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WWTP Effluent-borne Pathogen Regrowth Potential and Sediment Attachment Study

Detention Pond Storm Water and Sediment Effected

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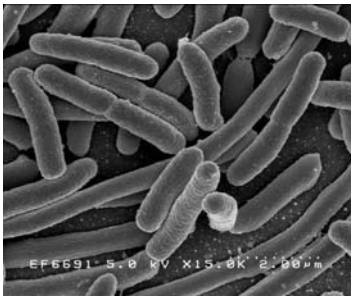

NSF International Engineering Research Services (ERS) was contracted by the Harris County Public Infrastructure Department to perform a series of investigations into the re-growth potential and adherence of waterborne pathogens to sewage treatment plant effluent and detention basin soils found in Harris County, Texas. The studies were performed in 2005 and 2006. The goals of this investigation were twofold: 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; and to assess the extent of attachment of the pathogens to the soil types found in the basins. These studies were developed by ERS and Harris County to shed some light on the potential environmental fate of a pathogen released into these types of waste/runoff detention systems.

Growth studies were carried out using sewage treatment plant effluent and sediment obtained from Harris County, TX as the potential growth substrate. A bench scale experiment was constructed in triplicate that possessed several test groups. The liquid effluent and solid sediment substrates were sterilized via autoclaving to kill any indigenous microbiological flora. Bacterial pathogens (*Escherichia coli* O157:H7 and *Shigella dysenteriae*) and the indicator organism *E. coli* were used as organism spikes for the studies. A starting concentration of 1,000 organisms per mL was targeted. For the effluent water studies, the experimental group possessed flasks with 160 mL of sterile effluent amended with 40 mL of organism challenge in phosphate buffered deionized water. For the experimental groups for the sediment studies, approximately 15 grams of sterile sediment were amended to 160 mL of sterile buffered water and challenge suspension. Negative controls (without challenge organism amendment), baseline controls (buffered water amended with challenge organism but no substrate) and positive controls (substrate plus organism plus 0.5% glucose to serve as a carbon source) were also setup. All flasks were incubated at 20-25°C while placed on a rotary shaker set at 25 rpm for light agitation. Liquid aliquots from the water column were removed for bacterial density determination every

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12 hours and 24 hours, for experimental and control flasks, respectively, over a 108-hour experimental period. Over the course of the studies, only the sewage water effluent displayed a significant difference in the concentrations of both *Shigella* and *E. coli* when compared to the baseline and negative control groups. *E. coli* density in the experimental group increased by a factor of 3 logs over the 108 hour study. *Shigella* increased less than 1 log but the baseline control densities of both challenge organisms decreased more than 1 log over the entire study. This is in contrast to the sediment experimental group where only *Shigella* displayed significantly increased growth (~3 logs) versus the baseline control. *E. coli* 11229 displayed an increase of only ~1 log. This indicated that the effluent plant water did indeed possess growth factors, carbon source and nutrients conducive for supporting the growth of the selected waterborne pathogens. The sediment may have lacked key organic growth constituents to support the growth of *E. coli*.

Attachment studies were also performed using silt, sand and clay from two Harris County detention basins. Soil was gathered by Harris County staff and sent to NSF International where it was dried at 75°C for 24 hours. The sediment samples were fractionated via sieves to separate out the sand (No. 230 sieve, mesh size of 63µm), clay and silt. As with the previous study, all sediment fractions were autoclave sterilized to eliminate the indigenous bacteria. Vessels containing 160 mL of buffered water were used as a basis for all control and experimental groups. The negative sediment control groups possessed only the buffered water and each individual sediment type. The negative organism control possessed only buffered water amended with the challenge organism (*E. coli* at a target concentration of 1,000 CFU/mL). Experimental flasks were setup for each sediment type and possessed 15 grams of the individual sediment in buffered water amended with *E. coli* at the concentration mentioned previously. All flasks were placed on a rotary shaker set at 25 rpm for 1 hour at 20-25°C. The flasks were allowed to settle for an additional hour at room temperature. At time 0 (setup) and at 1 hour post settling, aliquots were removed from the upper portion of the water column in the sample flasks and processed for determination of the challenge organism concentration. The removal/attachment efficiency was calculated by subtracting the log CFU of organism at time 0 by the log CFU observed at 1 hour. Percent removal was also calculated. For the negative organism control, an average of 0.07 log reduction (15%) was observed over the experimental period. This reduction is within the uncertainty of the plating method. Some of the sand samples did show a slightly significant difference when compared to the silt samples and control. The maximum reduction observed was 0.64 log (75%) and this was for a sand sample. Thus from this study it does appear that sediment type does have an effect on attachment of bacteria. This study further may suggest that, over a short term, *E. coli* abundance in storm water can be reduced by detaining runoff long enough for most sand and silt to settle, although longer detention times might actually foster bacterial regrowth.

To conclude, in the growth study the sewage water effluent displayed significant increases for *Shigella* and *E. coli* over the course of the study, indicating that the effluent plant water did possess growth factors or carbon sources and nutrients conducive for supporting their growth. Also of note, in the attachment study larger soil fractions did have an effect on the attachment of *E. coli*.

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