October 13, 2022

# Discovery and Tracking of a Novel Sulfolane-Degrading Bacterium through Laboratory and Field Studies

**Linda Eastcott**, Arujala Thavendrarasa (Imperial Oil Limited)

**Philip Dennis**, Ximena Druar, Melody Vachon, Jennifer Webb, and Sandra Dworatzek (SiREM)

Eva McLean, Sylvain Hains, and Andrew Madison (Golder Associates)

Trent A. Key (ExxonMobil Environmental and Property Solutions Company)

E&PS

# Agenda

Background

Remedial Activities

Microbiology & Molecular Biological Tools

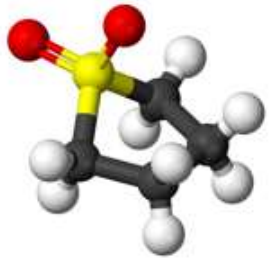
Conclusions

# Background

# Sulfolane – Background

- Industrial solvent used as a sweetening agent in sour gas processes in the upstream
- Historically used from the 1960s to the 1980s
- Cyclic ether similar to 1,4-dioxane
- $C_4H_8SO_2$

Biodegradation Pathways:



**Aerobic Degradation** → 1) $C_4H_8O_2S + 6.5O_2 \rightarrow 4CO_2 + 3H_2O + 2H^+ + SO_4^{2-}$ (Greene et al., 2000)

**Nitrate Reduction** → 2) $5C_4H_8O_2S + 18NO_3^- \rightarrow 20HCO_3^- + 9N_2 + 5HS^- + 7H^+ + 4H_2O$ (Greene et al.,1998)

Manganese Reduction → 3) $C_4H_8O_2S + 9MnO_2 + 5HCO_3^- \rightarrow 9MnCO_3 + 4H_2O + HS^- + 4OH^-$ (Greene, 1998)

Iron Reduction → 4) $C_4H_8O_2S + 10H_2O + 18Fe^{3+} \rightarrow S^{2-} + 18Fe^{2+} + 4HCO_3^- + 24H^+$ (Greene, 1999)

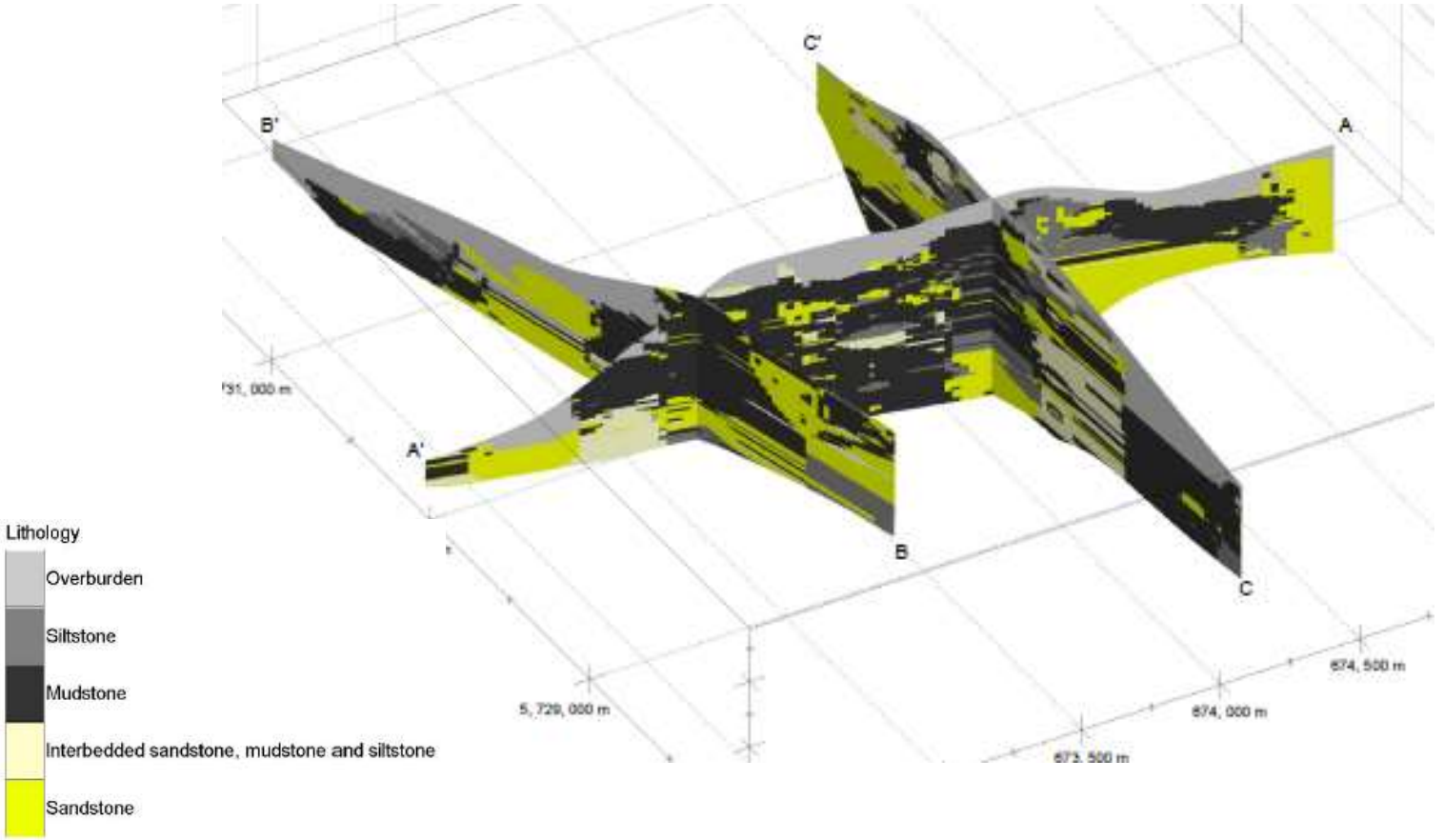
Sulphate Reduction → 5) $4C_4H_8O_2S + 4H_2O + 9SO_4^{2-} \rightarrow 13HS^- + 16HCO_3^- + 11H^+$ (Greene, 1999)

**Methanogenesis** → 6) $C_4H_8O_2S + 1.5H_2O \rightarrow 1.75CO_2 + 2.25CH_4 + H_2S$ (Greene et al.,1998)

E&PS GOLDER MEMBER OF WSP SiREM

# Conceptual Site Model – Geology

## 3D GEOLOGICAL MODEL

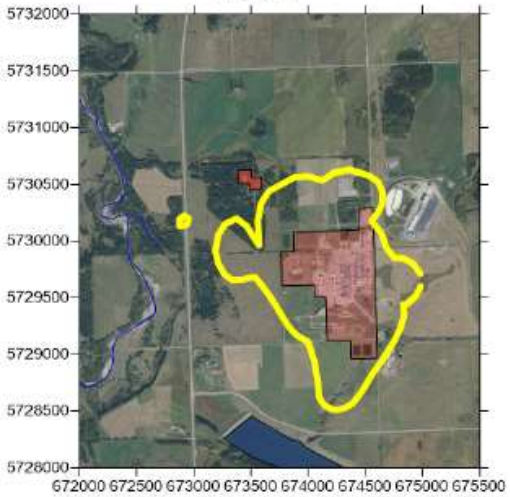

Lithology

- Overburden
- Siltstone
- Mudstone
- Interbedded sandstone, mudstone and siltstone
- Sandstone

