Microbiological Analysis of an Active Pilot-Scale Mobile Bioreactor Treating Organic Contaminants

by

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Submitted to:

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Attention: Dr. Regina S. Porter

Re: STC TASK ORDER 97-003 EVALUATION OF PILOT SCALE MOBILE BIOREACTOR DEMO

Submitted by:

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Introduction

Samples were obtained for microbiological analysis from a granular activated carbon fluidized bed bioreactor (GAC-FBR). This GAC-FBR was in operation at a former manufactured gas plant (MGP) Site in Augusta Georgia for in situ groundwater bioremediation of organics. The samples included contaminated site groundwater, GAC-FBR effluent, and biofilm coated granular activated carbon at 5, 9, and 13 feet within the GAC-FBR column. The objective of this analysis was to correlate contaminant removal with microbiological activity within the GAC-FBR.

Microbiological Analysis

Total bacterial densities were determined by the Acridine Orange (AO) direct count method (Hobbie et al., 1977). Acridine orange binds to nucleic acids with the RNA-AO complex fluorescing orange-red while the DNA-AO complex fluoresces green. The AO method has been used as an indicator of bacteria activity. Bacteria cells prepared this way that are labeled orange-red are considered "active" while those that are labeled green are considered "inactive." This technique stains bacterial RNA and DNA facilitating enumeration by epifluorescent microscopy.

Total viable aerobic bacteria were enumerated using the plate count technique. One percent Peptone-Trypticase-Yeast-extract-Glucose (PTYG) medium was used to culture these bacteria (Balkwill, 1989).

The number of viable aerobic bacteria capable of growth on petroleum as a carbon and energy source were enumerated using a minimal salts growth medium amended with

petroleum (Rodina, 1972). Petroleum fuel contains benzene, toluene, ethylbenzene, xylenes (BTEX), naphthalene and other organic compounds present in these MGP petroleum-contaminated soils (PCS).

Laser scanning confocal microscopy (LSM) was employed to examine the biofilm that developed on the GAC-FBR as previously described (Brigmon et al., 1997). GAC samples from the GAC-FBR were heat fixed on microscope slides and stained with AO. The samples were then examined with LSM.

Results

The bacteria densities inside the GAC-FBR were two orders of magnitude higher than the influent and effluent groundwater for AOs and four orders of magnitude higher for viable plate counts (Table 1). The results are expressed as colony forming units (CFU) gram dry weight^{-1} (gdw) for the carbon and CFU per milliliter (ml) for groundwater. The densities of total bacteria, viable bacteria, and petroleum fuel degraders were not significantly different at the three depths within the GAC-FBR column (Table 1). There were no petroleum fuel degraders detected in the effluent or influent water. Bacteria densities in both influent and effluent were several orders of magnitude less than the GAC-FBR.

While the densities of bacteria were similar in GAC-FBR at the three depths, different morphological characteristics in the GAC-FBR matrix were observed. Figure 1 from the 5 foot depth shows a diverse biofilm with filamentous bacteria. Many protozoans were present in this sample (i.e. large round organisms). Protozoans graze on bacteria. Figure 2 demonstrates a similar biofilm at 9 feet with fewer filamentous bacteria and very few protozoa. At the 13 foot depth of the GAC-FBR primarily bacterial rods were observed (Figure 3). A large percentage of the bacteria stained orange-red relative those

staining green with AO in the 13 foot sample indicating a high level of microbial activity (Figure 3). Total bacteria densities from the GAC-FBR samples were comparable to the number of viable aerobic bacteria. Effluent and influent samples plate counts or viable bacteria were two orders of magnitude lower compared to total counts (Table 1). The lower viablity in the influent could be due to site groundwater conditions. The lower viablity in the effluent could be due to dead or inactive cells sloughing off the biofilm.

Conclusion

The objective of this analysis was to correlate microbiological activity within the GAC-FBR with contaminant removal. Evidence that the process was working included GAC-FBR inlet and outlet concentrations for BTEX and Naphthalene demonstrating successful elimination of these compounds (Haselow, 1997). The microbiological analysis proved the presence of an active biofilm within the GAC-FBR and most importantly the high density of petroleum degraders within the system. The evidence of organic contaminant removal by the GAC-FBR and concomitant presence of specific microbiological activity on GAC suggests contaminant removal is a function of biodegradation. Microbial activity was likely influenced by compounds within the GAC-FBR since the influent groundwater had organic contaminants as well as additional oxygen, phosphate and nitrogen amendments not discussed here.

