



Discovery, and Characterization of a Novel Sulfolane-Degrading Microbe at a Former Gas Plant Site

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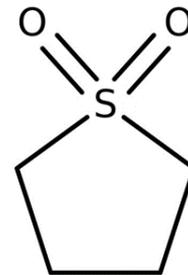


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Introduction to Sulfolane



- Industrial solvent used in:
 - Sour gas sweetening by Sulfinol process
 - Pharmaceutical production
 - Electronics for cleaning and etching circuit boards

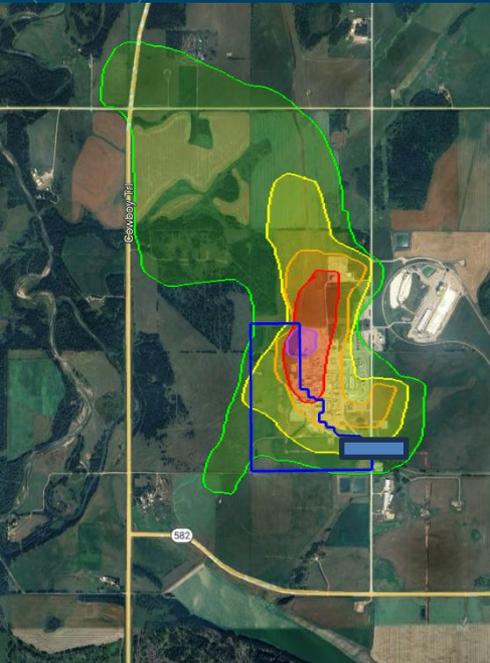


- Cyclic ether with some properties similar to 1,4-dioxane, is miscible in water

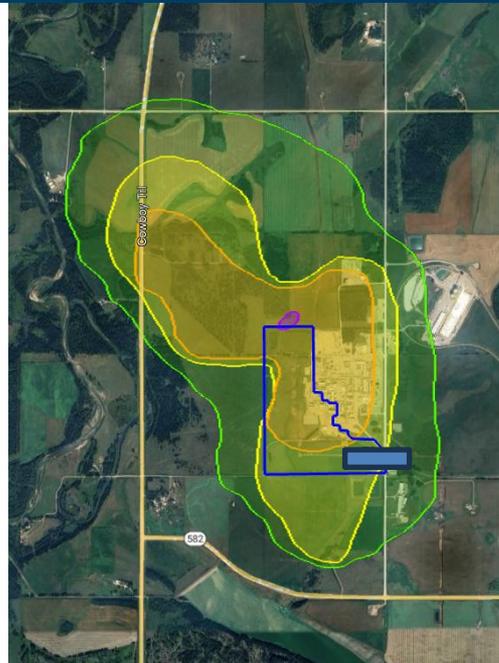


- Health impacts of sulfolane in drinking water are unclear:
 - No US-EPA Maximum Contaminant Level (MCL) for sulfolane,
 - Health Canada drinking water screening value of 0.3 mg/L.

Former Gas Plant Site – Sulfolane Distribution



Shallow Plume (~0 to 25 mbgs)



Mixed Plume (~25 to 50 mbgs)



Deep Plume (>50 mbgs)

