Microbiological Analyses of Samples from the H-Area Injection Well Test Site

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**MICROBIOLOGICAL ANALYSES OF SAMPLES FROM
THE H-AREA INJECTION WELL TEST SITE**

E. W. Wilde and M.M. Franck

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Executive Summary

Microbial populations in well water from monitoring wells at the test site were one to three orders of magnitude higher than well water from the Cretaceous aquifer (used as dilution water for the tests) or from a control well adjacent to the test site facility. Coupons samples placed in monitoring and control wells demonstrated progressive adhesion by microbes to materials used in well construction. Samples of material scraped from test well components during abandonment of the test site project revealed the presence of a variety of attached microbes including iron bacteria. Although the injection wells at the actual remediation facility for the F- and H-Area seepage basins remediation project are expected to be subjected to somewhat different conditions (e.g. considerably lower iron concentrations) than was the case at the test site, the potential for microbiologically mediated clogging and fouling within the process should be considered. A sampling program that includes microbiological testing is highly recommended.

Introduction

The H-Area injection Test Site was constructed in 1992 to test the physical capacity of the water aquifer to receive tritiated water from a groundwater remediation facility. The groundwater remediation facility that was partially simulated at the test site involves a pump-treat-inject process that is designed to remove radionuclides and other contaminants from groundwater under the F- and H-Area seepage basins.

Observations of "slimy" material on well components at the test site suggested biological fouling as one of the mechanisms for fouling/clogging of injection and monitoring well components. The SRTC Environmental Biotechnology Section was consulted and asked for assistance in late 1996. Methods and results of the subsequent microbiological tests are described below.

Methods

Sampling Activities

Cretaceous water samples from a deep aquifer below F- and H-Areas were collected 11/21/96 and 2/11/97. These samples were analyzed for total bacteria densities. In addition, the following samples were collected on 3/10/97 before and during disassembly of monitoring well pumping equipment at the test site:

- (1) Well water samples pumped from monitoring wells HIW-5MD, HIW-6MD, HIW-7MD, and control well HSB-133D
- (2) Stagnant pipe water from monitoring well HIW-5MD
- (3) Slurries of material scrapped off well piping diluted with entrapped water from the same well - This was done at HIW-5MD, HIW-6MD, and HIW-7MD.

Also, on 3/10/97, after the well assemblies were removed, coupons samples made of the same material (PVC) as the well casings were attached to string and hung to a depth below the water level in wells HIW-5MD, HIW-6MD, HIW-7MD, and HSB-133D.

Coupons were collected 4/22/97 and 5/6/97 to determine accumulation rates for attached microbes. These samples were diluted in the field with 25 ml of sterile buffer solution.

Lab and Analytical Procedures

Samples were preserved kept refrigerated and chemically preserved when received at the laboratory. Total bacteria densities were determined by performing Acridine orange direct counts (AODC's). Aliquots of water samples were stained with Acridine orange and filtered through 0.2 μm pore size Nucleopore filters. Portions of the filters were examined with a Zeiss Axioskop microscope at 100X magnification and the bacterial quantities were determined by the formula:

$$TC = NC \times EFA \div AC \div QC$$

Where: TC = Total quantity of bacteria per ml

NC = number of cells counted

EFA = the effective filtering area of the filter used

AC = the area of the filter counted

QC = the quantity (ml) of sample filtered

Coupon samples were analyzed quantitatively and qualitatively. Qualitative examinations were performed by preparing slides from scrappings from one edge of each coupon sample. the scrapped material was washed with dilute HCl to remove ferric chloride and counter stained wwith iodine. The slides were then examined at 100X using a Zeiss laser confocal microscope. The material from the remaining portion of each coupon was dislodged in the buffer solution by agitation and processed with Acridine orange as described above for the water samples. Quantities of bacteria per unit of surface area on the coupons was determined by the following formula:

$$TC = NCS \times DF \div AP \div ACS$$

Where TC = total number of cells per unit area of coupon

NCS = number of cells in the coupon scrape/buffer slurry
(determined as above for water samples)

DF = amount of buffer used to house coupon

AP = amount of sample processed for counts, and

ACS= area of coupon scraped for AODC analyses.

Results

Water Samples and scrapings from well materials

The quantities of bacteria in the water samples are shown in Table 1. The largest quantity, over six million per ml, occurred in the stagnant pipe water sample from Well HIW-5MD. However, quantities nearly as high were observed in the sample from Well HIW-7MD. Bacterial quantities in all of the samples taken from monitoring wells were substantially higher than those of control well HSB-133D. The lowest quantities were from the samples of Cretaceous water. These densities were only 10-17% as high as the levels in the control well.

Table 1 Bacteria Quantities in Water samples from the H-Area Injection Test Site.

Sample ID	Date Collected	Type Sample	Total Bacteria per ml	Density relative to control well
H/F Crete.	11/21/96	well water	4.64E+03	0.17X
H/F Crete.	2/11/97	well water (mean)	2.65E+03	0.10X
HSB-133D (Control)	3/10/97	well water	2.65E+04	---
5MD	3/10/97	well water	1.15E+05	4.34X
5MD	3/10/97	pipe water (stagnant)	6.60E+06	24.9X
6MD	3/17?/97	well water	2.22E+05	8.38X
7MD	3/17?/97	well water	5.96E+05	22.5X

Iron bacteria were observed in samples of the material scraped off the well piping from all wells.

Coupon Tests

Results of the coupon tests are summarized in Table 2. Substantial colonization of the coupons by bacteria was evident in all of the samples and an increase in microbes between the two sampling times, 31 days and 43 days after coupon installation, was observed in three out of the four wells sampled.

**Table 2 Bacteria concentrations on coupon samples
(placed in wells 3/10/97).**

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Sample ID	Date Collected	Time in well	Total Bacteria per mm ²
HIW-5MD	4/22/97	31 days	3.7E+04
HIW-5MD	5/6/97	43 days	3.2E+04
HIW-6MD	4/22/97	31 days	2.1E+03
HIW-6MD	5/6/97	43 days	6.9E+03
HIW-7MD	4/22/97	31 days	1.0E+04
HIW-7MD	5/6/97	43 days	1.3E+04
HSB-133D	4/22/97	31 days	1.9E+04
HSB-133D	5/6/97	43 days	6.5E+04

Conclusions

The study revealed that a consortium of bacteria, including iron bacteria are present in the aquifers underlying the F and H-Area seepage basins and that these bacteria are capable of adhering to well components. Coupon tests indicated that the time for bacteria to progressively accumulate on surfaces appears to be relatively short. Thus, despite expected differences between conditions at the test site and those of the actual pump/treat/inject project (e.g. considerably lower iron concentrations), we believe it would be remiss to ignore the possibility that biological materials might interfere with project operation. Microbiological testing could pay large dividends in helping to avoid potential costly problems involving microbiologically mediated clogging/fouling of process equipment