Note: This work was the result of sampling the GAC-FBR at one time point during operation. In light of these findings an additional short term study is recommended. This new study would determine the most efficient strategy for operation of the GAC-FBR. The questions to be answered would be: 1) what is the influence on the GAC biofilm of flow and or concentration change through the FBR, and 2) what is the stability of the biofilm and biodegradation rates?

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Table 1.

Total counts (AO), total heterotrophic aerobic bacteria (1% PYTG plates) and petroleum degraders.

SAMPLE	Total Counts Cells/gdw* or ml**	Heterotrophs CFU/gdw or ml	Petroleum Degraders CFU/gdw or ml
13 FT	1.47E+08 Cells/gdw	1.05E+08 CFU/gdw	6.21E+07 CFU/gdw
9 FT	4.53E+08 Cells/gdw	1.16E+08 CFU/gdw	3.14E+07 CFU/gdw
5 FT	2.66E+08 Cells/gdw	9.07E+07 CFU/gdw	7.58E+07 CFU/gdw
Influent	1.06E+06 Cells/ml	3.40E+04 CFU/ml	0.00E+00
Effluent	9.18E+06 Cells/ml	3.45E+04 CFU/ml	0.00E+00

*gdw=grams dry weight

**ml=milliliter (groundwater)

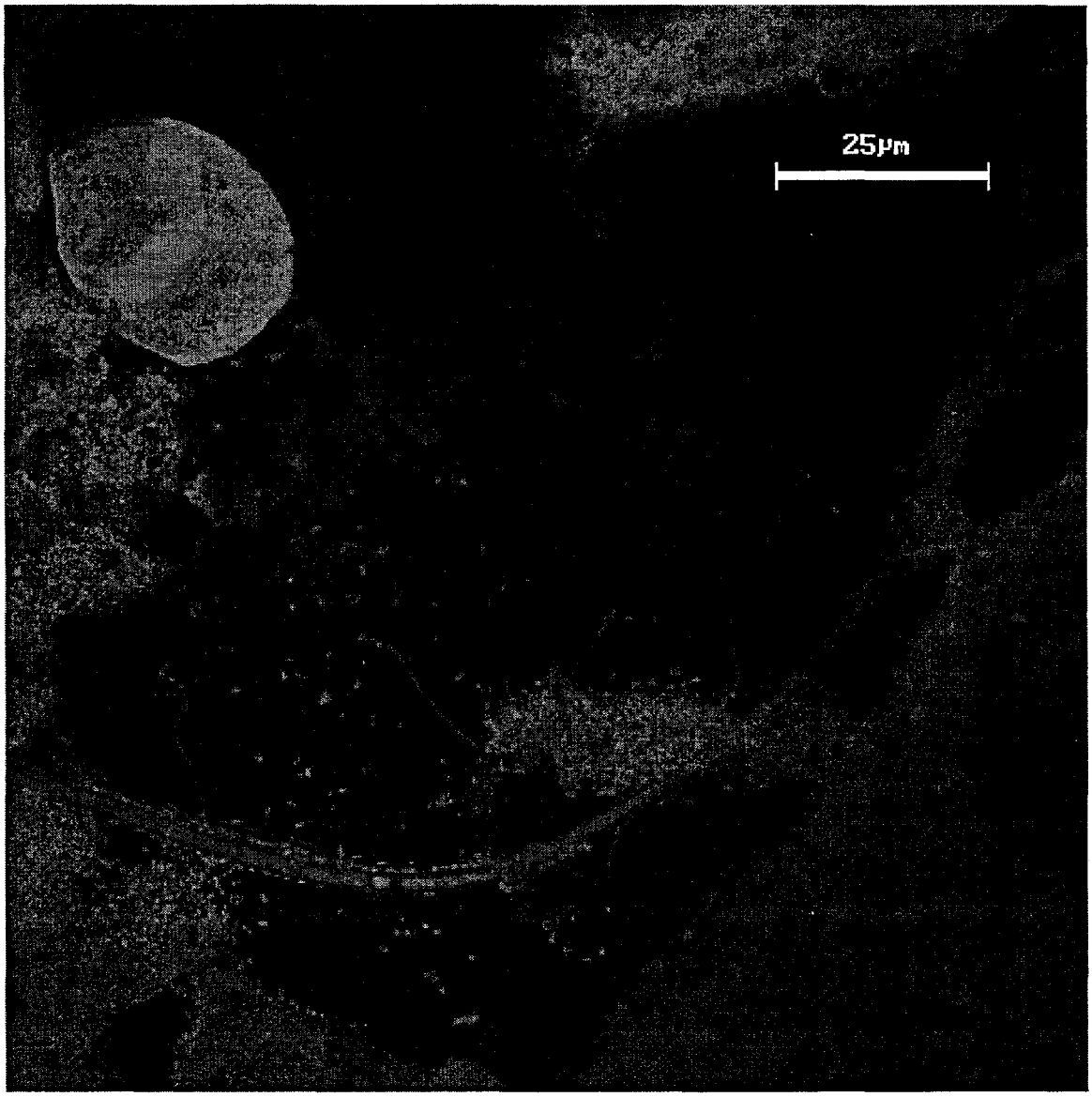

Figure 1. Biofilm from 5 ft depth in GAC-FBR.

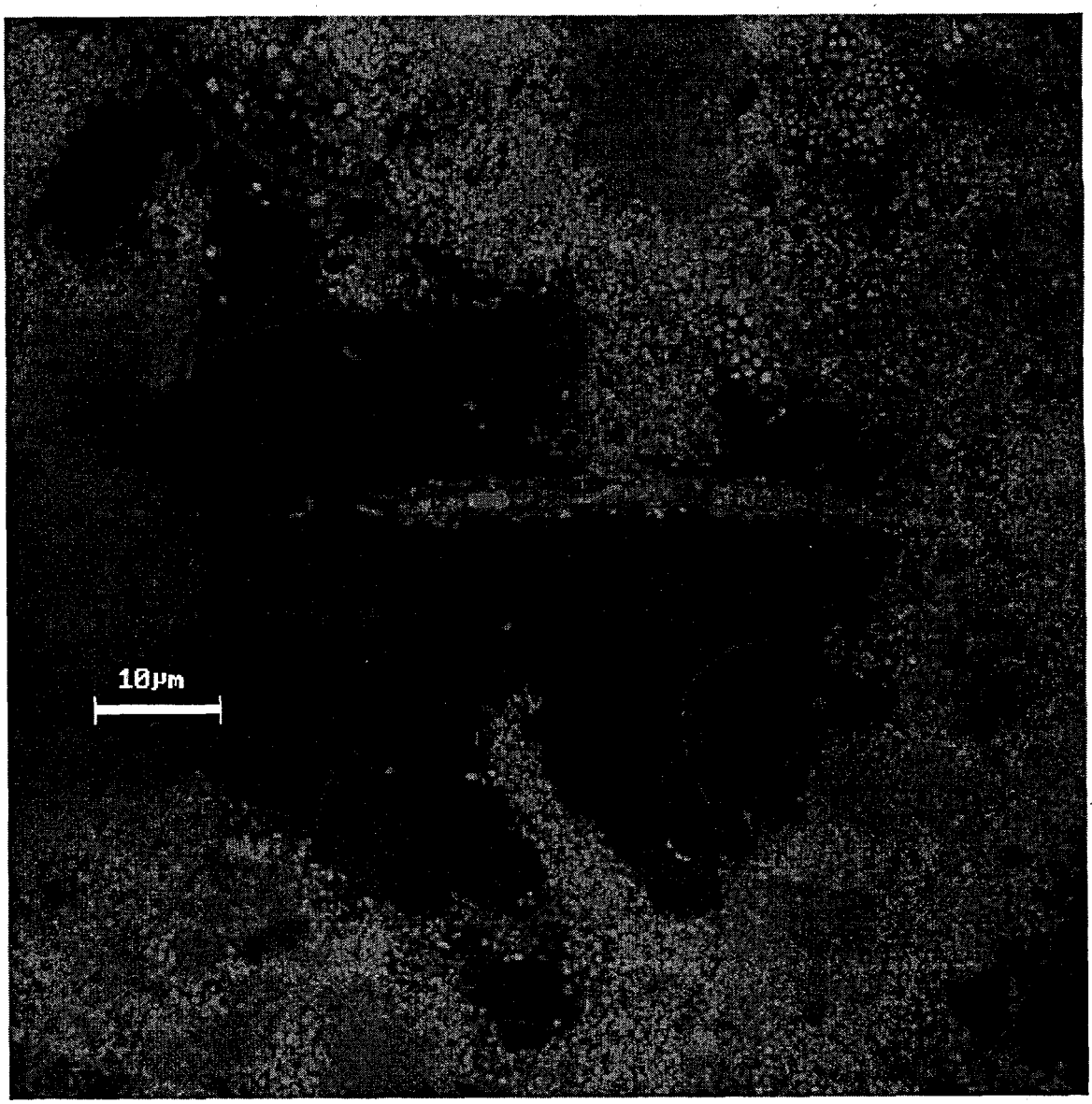

Figure 2. Biofilm from 9 ft depth in GAC-FBR.

