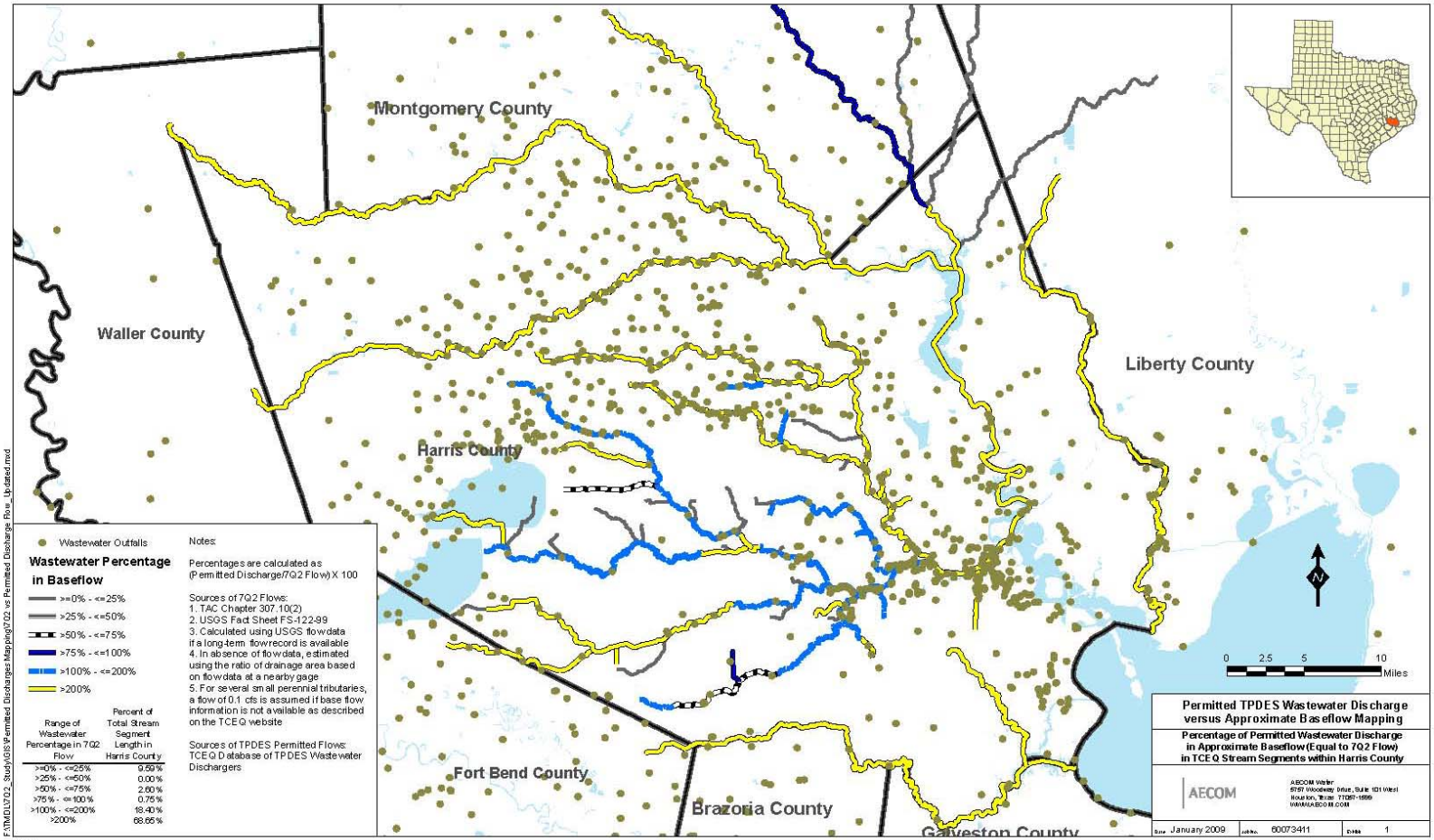
Harris County Texas



Background – Regrowth Potential

- Almost every stream in Harris County is listed by the EPA as “impaired” due to high bacteria (*E. coli*) levels.
- Data shows that adding up bacteria loads from all the sources of flow into the stream didn't come close to approximating the amount of bacteria that we see in the stream. In fact, when Harris County added bacteria loads, only 2-5% of the bacteria in the stream could be accounted for.
- In previous Harris County studies, high levels of *E. coli* were detected coming from sources that had no link to animal wastes. Among the highest numbers seen were in storm water runoff from mature pine forests. The County has documented high levels of bacteria from fresh water leaks, vehicle wash water, plant nurseries, ground water, and other generally-allowable sources. It appeared that *E. coli* would grow wherever there was a nutrient-rich, warm, moist environment.
- This contrasts sharply with the long-held view – still held by some – that *E. coli* and other indicator bacteria could not survive for long outside the host body.

Background – Regrowth Potential

- Harris County has 500 or so municipal wastewater treatment plants, so almost all streams are effluent-dominated. There was evidence to suggest that something in wastewater effluent might be causing bacteria to grow in the stream. This would help explain the shortfall between the bacteria in the stream and the loading into streams.
- Texas requires no nutrient limitations in its wastewater discharge permits. Limited sampling data from these plants indicates that they often discharge high levels of phosphorus and nitrogen.
- Harris County asked NSF to test the ability of *E. coli* and a few other pathogenic bacteria to grow in wastewater effluent.

Background – Sediment Attachment

- EPA issued Harris County a permit to discharge stormwater lakes and streams (MS4 permit). This MS4 permit required us to reduce pollutants running off from developed areas. The County addressed this requirement by requiring developers to install BMPs, such as detention basins, oil/grit separators, and grassy swales. Because it is the simplest and cheapest option, developers install detention basins designed to slowly discharge in 24-48 hours until completely dry. The County was interested in studying how effective this BMP was at reducing bacteria levels.
