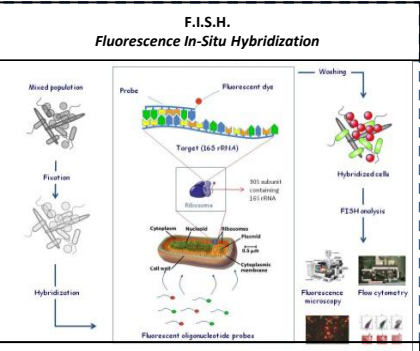
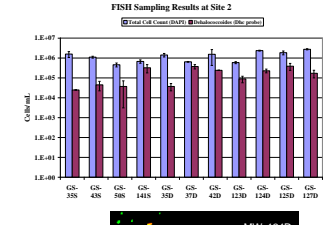
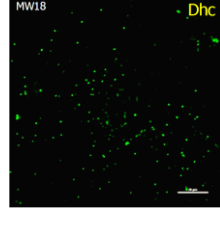
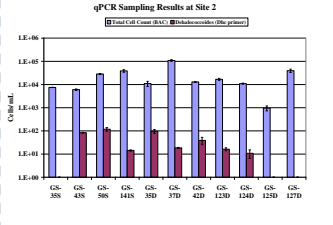
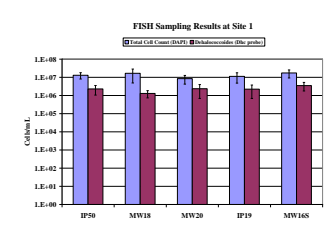
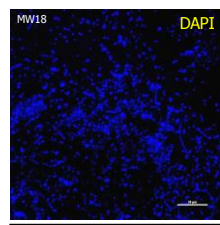
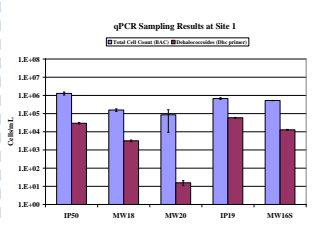


Field-Scale Comparison

- Microbial samples were received from two separate sites undergoing active bioremediation/MNA
- Various degrees of dechlorination observed
- Different sequences for primers/probe sets in FISH and qPCR



Experimental Protocol Requirements

- DNA Extraction from cells
- DNA Amplification and Hybridization with quenched fluorescently-labeled primers
- Cleavage of quencher by Taq polymerase activity releasing reporter
- Cycle repetitions to obtain sufficient amount of DNA
- Quantification of gene copies
- Correlation to number of cells through templates & standard curves

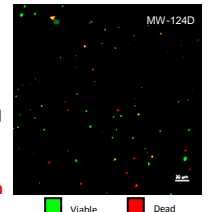
CONCLUSIONS

- qPCR results similar across sampling campaigns
- FISH >> qPCR results (2-3 orders of magnitude)
- FISH ~ qPCR on pure cultures
- Sets of primers and probes seem specific, based on positive/negative controls.
- >90% microbial viability in samples at Site 2.

⇒ FISH infers greater stability and quantity of *Dehalococcoides*

⇒ qPCR shows greater variations and lower inventories

➔ Specificity of primer/probe sets? Further study necessary to explain differences.



Drawbacks

- Indirect quantification method
- Destructive approach
- Several complex experimental steps
- Specificity of the primers

Experimental Protocol Requirements

- Fixation of cells *in-situ*, with paraformaldehyde
- Hybridization of cells with specific oligonucleotide probes
- Washing of unbound probes with stringent buffer
- Direct Enumeration by fluorescence microscopy and imaging

Drawbacks

- Microscopy-derived quantitation limiting high throughput use
- Knowledge of fluorescence microscopy and imaging required
- Specificity of the probes
- Washing step / non-specific binding

Advantages

- Automated rapid technique
- Widespread and commercially available
- Can target functional genes

Advantages

- Direct quantitation method
- Multi-parameter
- Can be used with other staining/microscopy techniques
- In-situ / non-destructive
- Fewer and simpler experimental steps
- Imaging:
 - 3D in situ rendition & spatial distribution
 - Colocalization
 - Morphology, size
- Automation capable – See Poster #587: on High-Throughput FISH