

Analysis of the Microbial Community in Trichloroethene Contaminated Bedrock using Fluorescent *in situ* Hybridization and Flow Cytometry

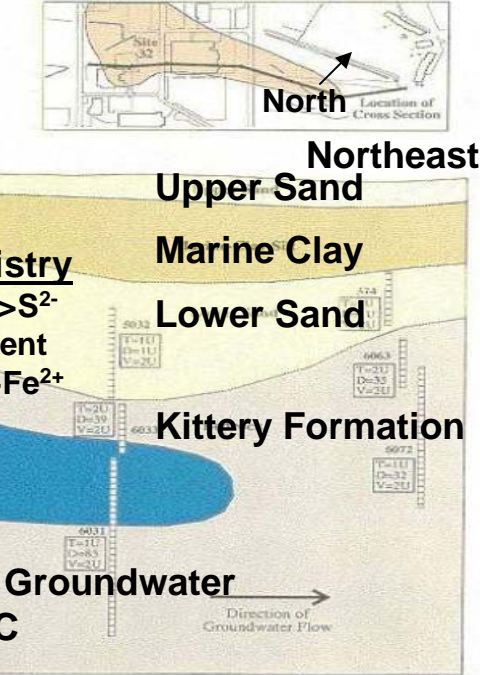
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was used to examine the microbial population (TCE) released from an overflowing (metasandstone) characterized in a bedrock Bioremediation Hampshire. Six reaching a maximum of the presence of *cis*-TCE, *trans*-DCE, suggesting that active *in situ* group- and species-level detection of the presence of TCE and its (M) was coupled with number of cells of enumeration. This structure will be essential shifts when

members of the microbial C boreholes using FISH cytometry.

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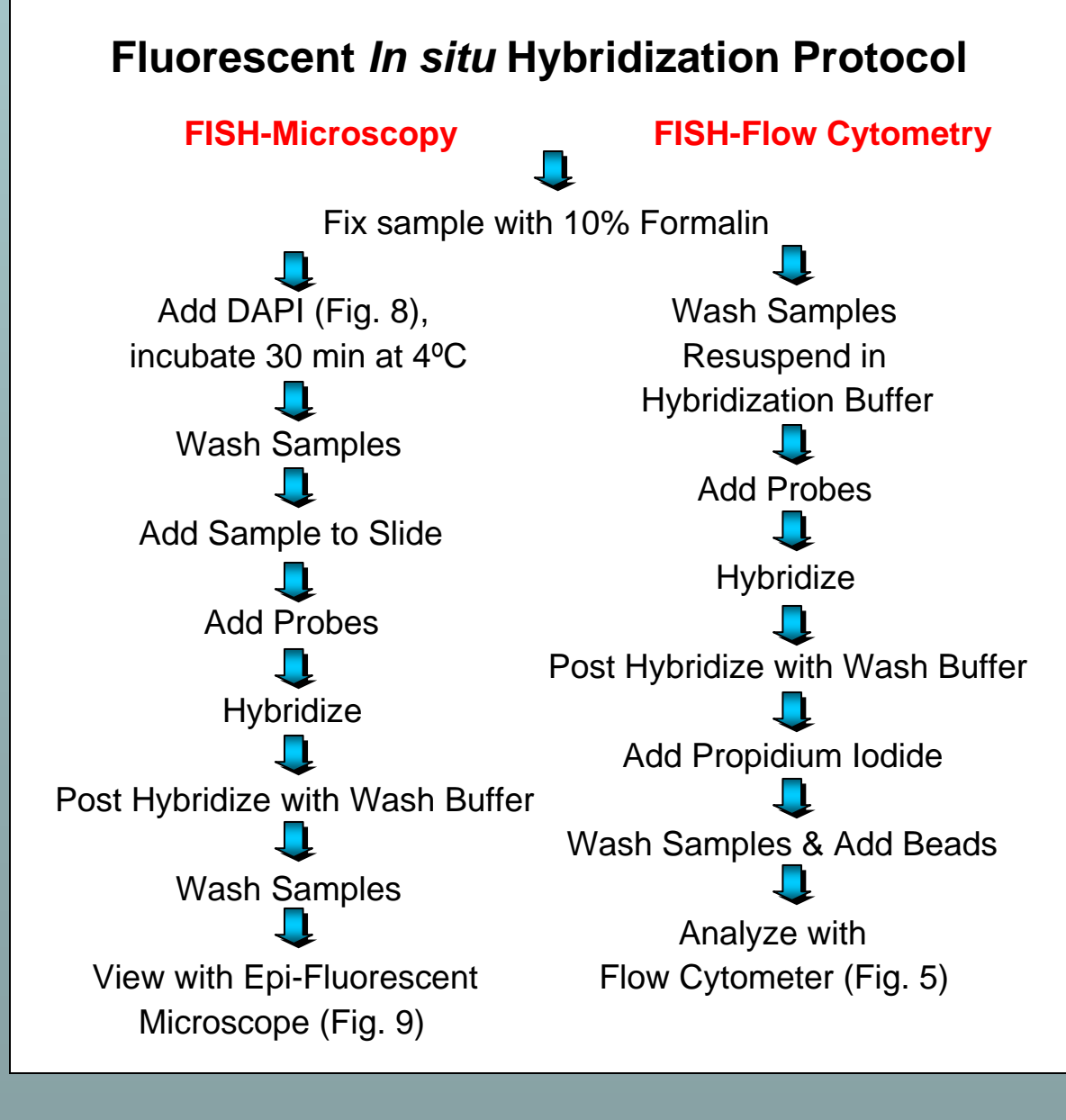