- County assumptions: sand in water settles at a rate of 1 foot per minute, silt at 1 foot per hour, and clay at 1 foot per day. Therefore detention of stormwater for 24 hours would result in most of the clay being discharged, while all of the sand and most of the silt would settle out.

Background – Sediment Attachment

- Since bacteria tends to attach to soil particles, allowing sand and silt to settle should reduce bacteria. However, if the bacteria attached to clay, or behaved like clay by not settling rapidly, was the water being detained long enough?
- The County developed a method to separate out sand, silt and clay from sediment samples taken from detention basins. The County put samples of the sand, silt, or clay into water and spiked it with bacteria, testing it immediately and at one hour after settling.
- Results indicated that by allowing sand and silt to settle out for at least one hour, good reduction of bacteria was achieved However, samples with clay showed little reduction or even regrowth of bacteria, suggesting that the clay particles provided substrate or even nutrients that the bacteria could use in survive.

Study Goals

- To determine if bacterial pathogens could utilize the inorganic and organic constituents present in the effluent water and basin soils to support metabolism and cell growth.
- To assess the extent of attachment of the pathogens to the soil types found in the basins.
- To shed some light on the potential environmental fate of a pathogen released into these types of waste/runoff detention systems.

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Study 1- Effluent Regrowth

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
  Sterilized
  De-chlorinated
  BOD measured at 2.5 mg/L, not adjusted due to lower than expected level
- Organisms inoculated at a concentration of ~ 20,000 CFU/100 mL each:
  E. coli (ATCC 11229)
  E. coli 0157 (ATCC 43890)
  Shigella dysenteriae (ATCC 12037)
  Vibrio parahaemolyiticus (ATCC 17802)

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Samples

- Sample points:
  - Control flasks: Every 24 hours for 96 hours
  - Test flasks: Every 12 hours for 108 hours

- Control flasks:
  - Negative controls:
    Sterilized effluent, 3 replicates, added carbon source (0.5% glucose)
  - Positive control:
    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

