1st Prize, 2010 and 2011: Physical Sciences and Engineering
USC Undergraduate Symposium for Scholarly and Creative Work

Investigating the Mechanisms of Arsenic Removal by Microbial Layer in a Bio-sand Filter used for Drinking Water Purification in Developing Countries

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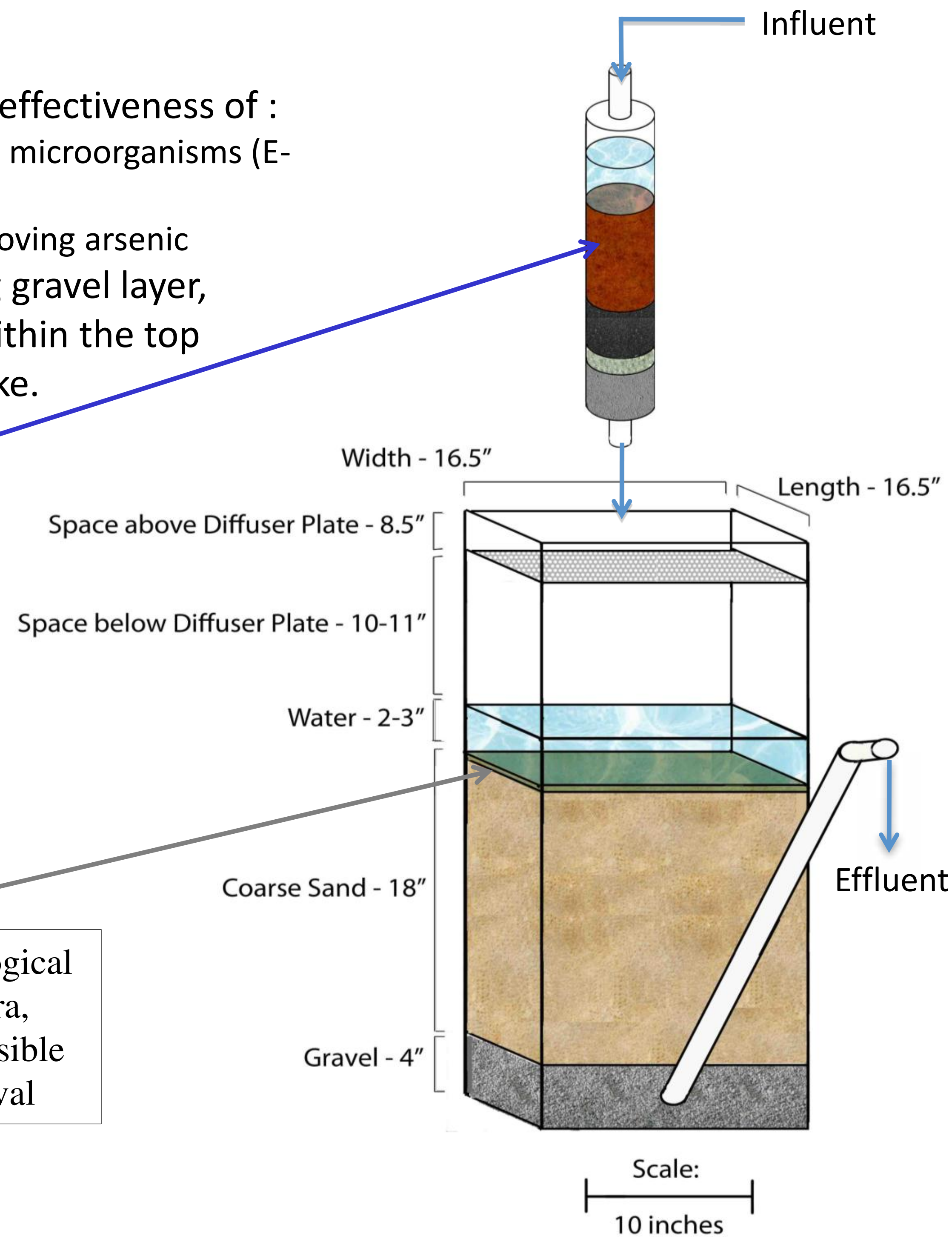
Health effects of pathogenic bacteria include:	Health effects of arsenic contamination include:
• Typhoid fever • Paratyphoid fever • Salmanellosis • Bacillary dysentery • Cholera • Gastroenteritis • Acute respiratory illness • Pulmonary illness	• Cancer: skin, lung, bladder, liver, and kidney • Cardiovascular disease • Peripheral vascular disease • Developmental effects • Neurologic & neurobehavioral effects • Diabetes Mellitus • Hearing loss • Portal fibrosis of the liver • Lung fibrosis • Anemia