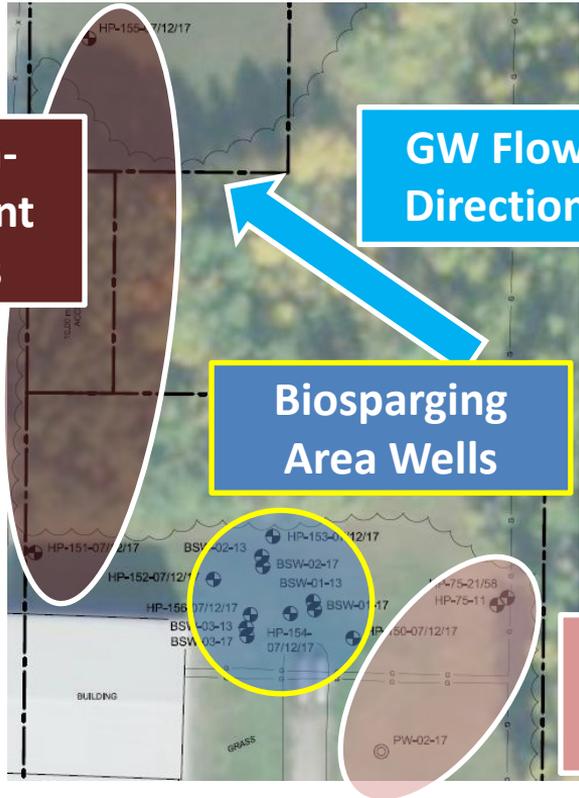
Sulfolane
Concentration
Contours

RDL to 0.09 mg/L
0.09 to 0.8 mg/L
0.8 to 10 mg/L
10 to 50 mg/L
>50 mg/L

Biosparging and Monitoring Well Network



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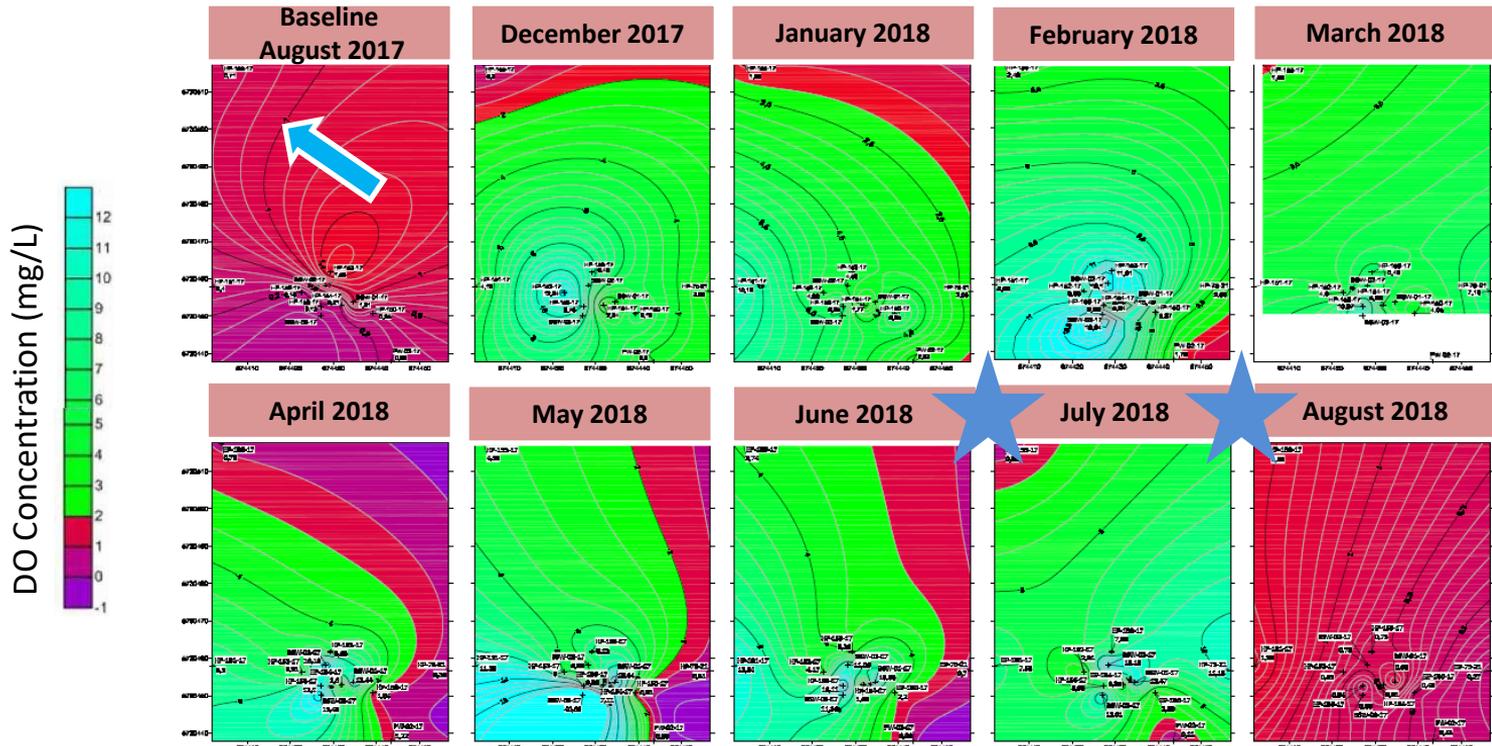


