



ENGINEERING & RESEARCH SERVICES

FINAL REPORT

RESEARCH STUDY:

A Measure of the Affinity of Escherichia coli (E. Coli)

to Attach to Sand & Clay

by

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Work Completed under Purchase Order # P098091

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, 2006

Introduction

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

Samples

KM-367-G – representing a sample from the Northwest side of the detention basin.

A-519-02 – representing a sample 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

The testing was performed according to the Test Plan attached as Appendix A.

Results

Microbiological results and summary for all samples are available in Tables 1-5. Results for the negative controls were acceptable, indicating that the sterilization by autoclave was effective. All test samples for sand and silt at both locations demonstrated less than a 1 log bacterial reduction. There was a statistically significant difference between the reduction seen from the A-512-02 Sand when compared to the KM-367-G Sand and to the KM-367-G Silt. Also, there was a statistically significant difference between the KM-367-G Sand and the Untreated Control. All other comparisons between location and soil types were not significant. The slight log reduction seen in the untreated control (0.07) may have been to settling or die off, despite the use of buffered water.

Table 1. KM-367-G (Northwest) Results

| Sample # | Sample Name | Sample Description | 0 hour | | 1 hour | | Reductions by replicate | |
|----------|-------------------------|--------------------|--------------|--------|--------------|------|-------------------------|-------------|
| | | | Ecoli/100 mL | | Ecoli/100 mL | | Log Reduction | % Reduction |
| | Northwest | | | | | | | |
| 1 | Sand #1 | Bacteria + water | 9.8E+04 | + sand | 2.5E+04 | 0.59 | 74.5% | |
| 2 | Sand #2 | Bacteria + water | 5.4E+04 | + sand | 1.9E+04 | 0.45 | 64.8% | |
| 3 | Sand #3 | Bacteria + water | 8.7E+04 | + sand | 1.2E+04 | 0.86 | 86.2% | |
| 4 | Silt #1 | Bacteria + water | 7.5E+04 | + silt | 1.0E+04 | 0.88 | 86.7% | |
| 5 | Silt #2 | Bacteria + water | 8.4E+04 | + silt | 1.7E+04 | 0.69 | 79.8% | |
| 6 | Silt #3 | Bacteria + water | 7.6E+04 | + silt | 3.6E+04 | 0.32 | 52.6% | |
| 7 | Negative Control – Sand | Water | <1 | + sand | <1 | - | - | |
| 8 | Negative Control – Silt | Water | <1 | + silt | <1 | - | - | |
| 9 | Untreated Control | Bacteria + water | 8.0E+04 | ---- | 6.5E+04 | 0.09 | 18.8% | |

Table 2. A-519-02 (Southeast) Results

| Sample # | Sample Name | Sample Description | 0 hour | | 1 hour | | Reductions by replicate | |
|----------|-------------------------|----------------------------------------|--------------|--------|--------------|------|-------------------------|-------------|
| | | | Ecoli/100 mL | | Ecoli/100 mL | | Log Reduction | % Reduction |
| | Southeast | | | | | | | |
| 1 | Sand #1 | Bacteria + water | 7.3E+04 | + sand | 4.9E+04 | 0.17 | 32.9% | |
| 2 | Sand #2 | Bacteria + water | 8.4E+04 | + sand | 5.1E+04 | 0.22 | 39.3% | |
| 3 | Sand #3 | Bacteria + water | 8.2E+04 | + sand | 6.7E+04 | 0.09 | 18.3% | |
| 4 | Silt #1 | Bacteria + water | 6.7E+04 | + silt | 4.0E+04 | 0.22 | 40.3% | |
| 5 | Silt #2 | Bacteria + water | 8.5E+04 | + silt | 3.0E+04 | 0.45 | 64.7% | |
| 6 | Silt #3 | Bacteria + water | 7.9E+04 | + silt | 2.8E+04 | 0.45 | 64.6% | |
| 7 | Negative Control – Sand | Water (Negative Control) | <1 | + sand | <1 | - | - | |
| 8 | Negative Control – Silt | Water (Negative Control) | <1 | +silt | <1 | - | - | |
| 9 | Untreated Control | Bacteria + water (Positive Control) | 6.5E+04 | ---- | 5.7E+04 | 0.06 | 12.3% | |

Table 3. KM-367-G (Northwest) average reductions

| | KM-367-G Sand | KM-367-G Silt | Combined Water Controls |
|---------------------------|----------------------|----------------------|--------------------------------|
| Replicate #1 | 0.59 | 0.88 | 0.09 |
| Replicate #2 | 0.45 | 0.69 | 0.06 |
| Replicate #3 | 0.86 | 0.32 | |
| Average | 0.64 | 0.63 | 0.07 |
| Standard Deviation | 0.21 | 0.28 | 0.02 |

Table 4. A-519-02 (Southeast) Average reductions

| | A-519-02 Sand | A-519-02 Silt | Combined Water Controls |
|---------------------------|----------------------|----------------------|--------------------------------|
| Replicate #1 | 0.17 | 0.22 | 0.09 |
| Replicate #2 | 0.22 | 0.45 | 0.06 |
| Replicate #3 | 0.09 | 0.45 | |
| Average | 0.16 | 0.38 | 0.07 |
| Standard Deviation | 0.07 | 0.13 | 0.02 |