Bio-Sand Filter – Phase 1

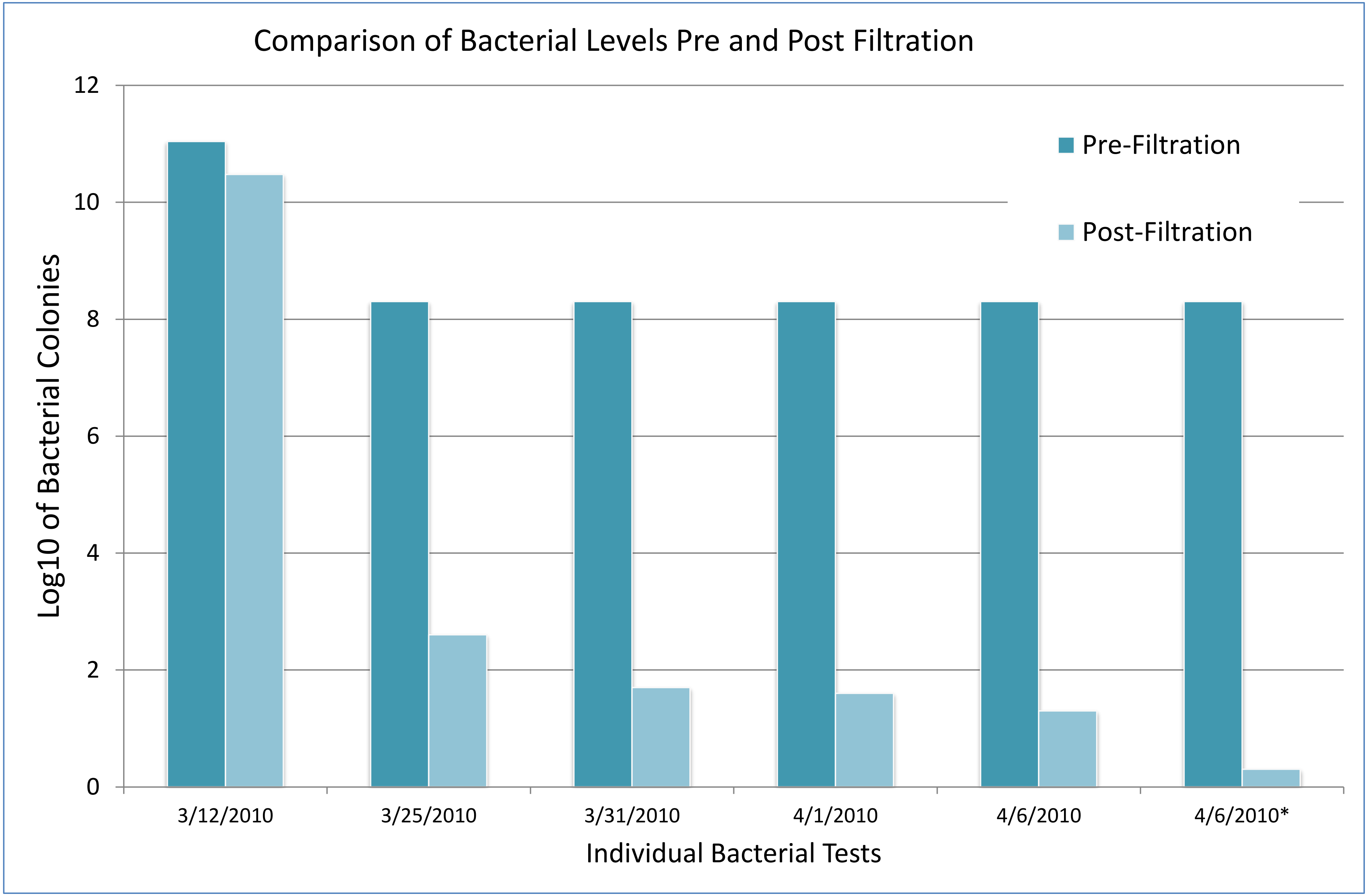
- Objective for Phase 1 included testing the effectiveness of :
  - The bio-sand filter in removing pathogenic microorganisms (E-coli)
  - The iron-oxide coated sand column in removing arsenic
- The bio-sand filter consists of a supporting gravel layer, a main sand layer, and microbial biofilm within the top centimeter of sand called the Schmutzdecke.

Iron-oxide coated sand column: Phase 1 of the research assumed that the column would be the primary mechanism of arsenic removal.

Bio-Layer (Schmutzdecke): This complex biological community containing bacteria, protozoa, rotifera, algae, fungi and other microorganisms is responsible for the majority of biological contaminant removal