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Shaker Set Up

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Methods

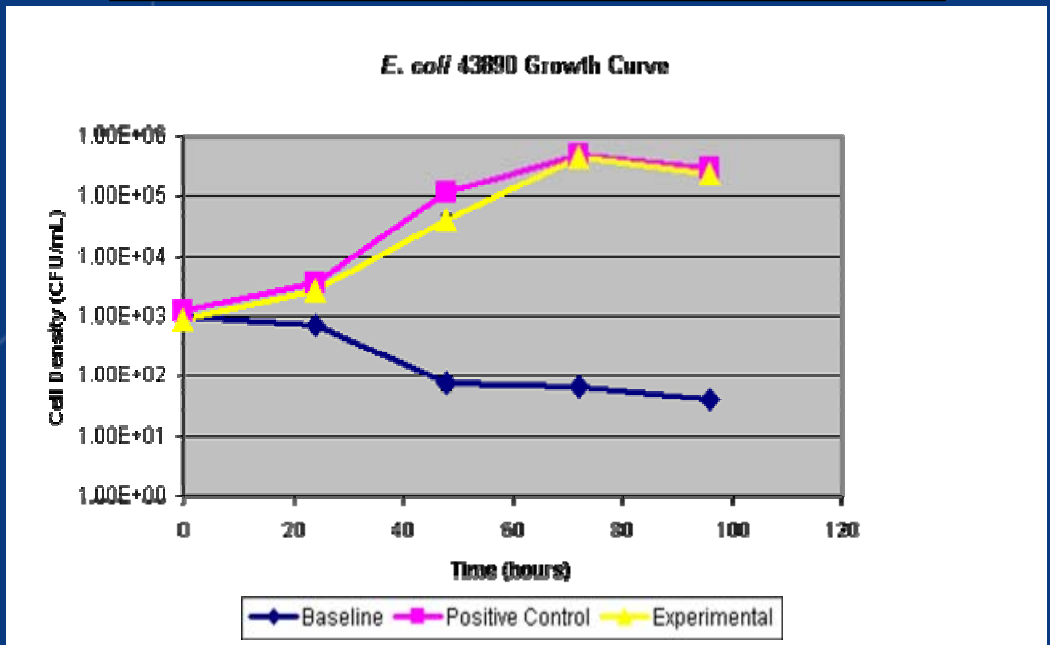
Samples were set up and enumerated separately per organism as follows:

- Add 40 mL of 100,000 CFU/100 mL organism to 160 mL of effluent or buffered water
- Place on rotary shaker at 25 rpm to provide slight continuous movement at 20-25ºC (68-77ºF)
- Sample, dilute and spread plate in duplicate
  - Growth media and conditions (used for all subsequent studies as well)
    - *E. coli* ATCC 43890 - Sorbitol Mackonkey Agar, 24 hours at 35ºC
    - *E. coli* ATCC 11229 - LES mEndo Agar, 24 hours at 35ºC
    - *S. dysenteriae* ATCC 12037- Hektoen Enteric Agar, 24-48 hours at 35ºC
    - *V. parahaemolyticus* ATCC 17802 - TCBS Agar, 48 hours at 35ºC