Biosparging Wells

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

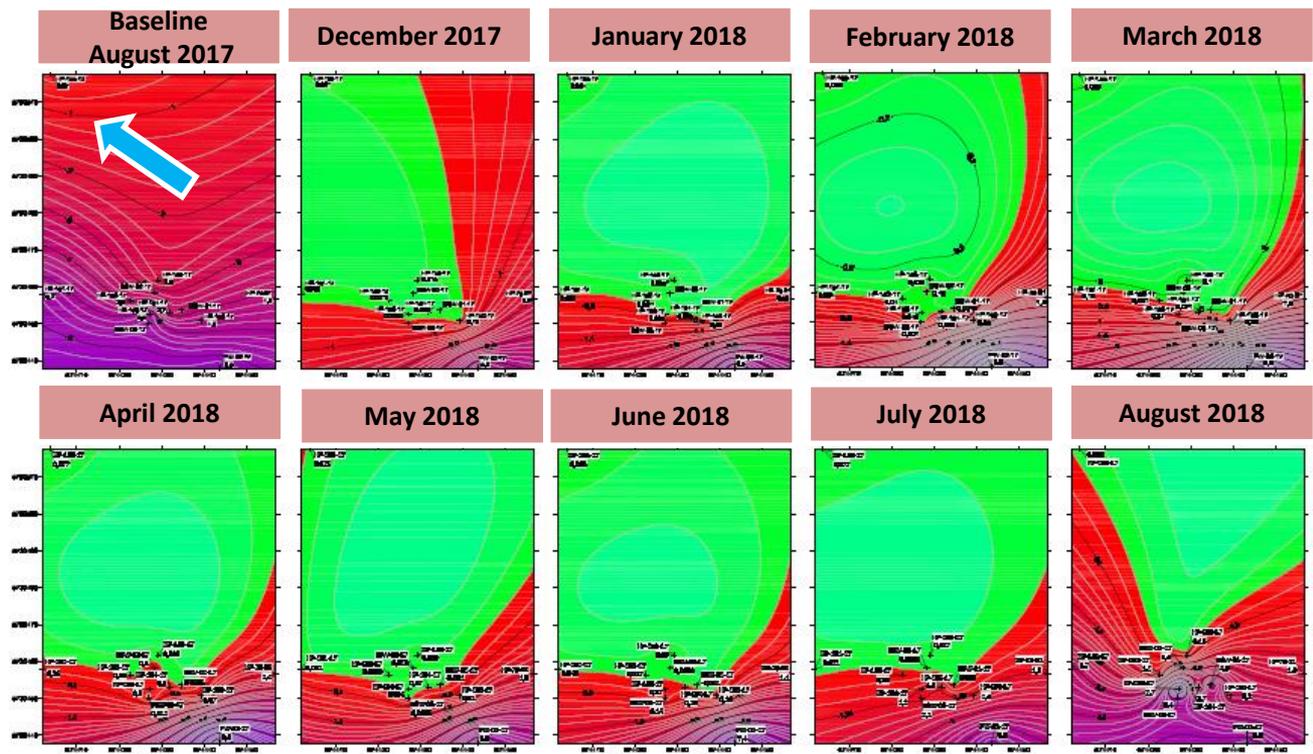
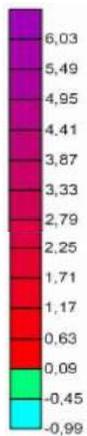