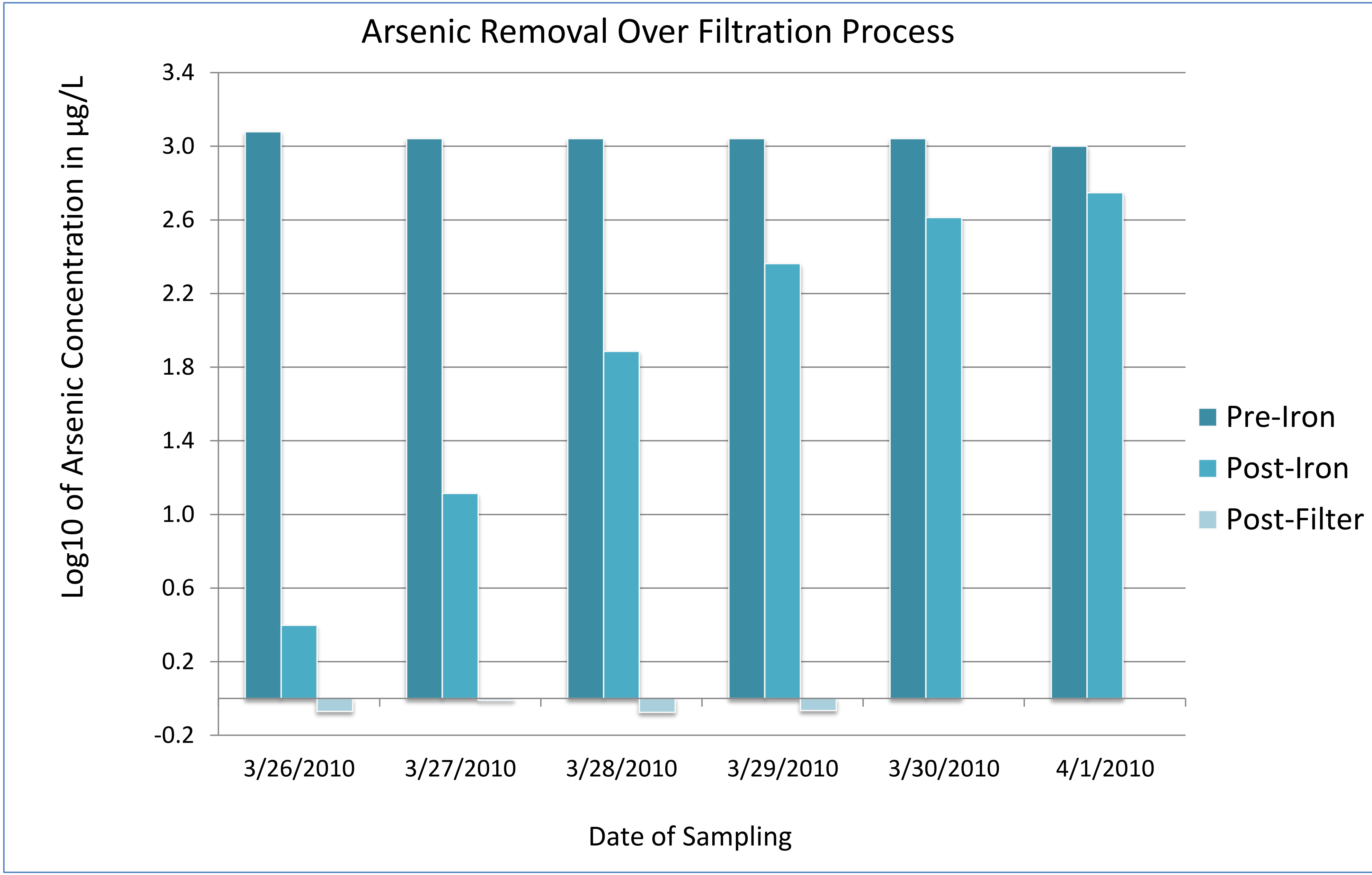


Phase 1: Filter Bacterial Removal Rates



After a month of testing, the biofilter reduced bacterial concentrations by over 99.99%. Test results show the improvement of removal rates over the time period, reflecting the development of the schmutzdecke.

Phase 1: Filter Arsenic Removal Rates



Test results show that the sand column was **initially** effective at removing arsenic, but quickly became saturated and almost completely ineffective. However, the biofilter removed nearly all arsenate from water regardless of the iron column's performance.

Transitioning from Phase 1 to Phase 2

- Phase 1 focused on optimizing removal of pathogens and arsenic using an iron-oxide sand column and the biosand filter
- Phase 2 investigated mechanisms of pathogen and arsenic removal and their specific locations within the filter

Phase 1	Phase 2
Iron Oxide Coated Sand	
•Placed in column in sequence to filter •Water contacts coated sand prior to Schmutzdecke	•Placed in a layer embedded in sand layer •Water contacts Schmutzdecke prior to coated sand
Arsenic	
•Only Sodium Arsenate tested •Smaller concentration used (1ppm) •Arsenic injected for 1 week of testing	•Both Sodium Arsenate and Sodium Arsenite tested •Larger concentration used (7ppm) •Arsenic injected for 5 weeks of testing
Location of Sampling Points	
•Influent to column, effluent from column, effluent from filter	•Influent to filter, between Schmutzdecke and iron sand, between iron sand and outlet, effluent from filter
Attention to Biofilm	
•E. Coli added to stimulate biofilm growth and provide coliform •Observed biofilm community behavior	•Only influent water used to form biofilm •Analyzed biofilm community for interactions with arsenic