E. Coli ATCC 43890 Results

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



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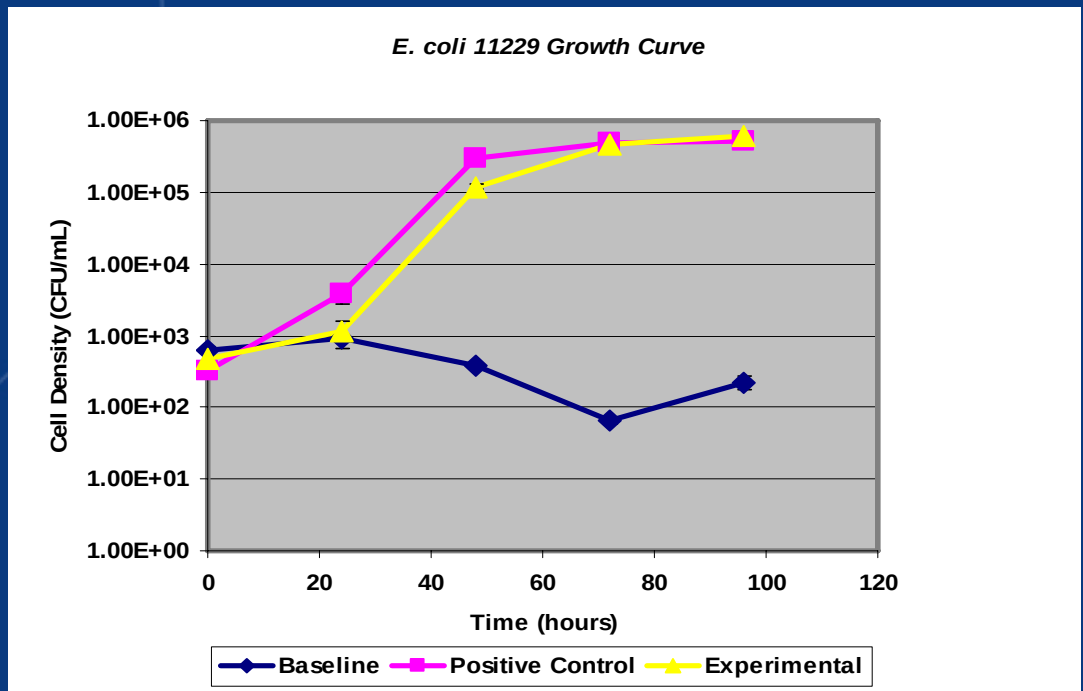
E. Coli ATCC 11229 Results

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



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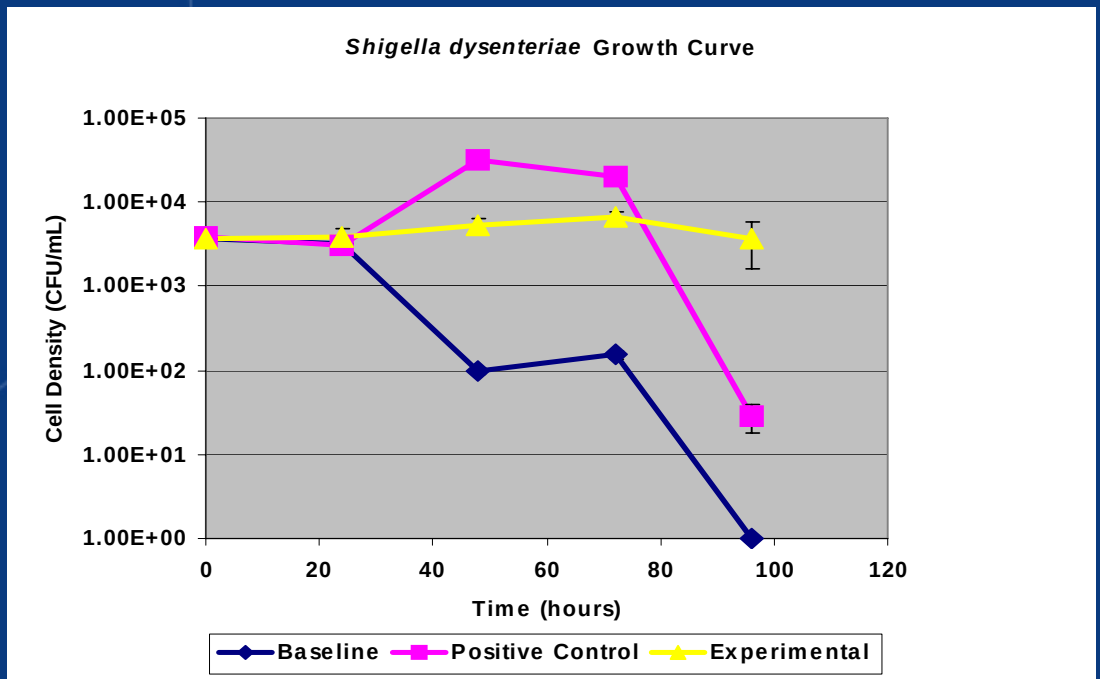
S. dysenteriae ATCC 12037 Results

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



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Results

- *E. coli* 43890:
  - 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:
  - 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:
  - 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:
  - No statistical analyses were performed due to non-detect results.