Dissolved Oxygen Concentrations – 17m Interval



Sulfolane Concentrations – 17m Interval

Sulfolane Concentration (mg/L)



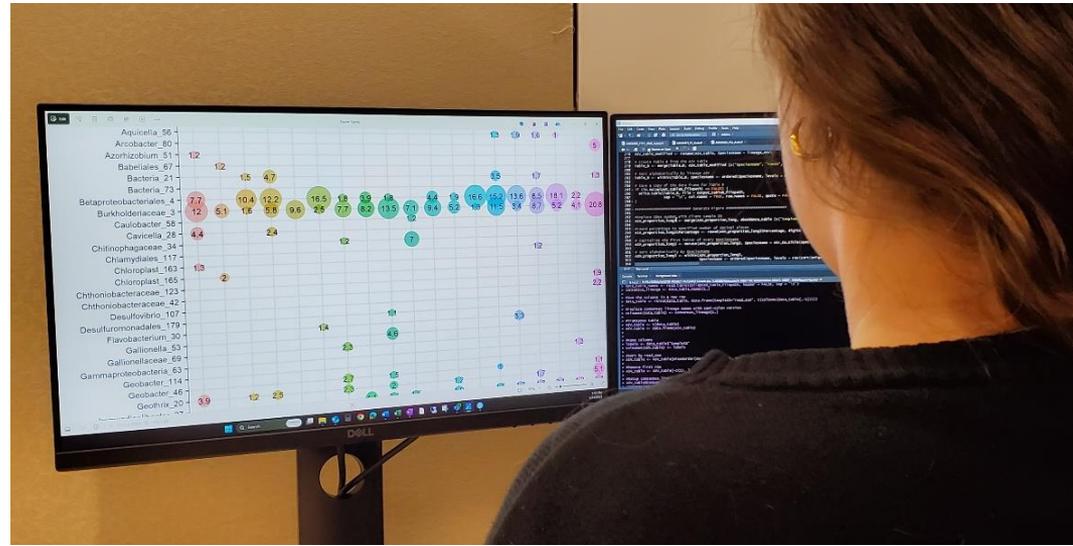
Key Questions /Study Goals



Biosparging decreased sulfolane concentrations in site groundwater

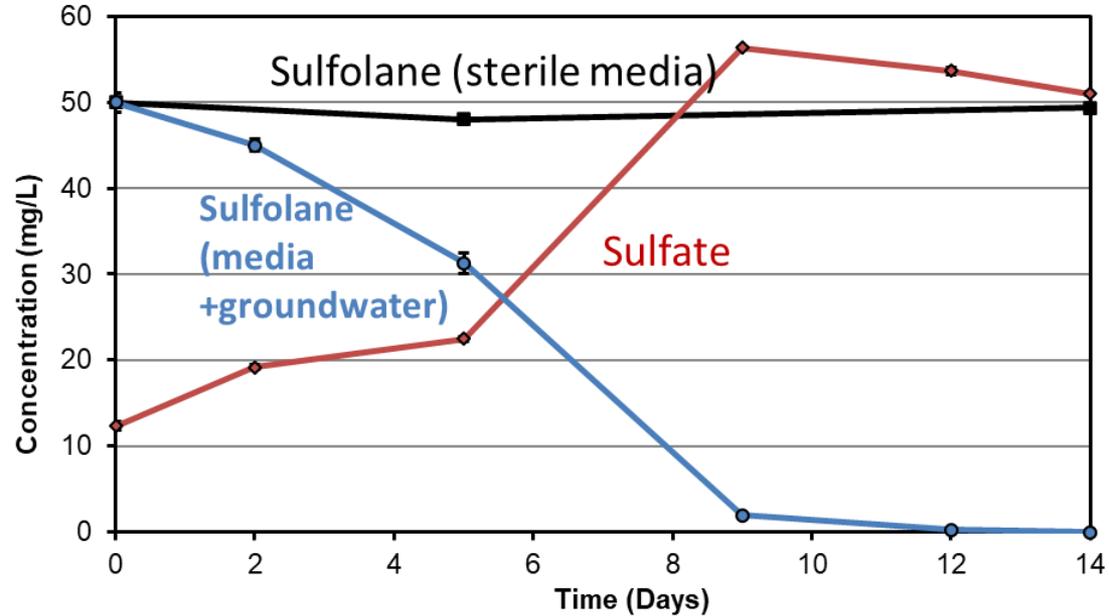
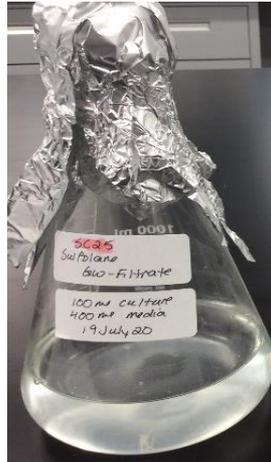
- Were the observed decreases under biosparging due to aerobic biodegradation or some other process? e.g. physical displacement
- Is sulfolane degradation occurring in the absence of biosparging? i.e., is MNA for sulfolane feasible?