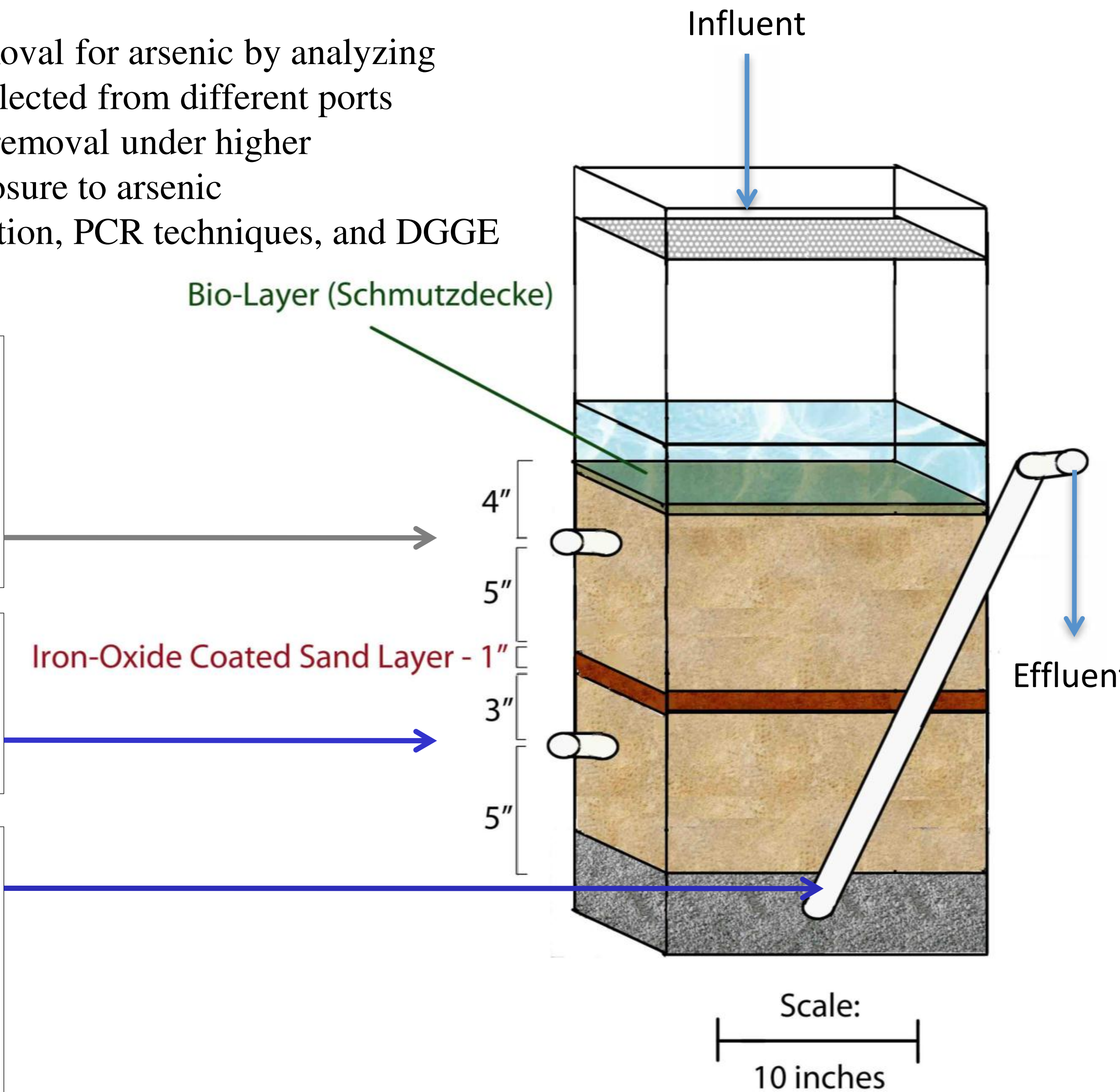
Bio-Sand Filter: Phase 2

- **Objectives** for Phase 2 included:
  - Determining the mechanisms of removal for arsenic by analyzing arsenic concentrations in samples collected from different ports
  - Verifying effectiveness of bacterial removal under higher