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Study 2 - Sediment Substrate Regrowth

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 inoculated at ~20,000 CFU/100 mL each:
  - *E. coli* (ATCC 11229)
  - *E. coli* 0157 (ATCC 43890)
  - *Shigella dysenteriae* (ATCC 12037)
  - *Vibrio parahaemolyiticus* (ATCC 17802)

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Samples

- Sample points:
  Control flasks: Every 24 hours for 108 hours
  Test flasks: Every 12 hours for 108 hours

- Control flasks:
  Negative controls: Sterilized sediment, 3 replicates, added carbon source (0.5% glucose)
  Positive control: 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

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Methods

Samples were set up and enumerated separately per organism as follows:

- Add 40 mL of 100,000 CFU/100 mL organism to 160mL of buffered water with ~1 tablespoon sediment in triplicate flasks
- Place on rotary shaker at 25 rpm to provide slight continuous movement at 20-25ºC (68-77ºF)
- Sample, dilute and spread plate in duplicate

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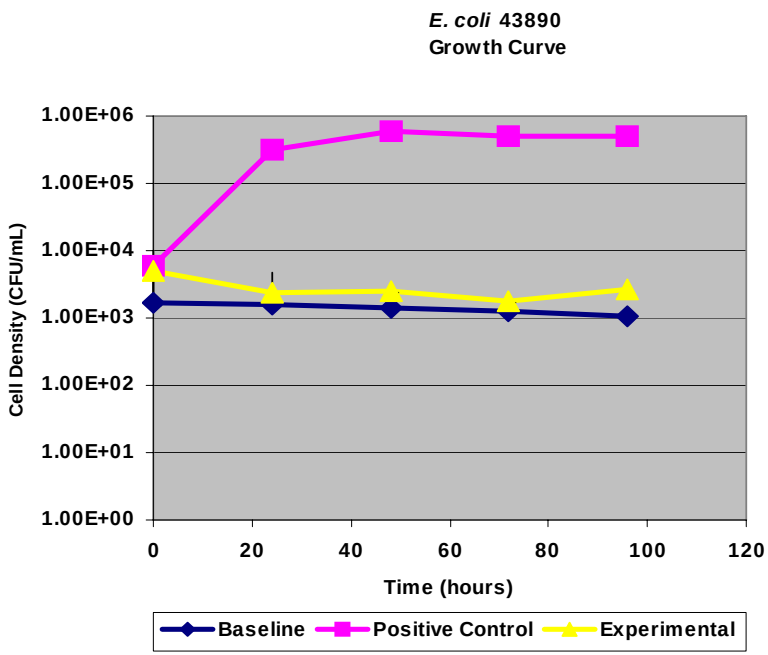
E. Coli ATCC 43890 Results

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



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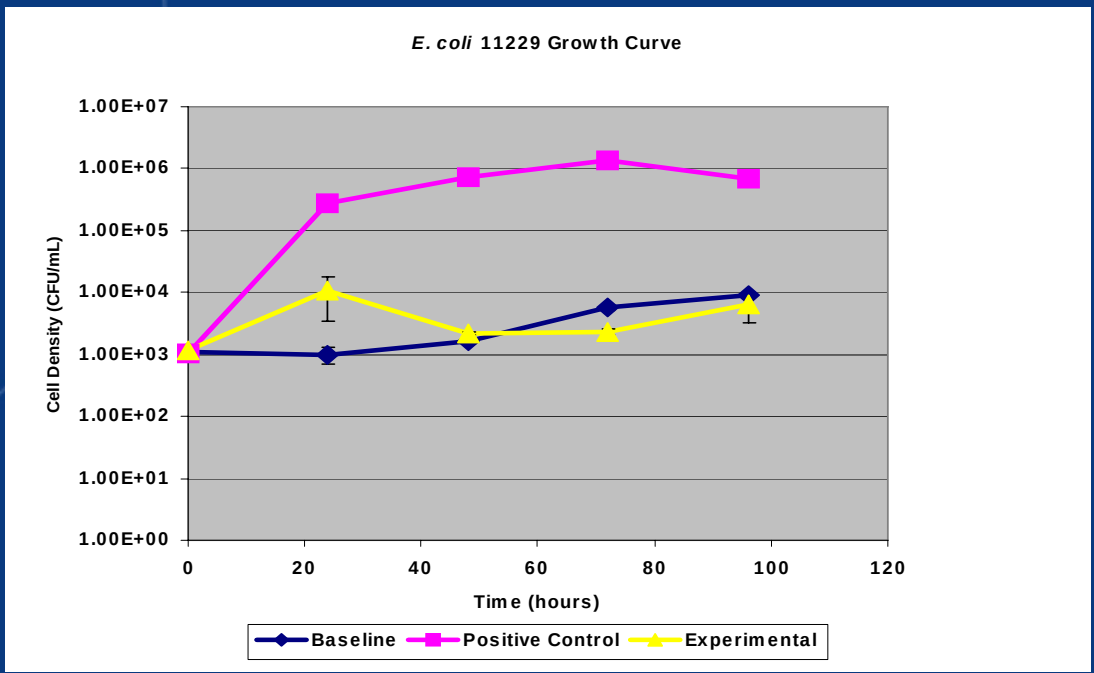
E. Coli ATCC 11229 Results