Table 5. Summary of T-tests. (P value (two tail) at 95% CI (alpha = 0.05))

| | KM-367-G Sand | KM-367-G Silt | A-519-02 Sand | A-519-02 Silt | Water Control |
|--------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| KM-367-G Sand | | | | | |
| KM-367-G Silt | 0.982777182 | | | | |
| A-519-02 Sand | 0.018986893* | 0.047017414* | | | |
| A-519-02 Silt | 0.139502934 | 0.226255442 | 0.063033849 | | |
| Untreated Control | 0.035791823* | 0.076255107 | 0.188274158 | 0.054904765 | |

* *statistically significant difference.*

SUMMARY

The following statements can be made based on the data and observations obtained in the study:

- All sand fractions demonstrated <1 log reduction at both locations.
- All silt fractions demonstrated <1 log reduction at both locations.
- There was statistical significance in log reduction for the following comparisons
 - A-519-02 Sand to KM-367-G Sand
 - A-519-02 Sand to KM-367-G Silt
 - Water Control to KM-367-G Sand

Appendix A – Study Plan

STUDY PLAN

**A Measure of the Affinity of *Escherichia coli* (*E. Coli*)
to Attach to Sand & Clay**

January 9, 2006

**NSF International
789 Dixboro Road
*Ann Arbor, Michigan 48105***

General

The purpose of this test is to evaluate the affinity of *Escherichia coli* (*E. coli*) to attach to sand and clay collected from a detention basin in Harris County, TX.

Test Sample

Southeast Sediment Sample
Northwest Sediment Sample

Sponsor

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

Representative: Trent Martin
(713) 290-3092

Test Facility

NSF International
789 Dixboro Road
Ann Arbor, MI 48105

Project Manager : Nikki Beetsch
Phone: 734-913-5718
Fax: 734-827-6145

Experimental Start Date: January, 2006

Materials

No. 10 sieve

No. 230 sieve

E. coli solution at a concentration of 100,000mpn/100ml

Buffered water

Rotary shaker

Autoclave

Laboratory oven

Method

1. One soil sample from the bottom of detention basins in both northwest and southeast will be gathered by Harris County staff and sent to NSF.
2. NSF technician will dry the sediment samples at 75 C for 24 hours.
3. Fractionate Northwest sediment sample into sand, silt and clay.
 - Screen through NO. 10 sieve to remove organic debris, stones, *etc.*
 - Screen the sample through a NO. 230 sieve (mesh size = 63µm) to separate out the sand.
 - Thoroughly mix the remaining silt/clay mixture with water. Allow silt/clay mixture to settle for one hour. Decant remaining liquid. The settled solids should consist of primarily silt, while the majority of clay is removed with the liquid.
 - Autoclave the sand and silt to eliminate resident bacteria.
4. Prepare bacterial solution with a concentration of 100,000mpn/100ml *E. coli*
 - **Samples 1-6:** Add 40mL bacterial solution and 160 mL of buffered water into each of six 250mL beakers
 - **Samples 7-8 (Negative Controls):** 160 mL buffered water only.

- **Sample 9** (Untreated control): 40mL bacteria solution and 160mL of buffered water.
5. Sample water from each of the beakers and quantify the *E. coli* bacteria for each beaker
 - *(Method: Standard Methods for the Examination of Water and Wastewater 20th Edition, 1998, Method 9222 B. The media used was LES mEndo from BD Biosciences.)*
 6. Addition of sand and silt to beakers
 - Add 15mL (~1 tablespoon) of sand to each of three *E. coli* beakers (Samples 1-3).
 - Add 15mL of silt to each of the remaining three *E. coli* beakers (Samples 4-6).
 - Add 15mL sand to Sample # 7 (Negative Control – sand)
 - Add 15mL silt to Sample #8 (Negative Control – silt)
 - DO NOT ADD sand nor silt to Sample #9 (Untreated control)
 7. Place sample in a rotary shaker for one hour at room temperature.
 8. Let settle for one hour at room temperature.
 9. Sample from each beaker to quantify the bacteria.
 10. Repeat steps 3-10, for Southeast sample.
 11. The following bacterial results will be reported
 - Experimental groups:
 - Northwest basin sand – 3 replicates
 - Northwest basin silt – 3 replicates
 - Southeast basin sand – 3 replicates
 - Southeast basin silt – 3 replicate
 - Negative Controls

- Buffered Water and Sand
 - Northwest
 - Southeast
- Buffered Water and Silt
 - Northwest
 - Southeast
- Untreated control – E. coli and buffered water
 - Northwest
 - Southeast

Reporting

A report will be submitted, including the methods used in the study, and data analysis.

All records that would be required to reconstruct the study and demonstrate protocol compliance will be maintained. All records will be maintained in laboratory notebooks, and NSF Standard Operating Procedures will be used for all laboratory operations.

A final report shall be prepared for the study, which will include the following:

1. The name and address of the test facility and the dates on which the study was initiated and completed.
2. Objectives and procedures from the approved protocol, including any modifications to the original protocol.
3. A description of all circumstances that may have affected the quality or integrity of the data.
4. Methods used in the study.
5. Name of Study Director, and others involved in the study.
6. Summary and analysis of the study data.