concentrations of and prolonged exposure to arsenic
  - Analyzing bacteria using DNA isolation, PCR techniques, and DGGE fingerprinting

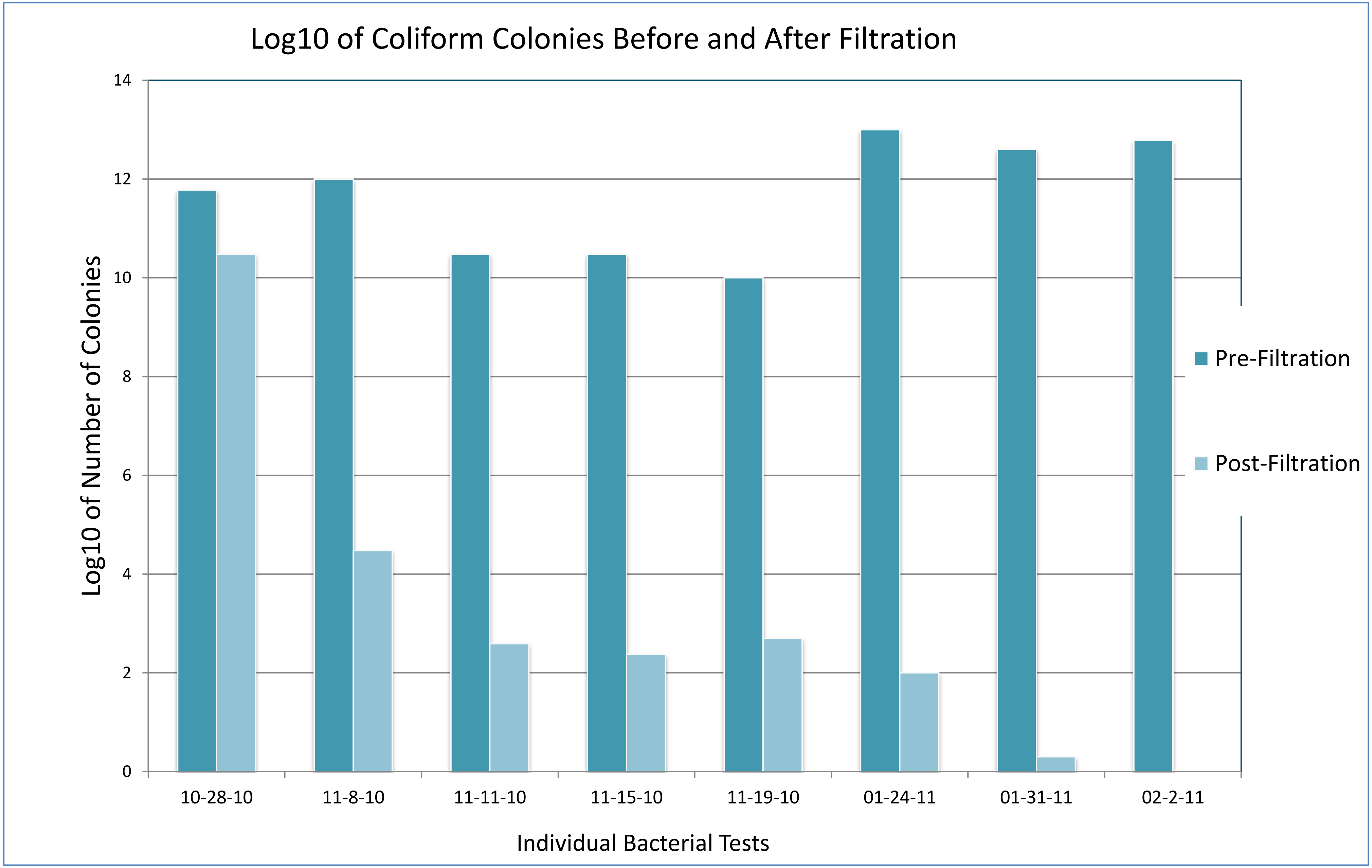
Sampling Port 1 –
Concentrations of arsenic measured in samples from this port indicates removal within bio-layer (Schmutzdecke)