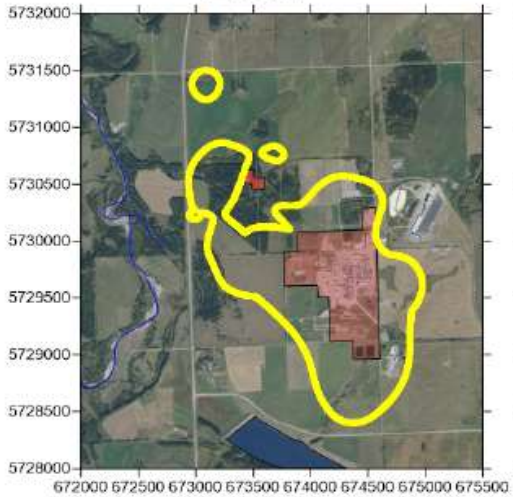
E&PS GOLDER MEMBER OF WSP SiREM

# Conceptual Site Model – Sulfolane Distribution

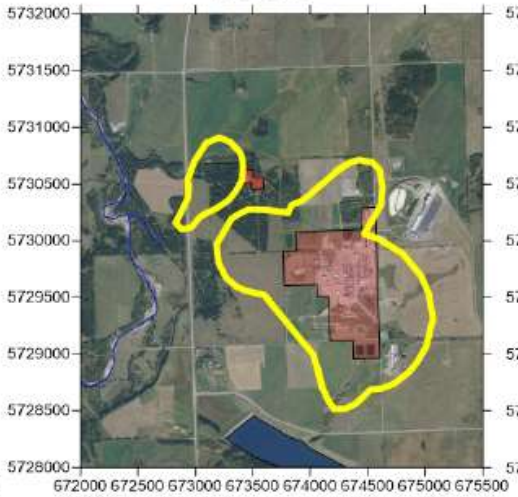
2004



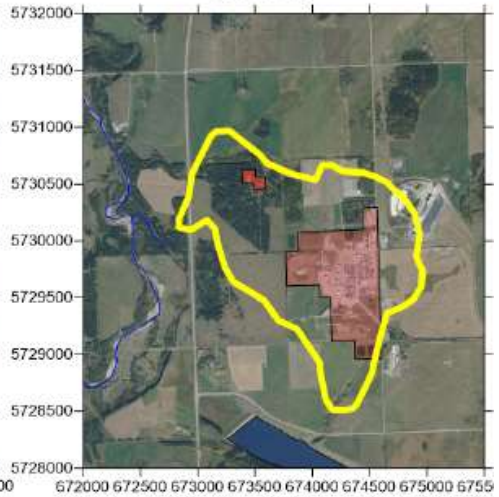

2007



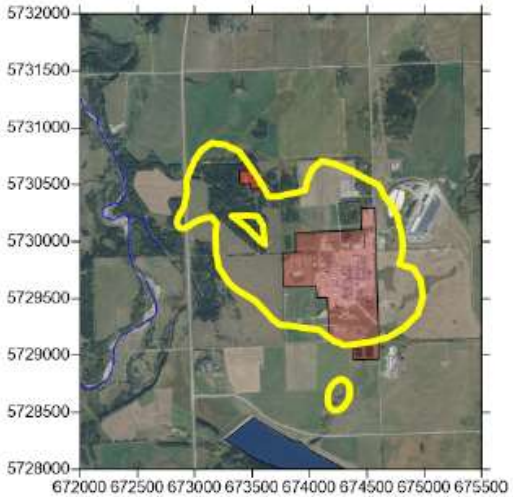

2008



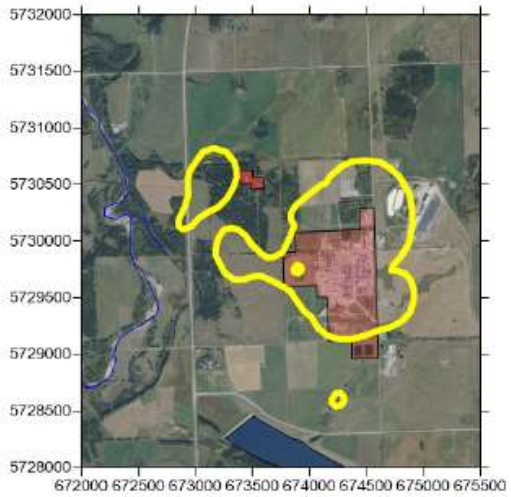

2010



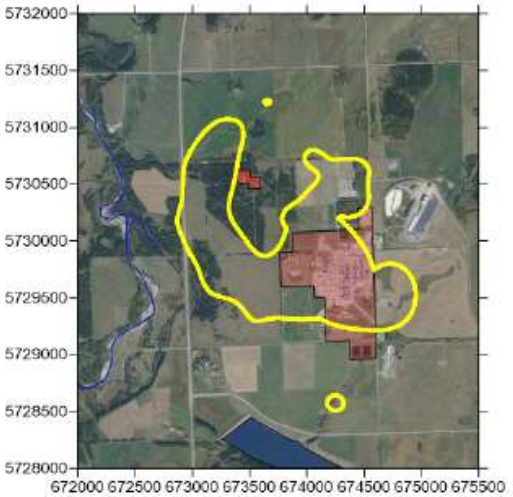

2012




2014




2018

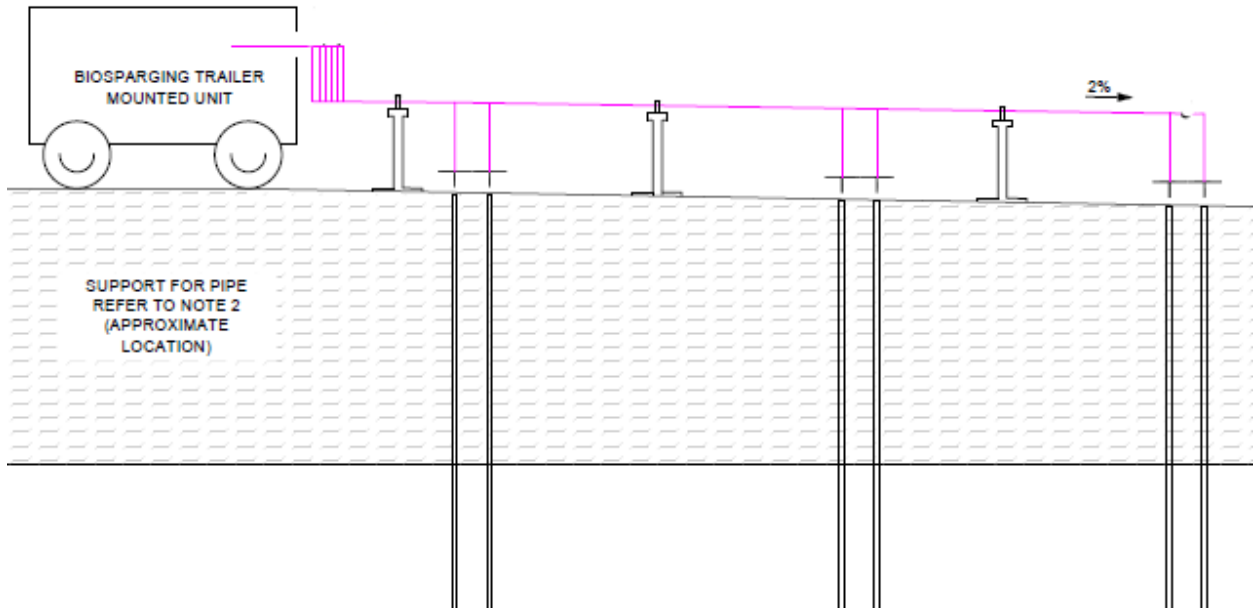