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



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Results

- *E. coli* 43890:
  - Baseline and experimental groups were similar through out the test.
- *E. coli* 11229:
  - Growth spike (~1 log) was observed at 24 hours but bacterial concentrations returned to levels similar to those of the baseline control for all subsequent points
- *S. dysenteriae* 12037 and *V. parahaemolyticus* 17802:
  - No statistical analyses were performed due to non-detect results or inhibition of growth observed in positive controls.

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Study 3 - *E. coli* Affinity to Sand, Silt, and Clay

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

Materials

- Samples - collected by Harris County personnel
  - 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
  - Sieves - No. 10 and No. 230
  - Buffered water
  - Laboratory oven
  - Sterilized effluent - BOD measured at 2.5 mg/L, not adjusted due to lower than expected level
- Organism solution at a concentration of ~100,000 CFU/100 mL each:
  - *E. coli* (ATCC 11229)

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Experimental Design

Due to space limitations testing was split into 3 groups:

Group 1: Sand/Silt,

Group 2: Sand/Silt/Clay,

Group 3: Clay only

- Experimental (organism spiked) groups:
  - Northwest basin soils from each group
  - Southeast basin soils from each group
- Negative controls
  - Buffered Water plus soil type from each group
- Background Sample
  - E. *coli* (ATCC11229) and buffered water

- Triplicate replicates were performed for all experimental and control groups

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Shaker Set Up

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Methods

- Fractionated samples sterilized by autoclaving then dried in oven
- Samples were set up on a rotary shaker at 25 rpm and 20-25ºC (68-77ºF) and enumerated separately per organism at 0 and 1 hour of exposure
- Sample, dilute and spread plate in duplicate