Sampling Port 2 –
Concentrations of arsenic measured in samples from this port indicates removal within iron-oxide coated sand layer

Sampling Port 3 –
- Concentrations of arsenic measured in samples from this port indicates any additional removal resulting from adsorption to sand.
- Bacteria removal was measured from this port

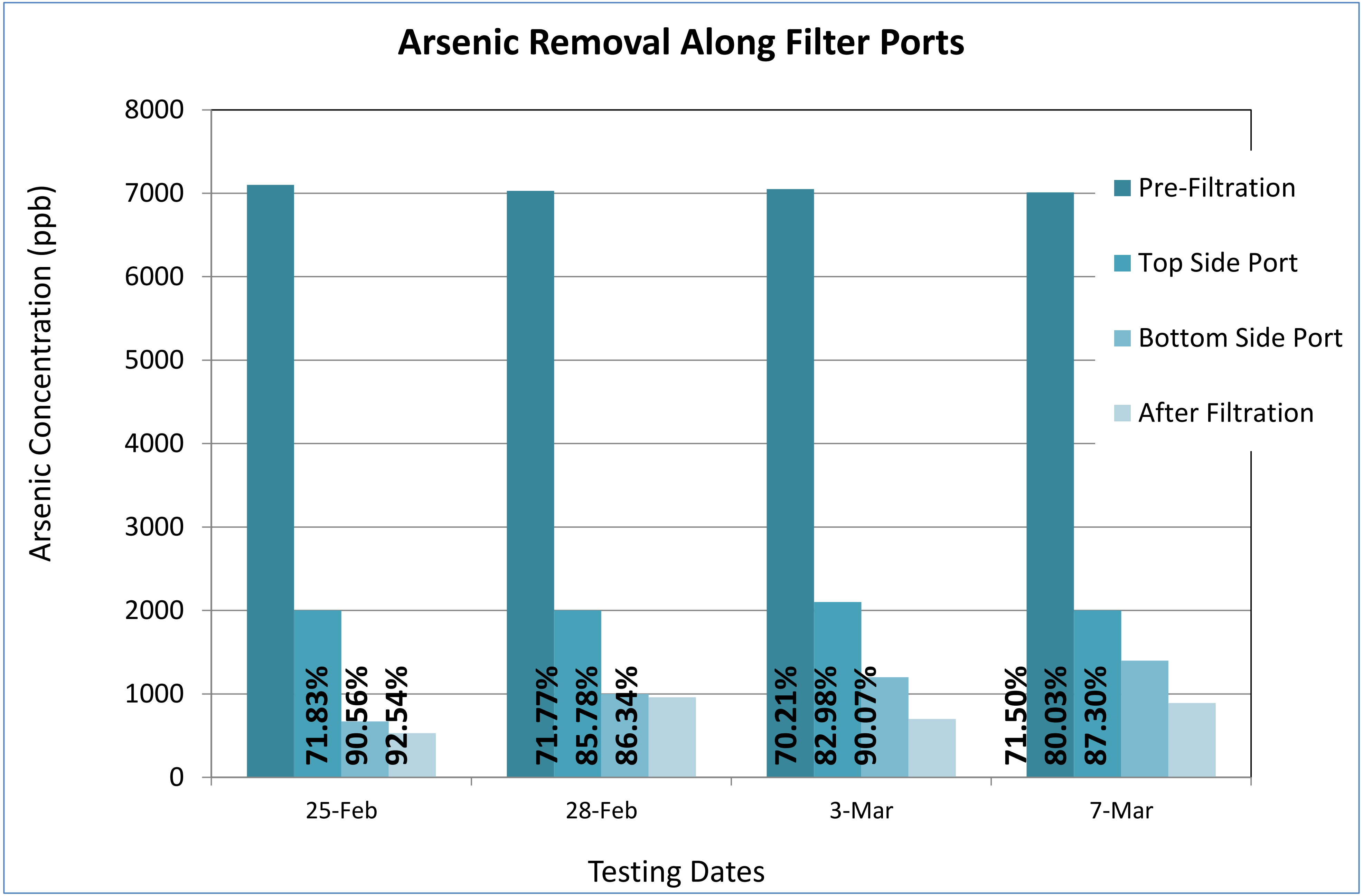