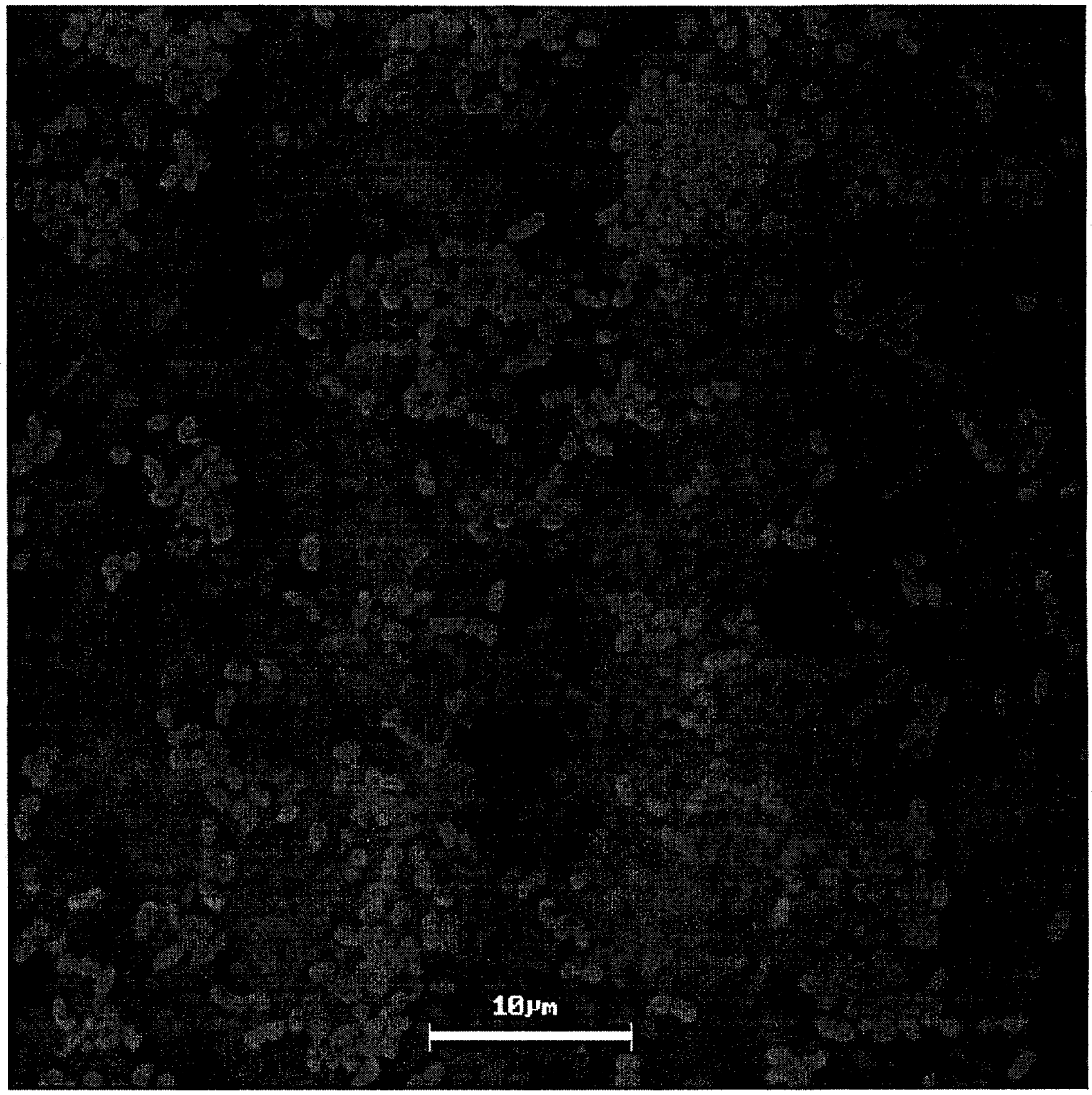

Figure 3. Biofilm from 13 ft depth in GAC-FBR.