extends 0.5 km into the

Parameters	Units	BBC1				BBC3	BBC5
		13m	15m	27m	30m		
TCE	µg/L	330	200	10	43		
<i>cis</i> -DCE	µg/L	430	330	73	320		
<i>trans</i> -DCE	µg/L	170	110	9	45		
VC	µg/L	31	14	4	14		
ethene	µg/L	32	10	2	11		
SO ₄	mg/L	74	63	150	110		
DO	mg/L	0.7	5	1	2.5		

Table 1: Chemical composition of BBC boreholes.

METHODS



Probe name	Target organism	Reference
EUB338	<i>Eubacteria</i>	1
ARCH915	<i>Archaea</i>	3
SRB385Db	<i>Desulfobacteriaceae</i>	2

Table 2: Oligonucleotide Probes

RESULTS

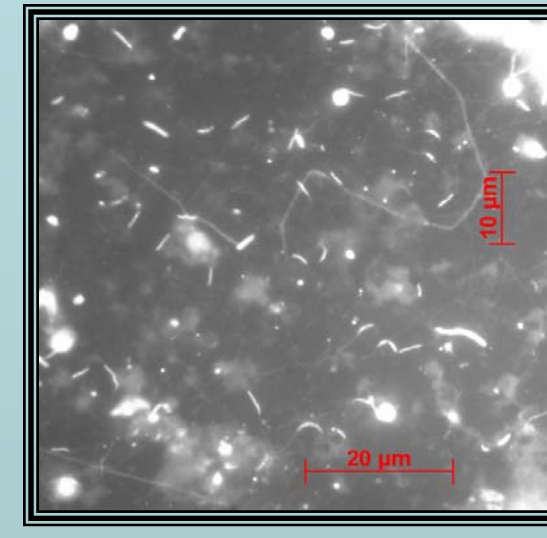


Figure 2. BBC groundwater sample stained with DAPI, a general DNA stain and is used to determine total cell counts.