E&PS GOLDER MEMBER OF WSP SiREM

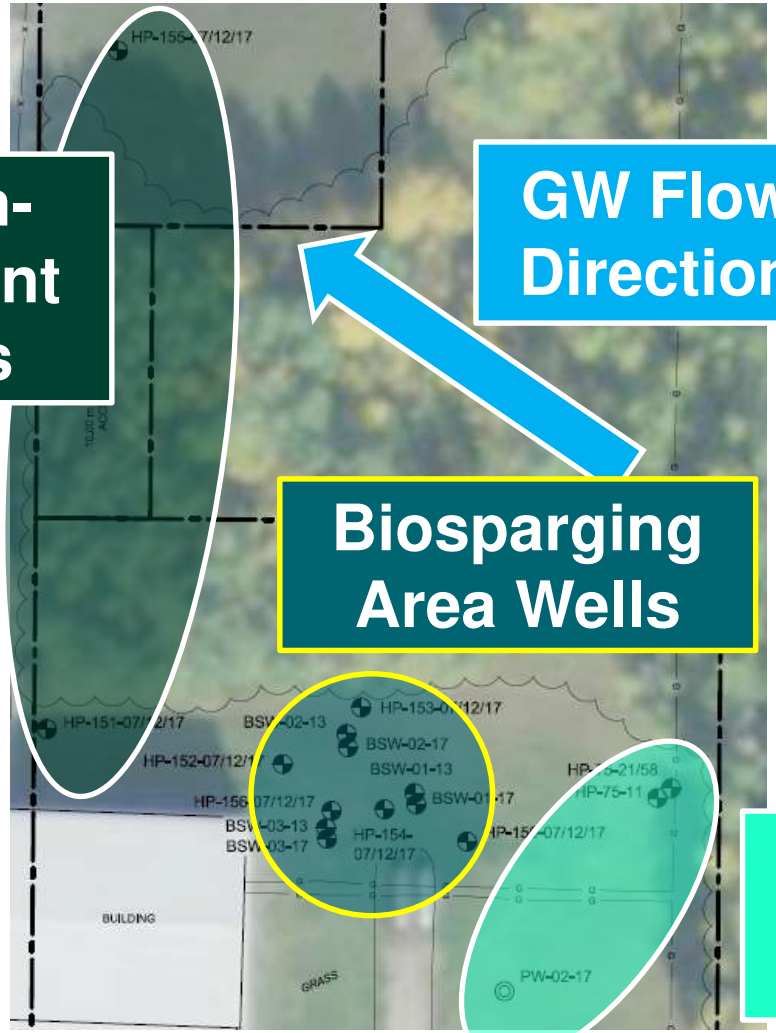
# Remedial Activities

# Mobile Unit System Design

## BIOSPARGING




# Biosparging and Monitoring Well Network




Biosparging Wells

- 12m Interval - 3 wells
- 17m Interval – 3 wells

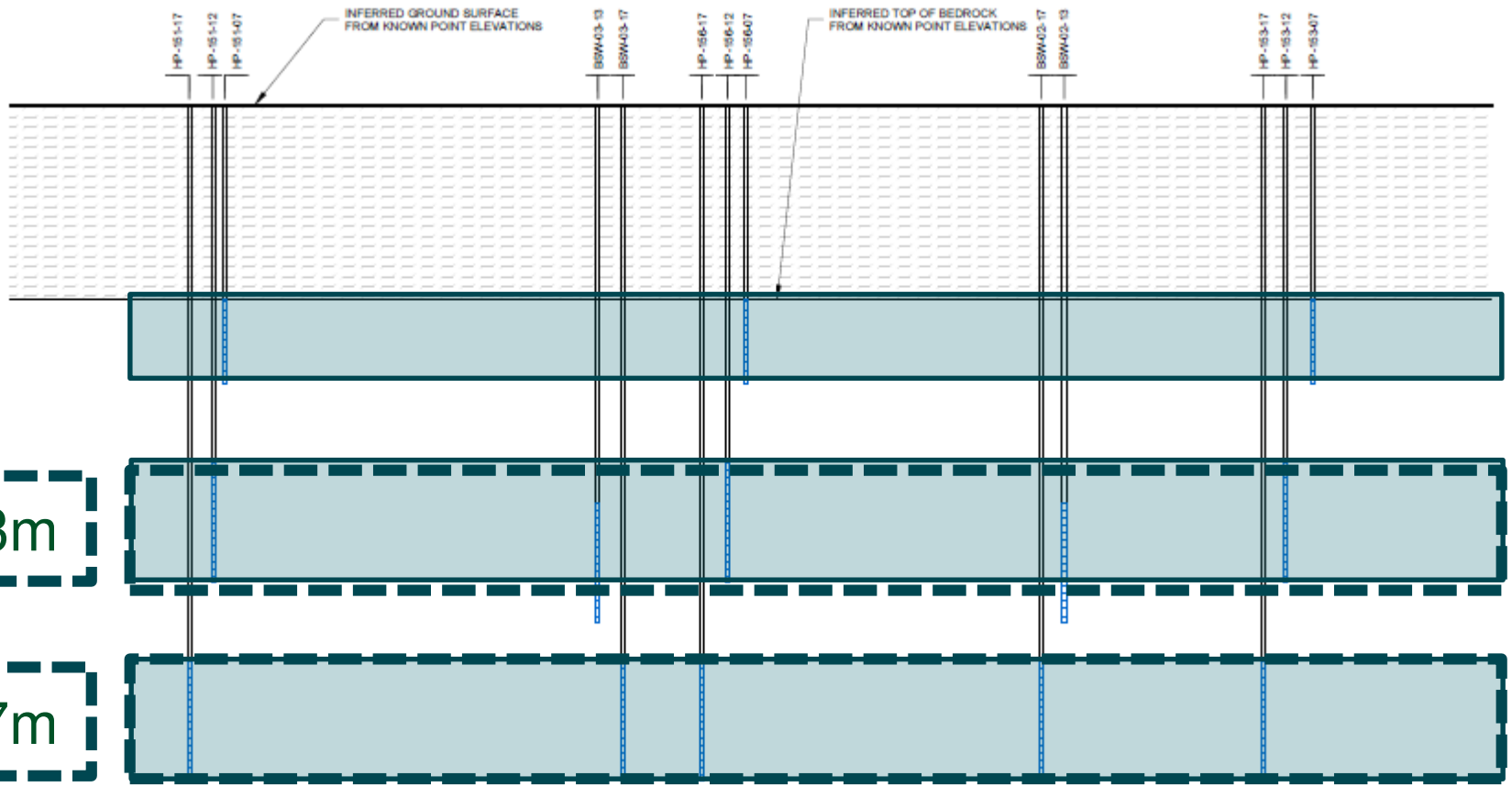
Monitoring Network

- 7m Interval – 8 wells
- 12m Interval – 14 wells
- 17m Interval – 14 wells