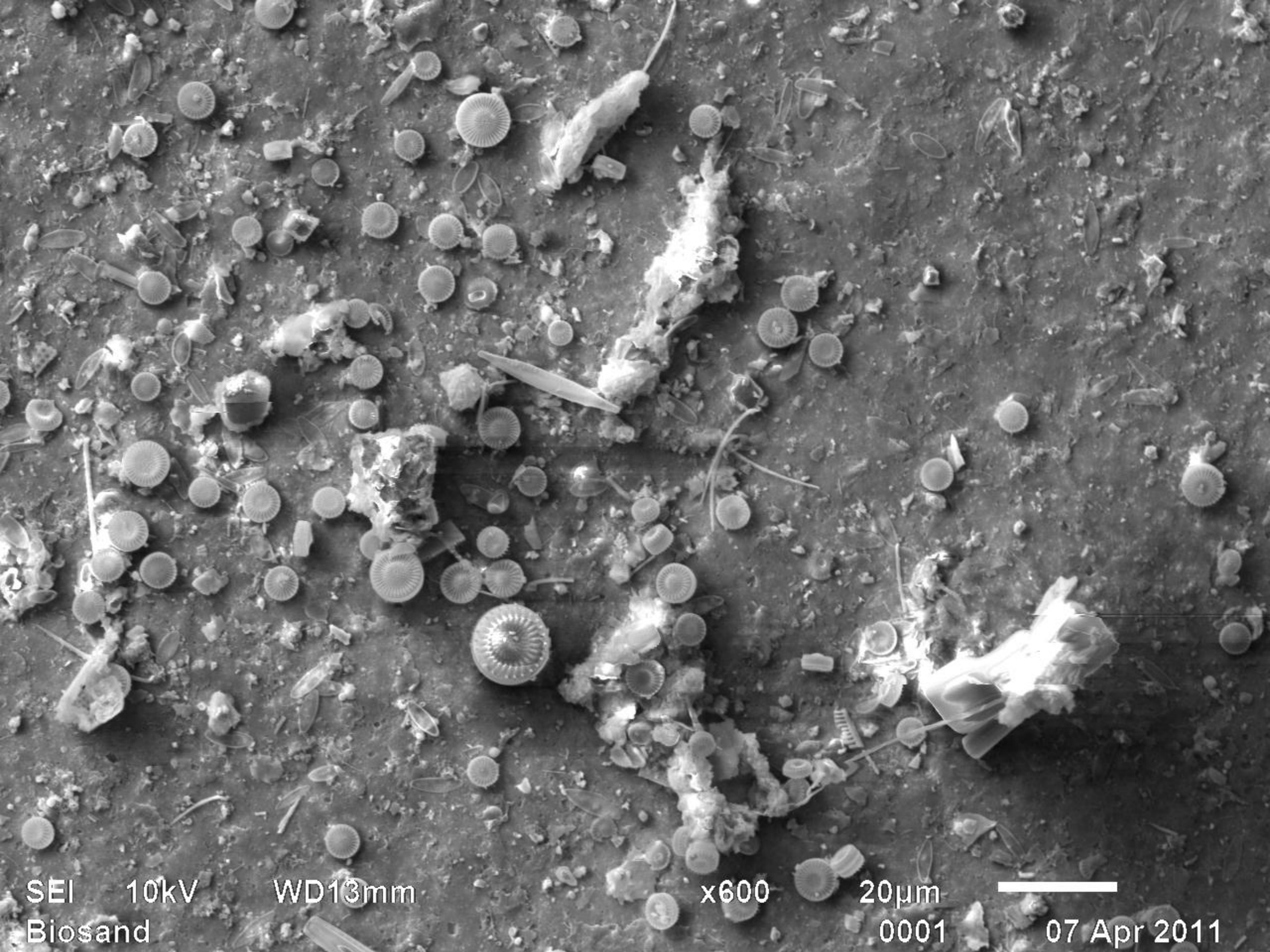
Phase 2: Bacteria Removal Rates

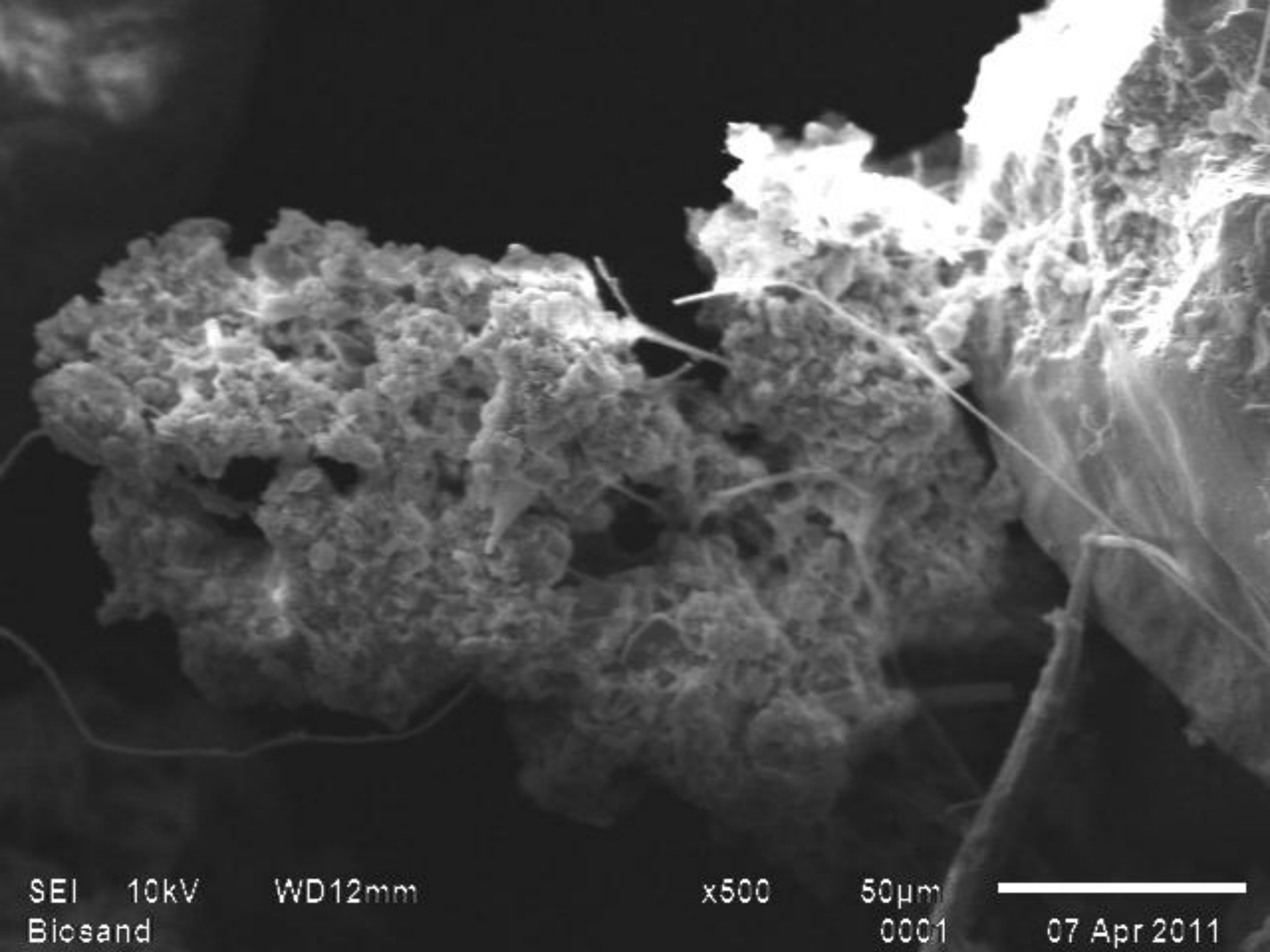


In the second phase of testing, coliform colony removal demonstrated higher removal efficiency over time, particularly after a periodic cleaning on January 20th. Even with extremely high concentrations of coliform and arsenic entering , the filter effectively removes over 99.999999%.