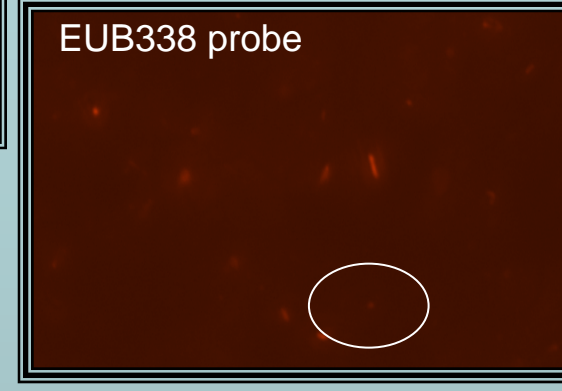
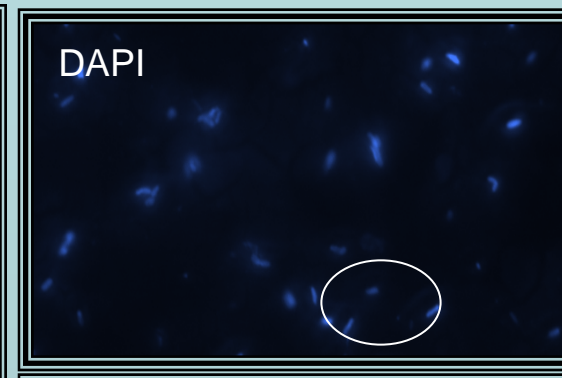


Figure 3. Visualization of a groundwater sample (1000X) taken from BBC5 at a depth of 30m. Comparison of total cells stained by DAPI with cells hybridized to the Eubacterial probe.

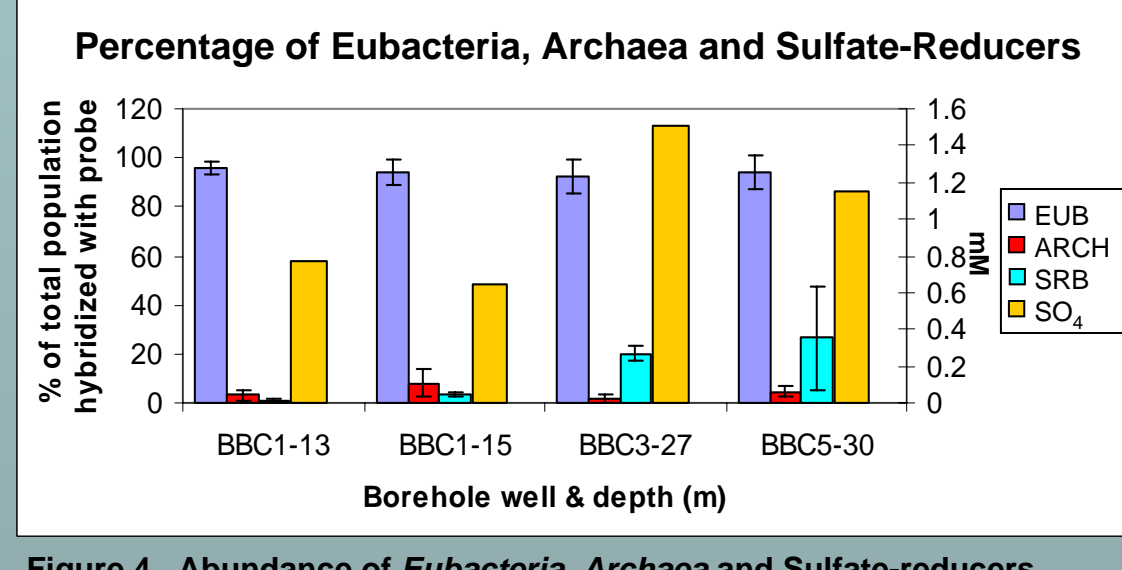


Figure 4. Abundance of *Eubacteria*, *Archaea* and Sulfate-reducers. Two domain level probes were used to detect the presence of all Bacteria. *Eubacteria* comprised between 93-96%, and *Archaea* comprised between 3-8%. Sulfate reducing bacteria were found to comprise between 1-28% of *Eubacteria*. Error bars represent standard deviation.