# Biosparging and Monitoring Well Network (Cont.)

**Injection Depths**

**Monitoring Depths**




10m to 13m

14m to 17m

4m to 7m

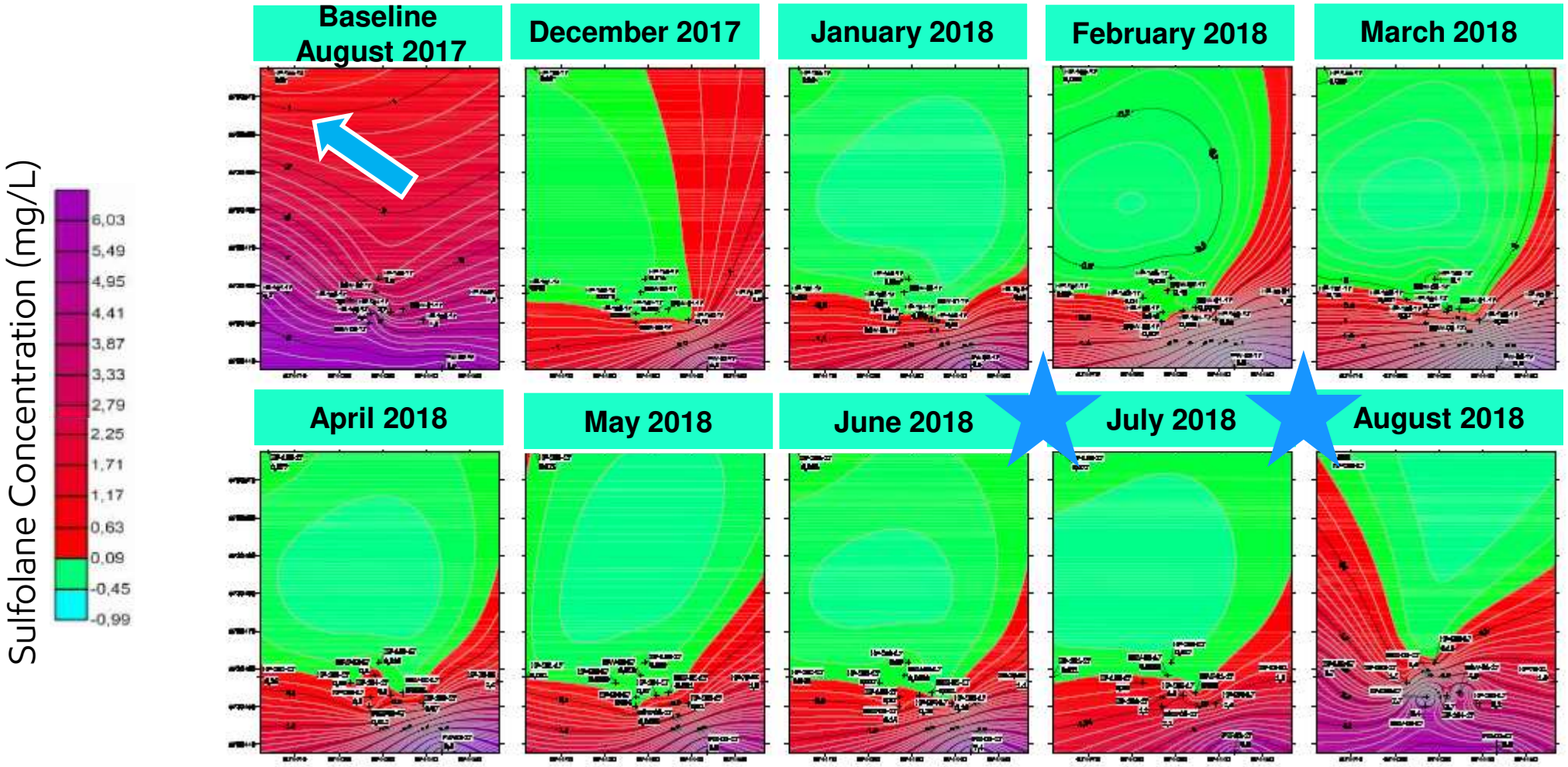
9m to 12m

14m to 17m

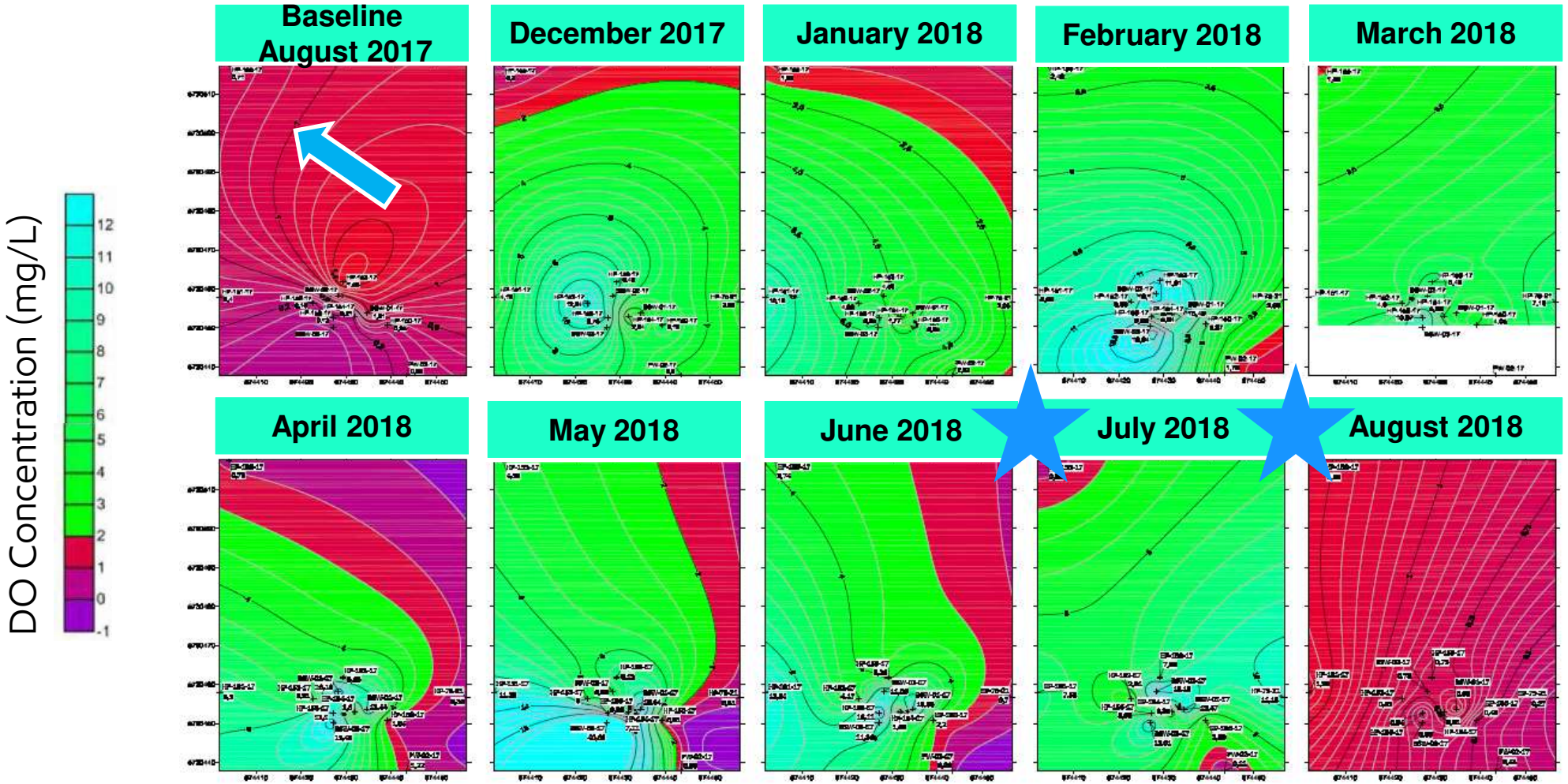
# Groundwater Monitoring Program

- Sulfolane
- Geochemistry
  - Electron acceptors (e.g., dissolved oxygen), nutrients, and biodegradation by-products
- Microbiology & Molecular Biological Tools