Phase 2 Results: Arsenic




SEI 10kV WD13mm x600 20µm
Biosand 0001 07 Apr 2011

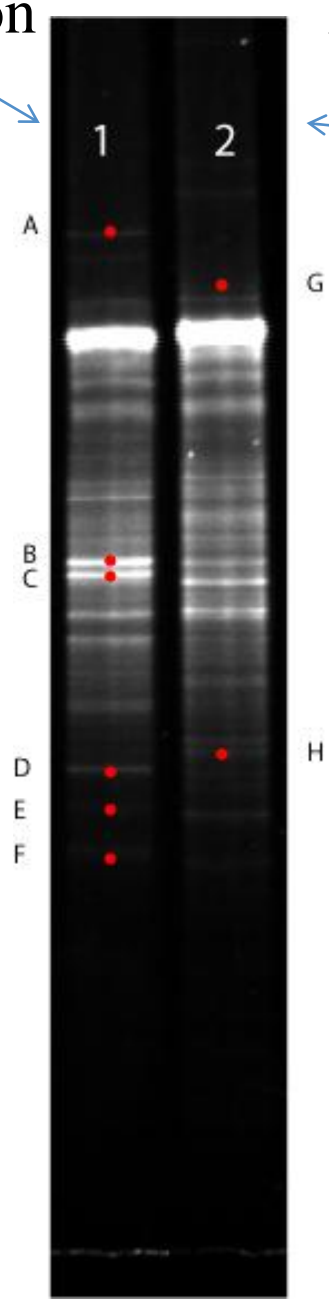

SEI 10kV WD12mm x500 50µm
Biosand 0001 07 Apr 2011

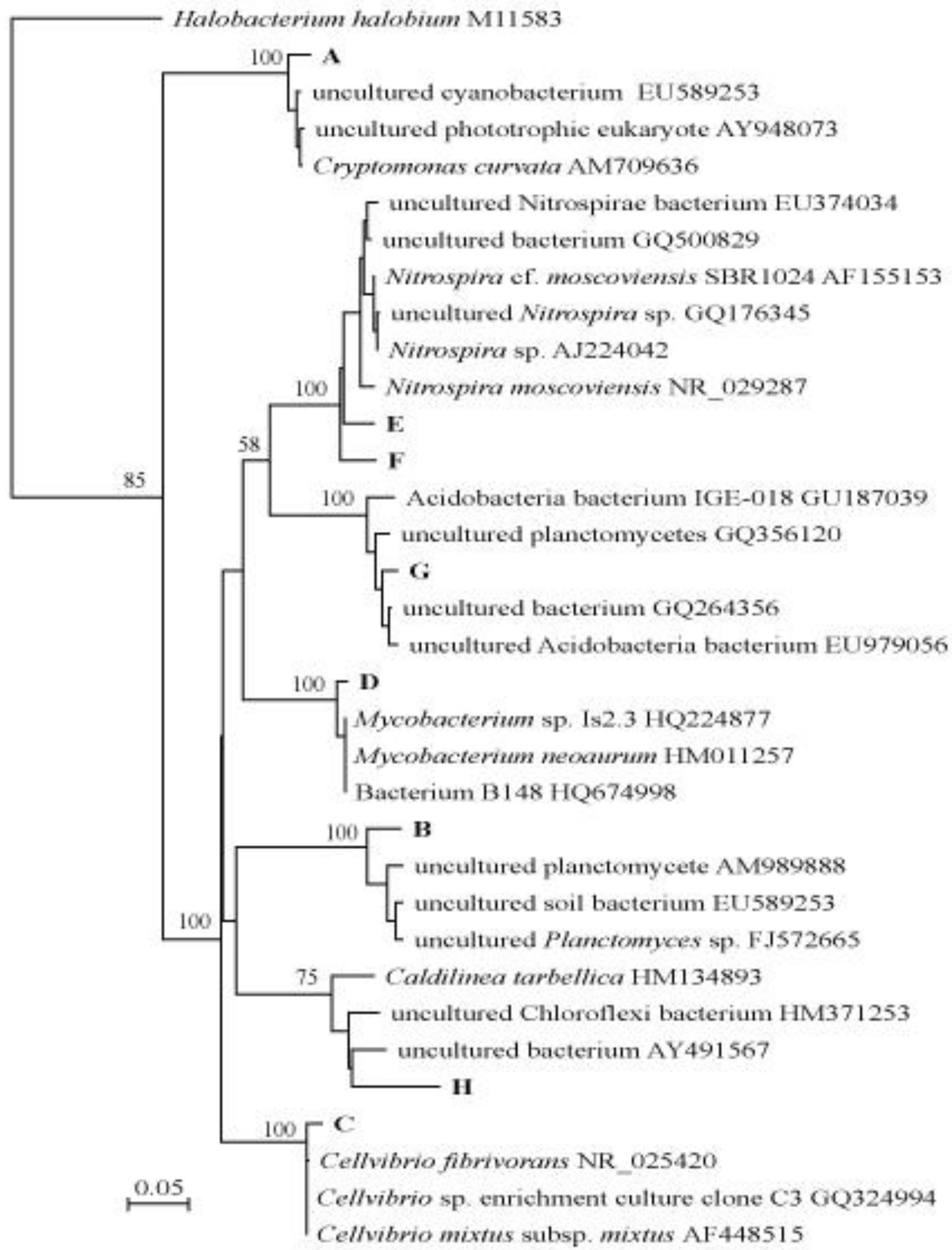
DGGE/DNA Analysis

- DNA isolated from schmulzedecke layer
  - 30 days after start of filter
  - At the end of the experiment
- 16S Ribosomal RNA gene amplified by PCR using universal primers (1070F, 1392R)
- DNA separated by Denaturing Gradient Gel Electrophoresis (DGGE)
  - 40% - 65% gradient
- Bands excised and sequenced
  - Alignment with ClustalW
  - Jukes-Cantor distance matrix used to create neighbor-joining tree
    - Bootstrap values from 1000 resampling events of the data set

30 days operation

End of Experiment





DGGE Analysis Cont.

- Indication of highly diverse microbial community
- Community after 30 days of operation
  - Major Bands
    - *Planktomyces* (associated with algae and cyanobacteria)
    - *Cellvibrio* (cellulose decomposition)
  - Minor Bands
    - Cyanobacteria (*Cryptomonas curvata), Nitrospira, Mycobacterium*
- Community at end of experiment
  - Acidobacteria, Chloroflexi (*Caldilinea tarbellica)*
    - *aoxB* genes associated with Chloroflexi suggest capabilities of arsenite oxidation (Quemeneur *et al.* 2010)

Possible fates of Arsenic?

- Bacterial species show arsenate reductase genes for reduction to arsenite
  - Cellular detoxification pump exists specific to arsenite
- Arsenic fate?
  - Reduction to As^0 unclear
  - Genes also exist of methylation of arsenite to form less toxic organo-arsenic species
    - Association in hydrophobic cellular components
      - Membranes, lipids, polysaccharide, etc.
- More in depth metagenomic studies are needed
  - Clone library
  - Pyrosequencing

Conclusion

- Effective removal of both pathogens and arsenic attributed to biofilm (first outlet port)
- High arsenic removal in the schmutzdecke suggests that the expensive iron-oxide sand layer may not be required
- Current WHO and EPA standards for arsenic in safe drinking water is 10 ppb
  - In Bangladesh 35% of wells contain arsenic concentrations above 50 ppb, 8.4% over 300 ppb, both significantly **less** than our concentration
  - Results indicate the biofilm in the biofilter would be able to significantly reduce these levels to within safe drinking standards
- After two years of successful laboratory research, the next important step involves testing and implementing the biosand filter in developing countries for practical applications
- Implementation of biosand filters in communities must be accompanied by education and follow-up after process evaluation.

Researchers in the Laboratory

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