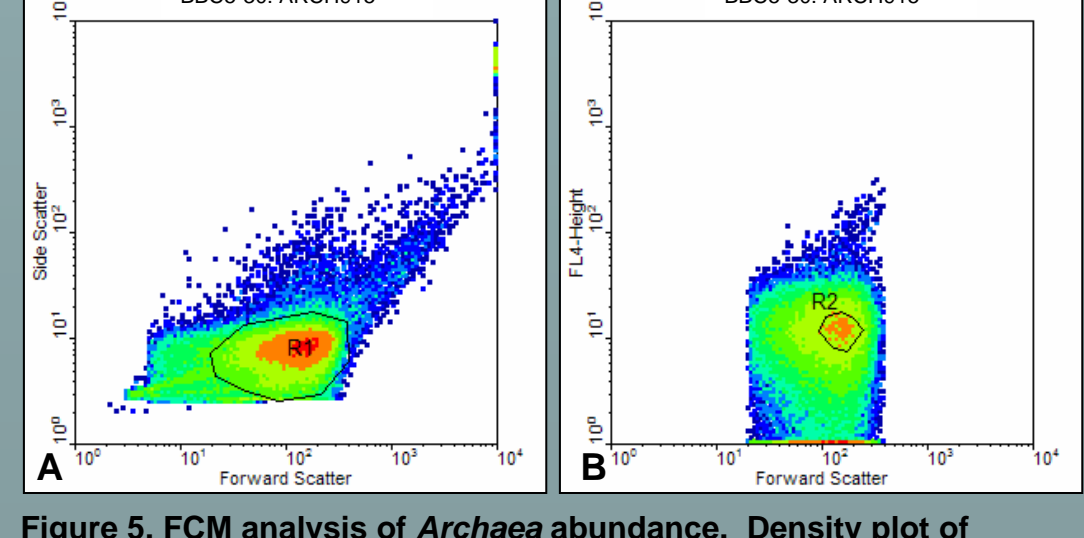


Figure 5. FCM analysis of *Archaea* abundance. Density plot of BBC5-30 probed with ARCH915. A) Forward scatter/side scatter plot depicting total cells (R1). B) Forward scatter/FL4 plot gated by R1, depicting cells positive for probe (R2).

Sample	Microscopic Counts	
	Total cells/mL	% Archaea
BBC1-13	1.22 x 10 ⁵	3
BBC3-27	4.50 x 10 ⁴	2
BBC5-30	1.29 x 10 ⁵	4

Table 3. Comparison of abundance between mic and FCM counts

SUMMARY

Morphologic diversity was revealed in groundwater samples (Fig. 2). The number of cells ranged between 10⁴ and 10⁵ cells/mL.

FISH coupled to microscopy revealed that *Eubacteria* were more abundant than *Archaea* in all samples, comprising between 93-96% of the total microbial community. Sulfate-reducers comprised only 3-8% of the total (Fig. 4). Sulfate-reducers were not limited by the amount of sulfate, however sulfide was detected indicating that the

CONCLUSIONS

FISH coupled to FCM revealed the abundance of *Archaea* in groundwater samples. The heavy debris within the boreholes prevented the use of FISH for enumeration of particles was subtracted. Phylogenetic probes can be implemented in characterizing microbial communities in groundwater.

REFERENCES

- Amann, R.L., B.J. Binder, R.J. Koster, H. Ludwig, R. M. H. Ormrod, and H. Stahl. 1990. Fluorescent-oligonucleotide probes for visual confirmation of microorganisms. *Environ. Microbiol.* 56:19-26.
- Rabus, R., M. Fukui, H. Wilke. 1998. Degradative capacities and 16S rDNA-based phylogenetic analysis of sulfate-reducing bacteria in an anaerobic enrichment culture from crude oil. *Appl. Environ. Microbiol.* 64:100-105.

ACKNOWLEDGMENTS

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