# Sulfolane Concentrations – 17m Interval



# Dissolved Oxygen Concentrations – 17m Interval



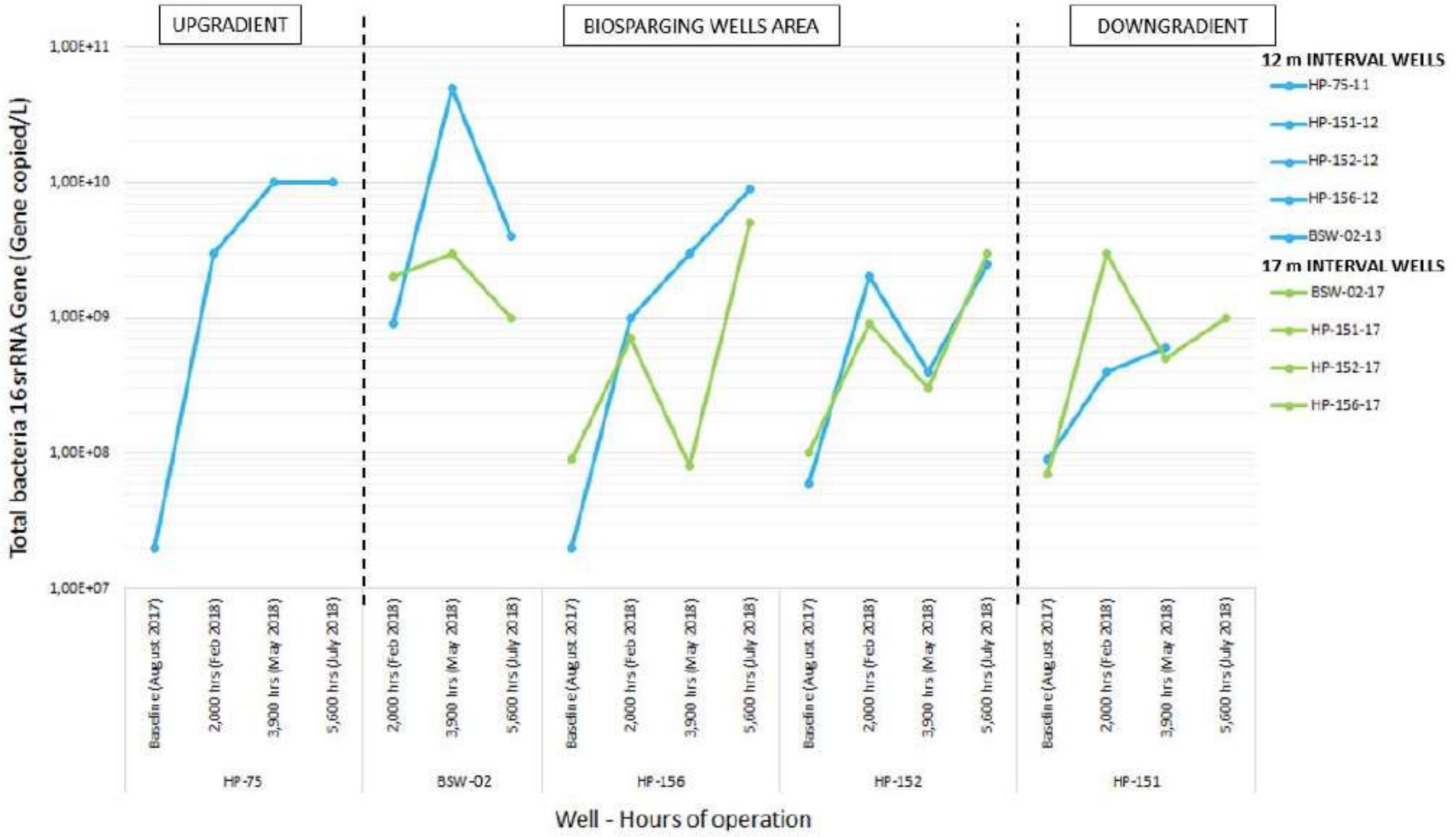
# Microbiology & Molecular Biological Tools

# Application of Microbiology & Molecular Biological Tools

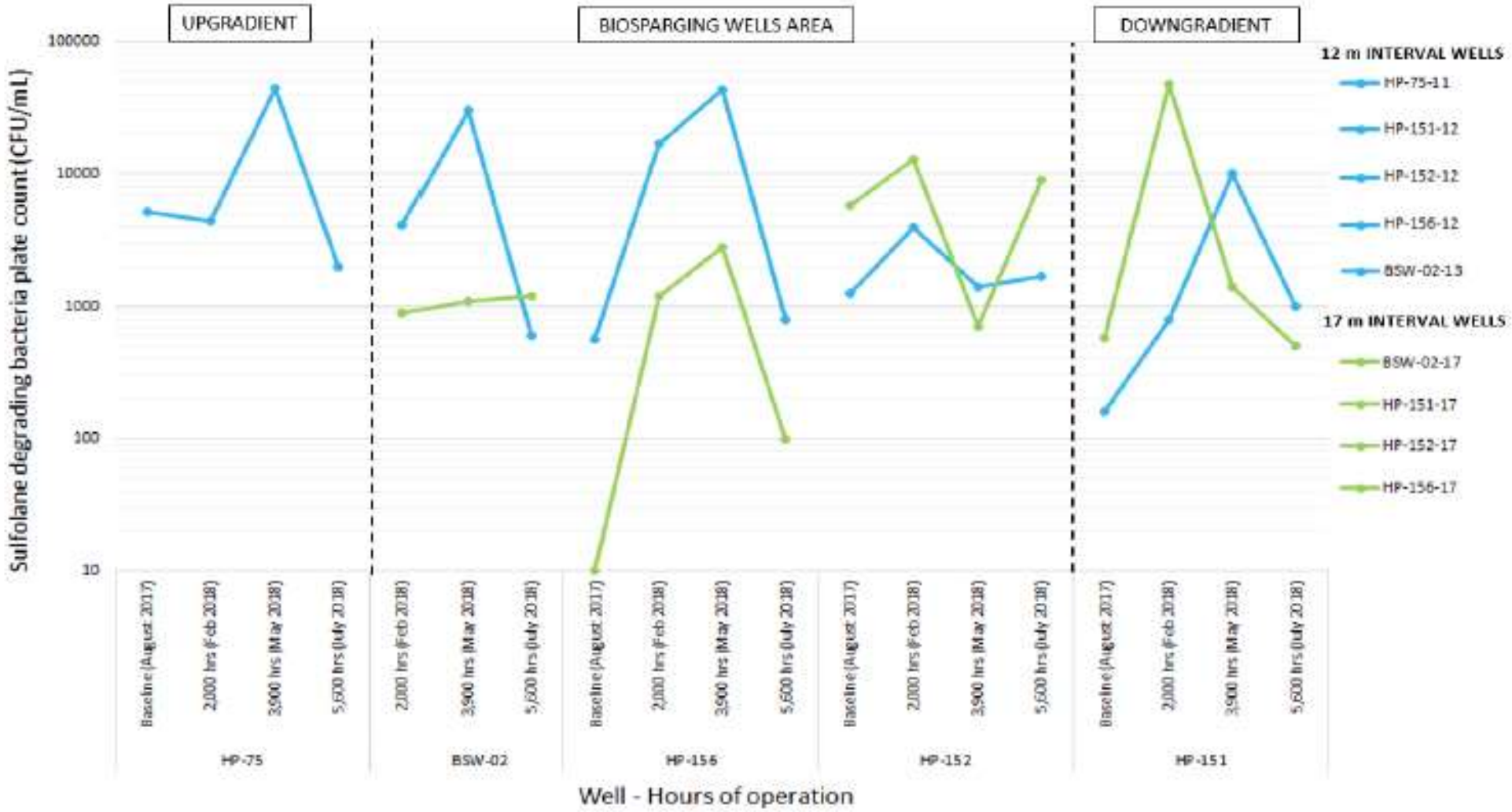
## BIOSPARGING RESULTS

- Provide supporting evidence that *in situ* biodegradation occurred due to biostimulation with oxygen
- Multiple lines of evidence approach was needed to showcase sulfolane biodegradation was a result of biosparging
  - ❑ Sulfolane media plate counts
  - ❑ Microcosm treatability study
  - ❑ Quantitative (q)PCR
  - ❑ Microbial Community Analysis (NGS)