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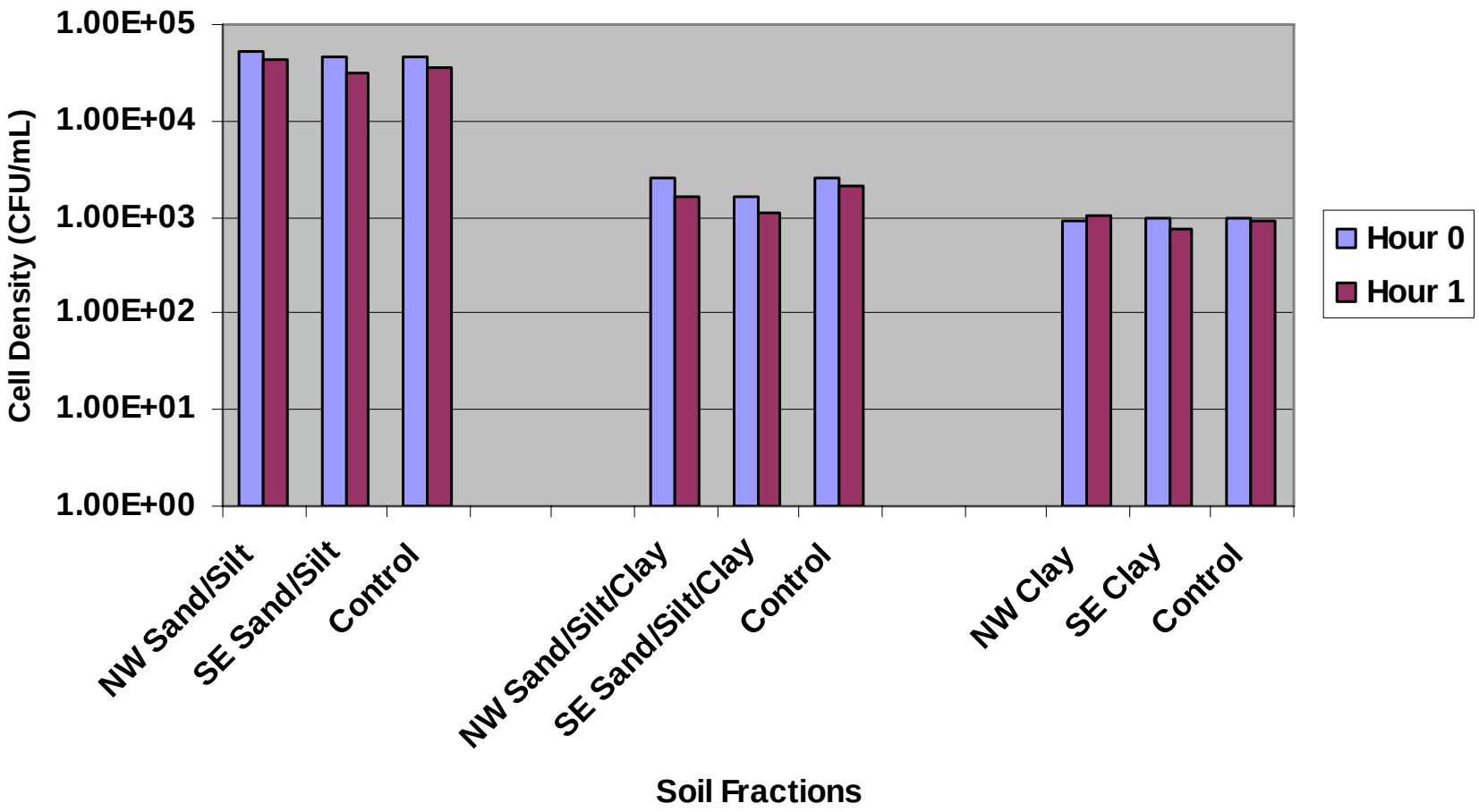
E. coli Affinity



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E. coli Affinity to Sand, Silt, and Clay

Conclusions:

- All test samples for sand/silt, sand/silt/clay, and clay demonstrated less than 90% bacterial reduction over the 1 hour period.
  - 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.
- Greatest difference compared to the control was observed for the sand/silt/clay group

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Conclusions

- In the regrowth study, the sewage water effluent displayed significant increases for *Shigella* and *E. coli* over the course of the study.
  - indicates that the effluent plant water did possess growth factors or carbon sources and nutrients conducive for supporting organism growth.
- These studies indicate that sediment type does have a minor effect on attachment of bacteria.
- This further suggests that over long term detention times may foster bacterial growth.

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Conclusions

- *E. coli* is limited as an indicator organism, at least in a sub-tropical climate like Houston.
- Based on the results of the study, Harris County has been lobbying to revise Total Maximum Daily Load allocations from WWTPs, and to have Texas follow the lead of other states to include nutrient limits in WWTP discharge permits.
- Harris County is continuing research efforts with NSF to test disinfection technologies that reduce bacteria regrowth. The County researching the life history of *E. coli*, how it lives, dies, what makes it grow.
- Future research needs to be done on finding a better indicator organism or suite of organisms, determining what is the limiting nutrient for bacterial growth in this area.

Conclusions

Link to complete study report:

http://pno.hcpid.org/research/research.htm

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Questions & Answers

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