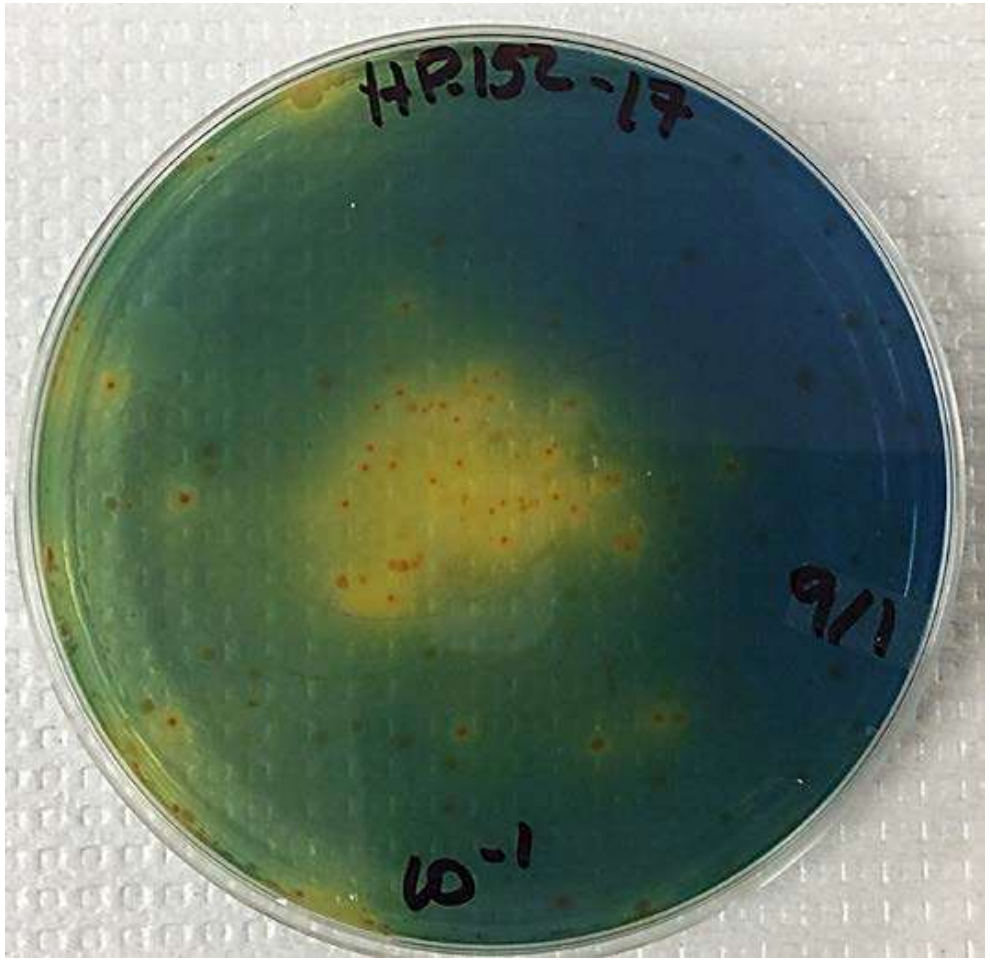
# Site Groundwater -Total Bacteria via qPCR (16S rRNA)



# Groundwater -Sulfolane Degrading Bacteria Plate Counts

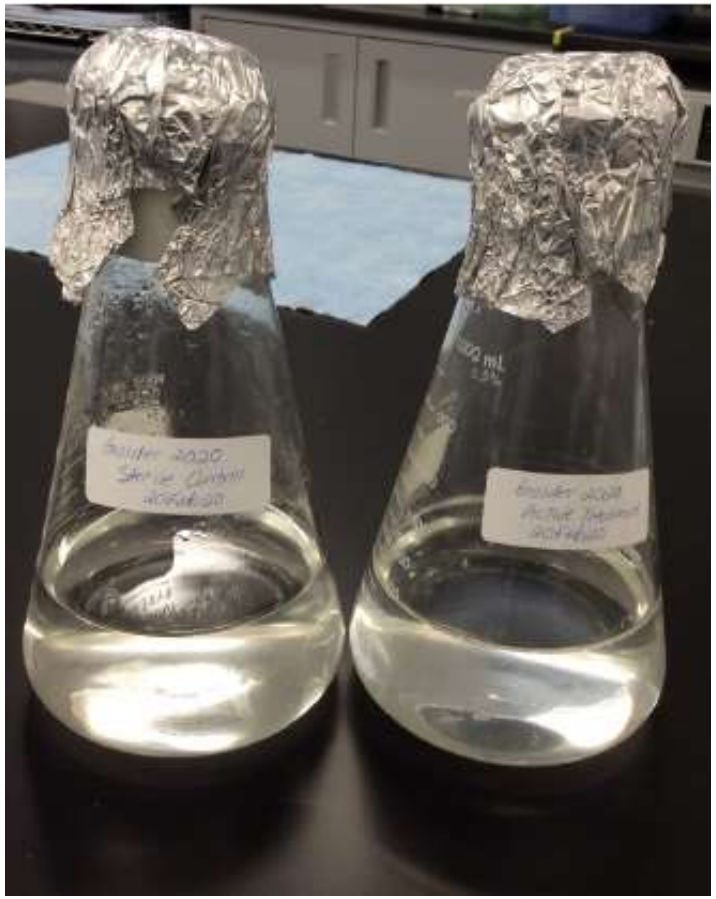


# Biodegradation of Sulfolane – Colony Identification




# Bioprospecting Overview

- **Stage 1: Treatability study** to assess biodegradation capacity of sulfolane from natural microorganisms present at site
- **Stage 2: Enrichment culture** study to select for sulfolane-degrading microorganisms ("weed out" organisms not of interest)
- **Stage 3: DNA sequencing** (NGS) of microbial community to aid in the identification of sulfolane-degrading microorganisms
- **Stage 4: Isolation and pure culture** study to confirm if isolated microorganisms biodegraded sulfolane and performed DNA sequencing on colonies to identify.
- **Stage 5: qPCR method development** (biomarker) to directly measure sulfolane-degrading microorganism in site samples

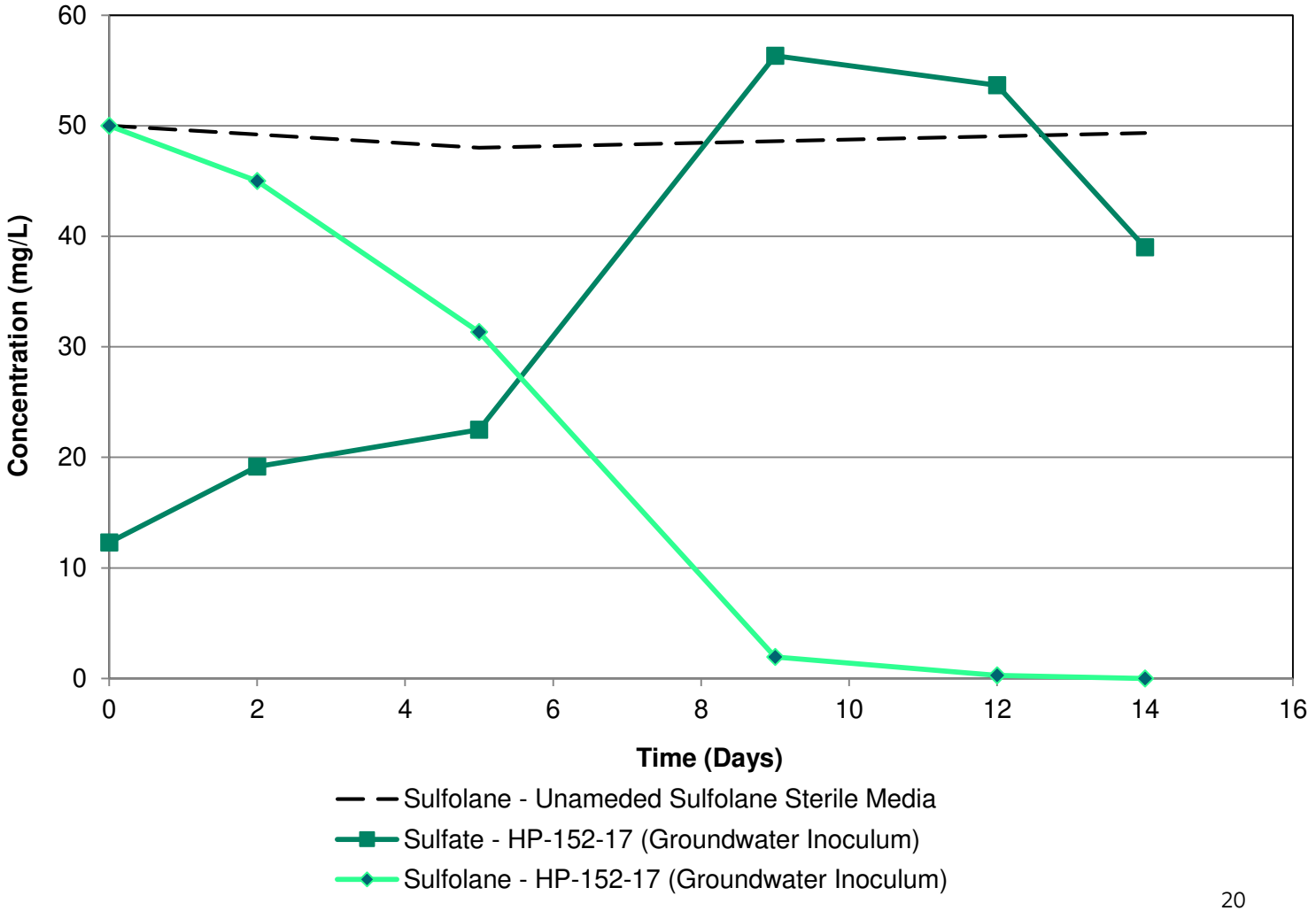


E&PS GOLDER MEMBER OF WSP SiREM

# Stage 1: Treatability Study

- Microcosm bench study completed under aerobic conditions
- Sulphate release quantified as a surrogate for aerobic sulfolane degradation
- Controls set up and sterilized with mercuric chloride and sodium azide
- Groundwater inoculum collected from a well located downgradient to the biosparging injection zone
- Treatment microcosms were sampled on Days 2, 5, 9, 12 and 14

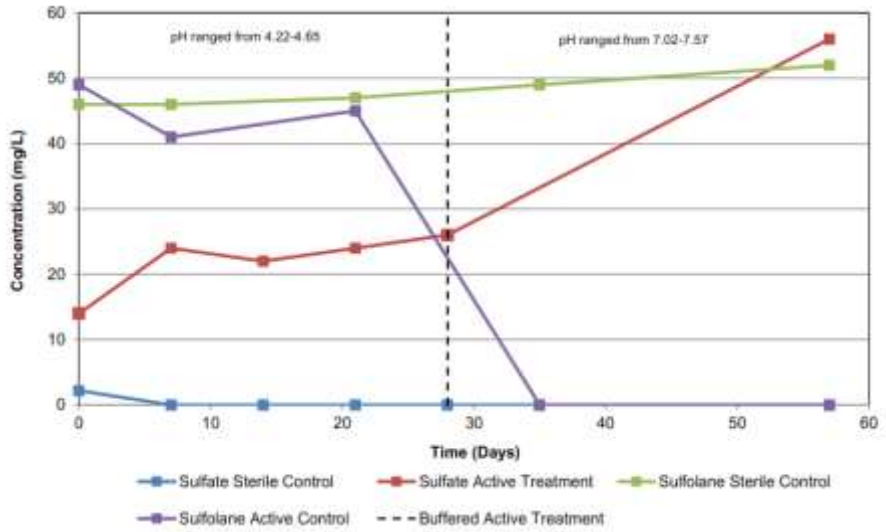
$$C_4H_8O_2S + 6.5O_2 \rightarrow 4CO_2 + 3H_2O + 2H^+ + SO_4^{2-}$$ (Greene et al., 2000)

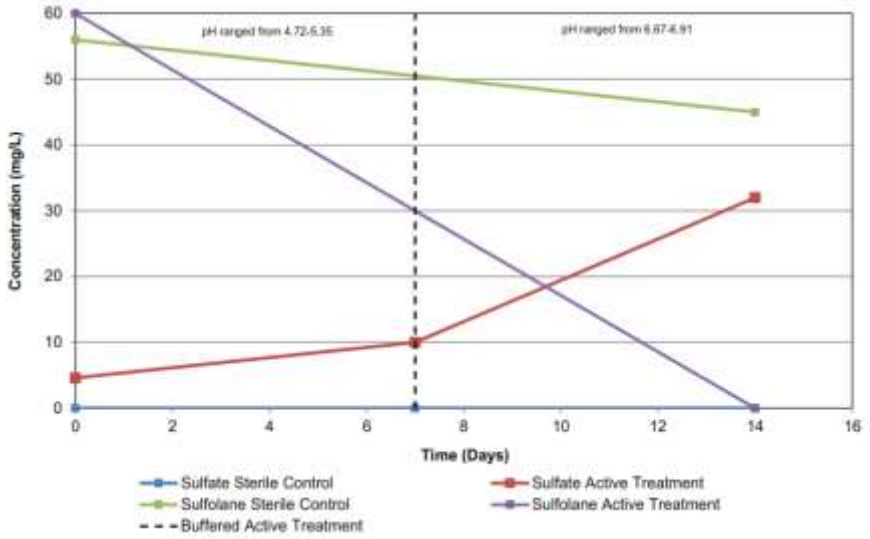


# Stage 2: Enrichment Culture Study

$$C_4H_8O_2S + 6.5O_2 \rightarrow 4CO_2 + 3H_2O + 2H^+ + SO_4^{2-}$$ (Greene et al., 2000)

- Treatability study was completed again enrich for sulfolane degraders
- Sulfolane was the only organic carbon source in these incubations
- Repeated sulfolane decrease from 50-60 ppm down to non-detect in 2-4 weeks in aerobic culture inoculated from biosparge area



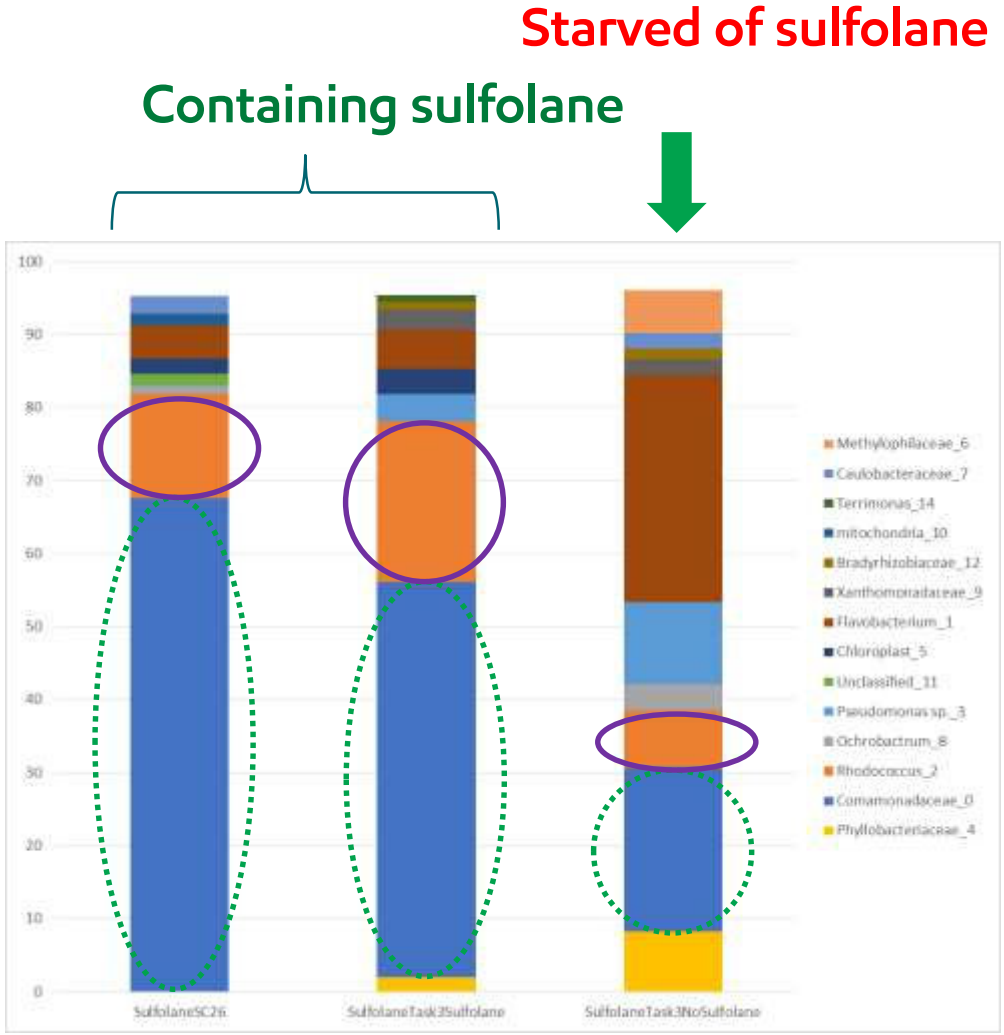



# Stages 3-5: Biomarker Development

- Created enrichment cultures in media containing sulfolane as the only organic carbon source

- Reduced total microbial community diversity down to 14 organisms with 2 putative taxa identified as potential sulfolane degraders based on increased relative abundance when incubated with sulfolane
  - *Comamonadaceae*: Family contains several previously described sulfolane degraders (*Acidovorax*, *Variovorax*, *Rhodoferax* spp.). **Isolate unable to degrade sulfolane**
  - *Rhodococcus*: Species contains previously described to desulfinate other cyclic organosulfur compounds (dibenzoethiophene sulfone). **Confirmed to be sulfolane-degrading isolate**

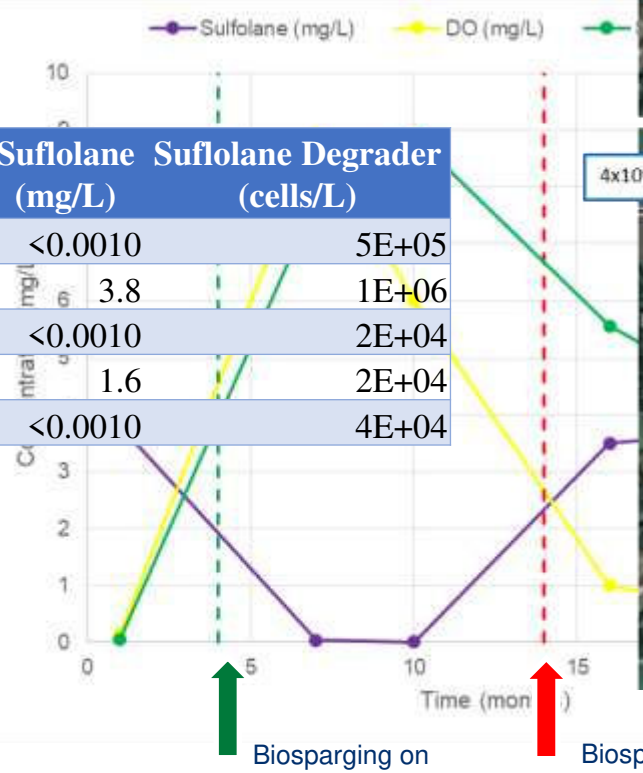
- qPCR assay and associated primers designed to target *Rhodococcus* isolate

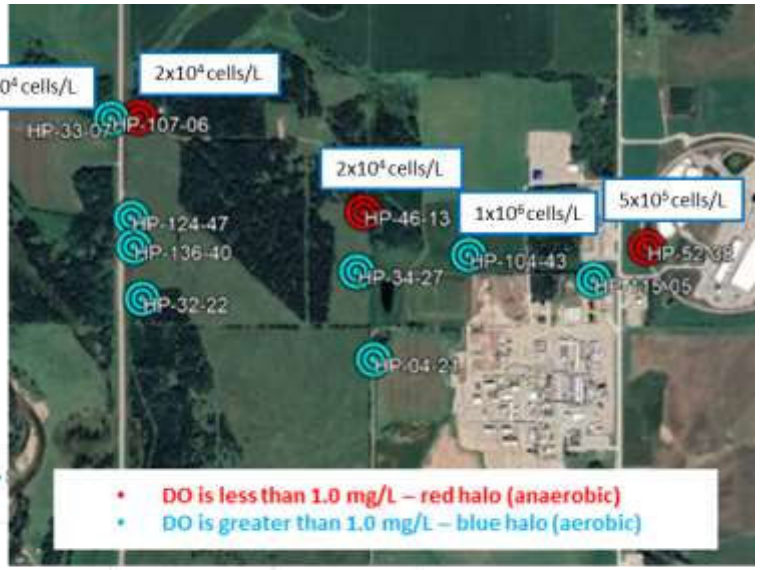


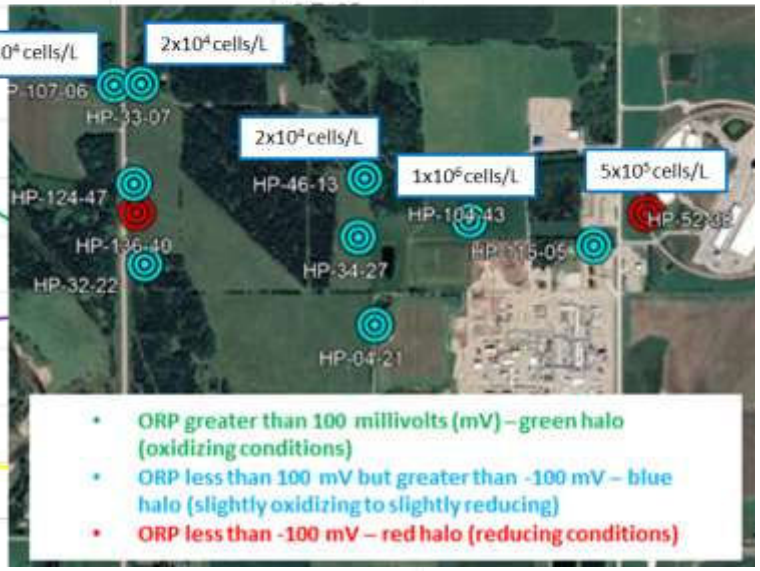
# MBT Method Development & Validation

- MBT (qPCR) method was developed for a sulfolane-degrading *Rhodoccocus* sp. present at the site and ground-truthing was completed against 10 site samples
  - 5 time-series samples in one well within the biosparging area
  - 5 samples across various wells
- qPCR method was applied to 10 site samples
  - 5 time-series samples in one well within the biosparging area
  - 5 samples down the plume length

| | Well ID | DO (mg/L) | ORP (mV) | Suflolane (mg/L) | Suflolane Degrader (cells/L) |
|---|---|---|---|---|---|
| **Upgradient** | HP-52-38 | 0.24 | -147.7 | <0.0010 | 5E+05 |
| **Source Area** | HP-104-43 | 2.75 | -65.3 | 3.8 | 1E+06 |
| **Downgradient** | HP-46-13 | 0.19 | -90.2 | <0.0010 | 2E+04 |
| | HP-33-07 | 0.86 | 54.8 | 1.6 | 2E+04 |
| | HP-107-06 | 1.89 | 93.1 | <0.0010 | 4E+04 |



# Conclusions & Future Efforts

# Conclusions & Future Efforts

**Conclusions**

- Biosparging successfully demonstrated for sulfolane remediation
- An increased understanding of site microbiology has increased our understanding of the potential for intrinsic sulfolane biodegradation at the site
- Molecular Biological Tools (e.g., qPCR) can improve conceptual site models, performance monitoring, and optimization of remediation technologies

**Future Efforts**

- Analyze additional field-samples with developed qPCR assay for sulfolane-degrader to gauge assay robustness for field-scale monitoring [in progress]
- Sequence genome of *Rhodococcus* isolate [in progress]
- Complete treatability study and track organism growth during sulfolane degradation to gauge potential use of qPCR data for rate estimation

E&PS GOLDER MEMBER OF WSP SiREM

# Thank you!
# Questions or Comments?

Linda Eastcott: linda.j.eastcott@esso.ca
Phil Dennis: pdennis@siremlab.com

E&PS