Characterization of the structure and function of the microbial community needed to clarify the above



Stage 1: Does Site Groundwater Harbour Sulfolane Degraders?

- Degradation of sulfolane demonstrated in batch reactors inoculated with groundwater
- Sulfolane degrading enrichment culture SC25 developed

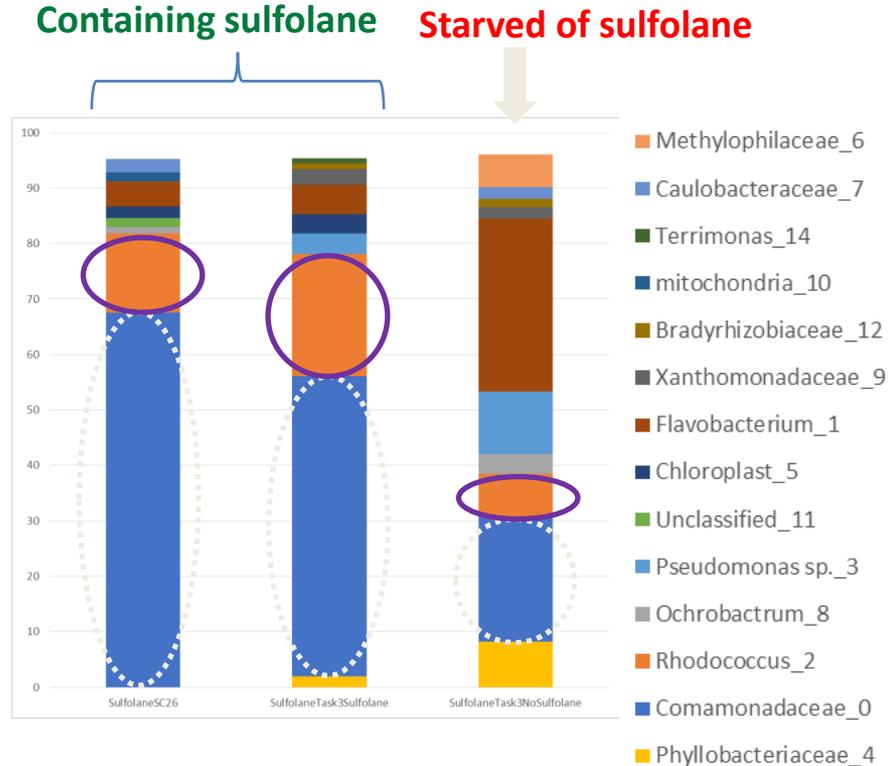


Stage 2: Characterization of Enrichment Culture

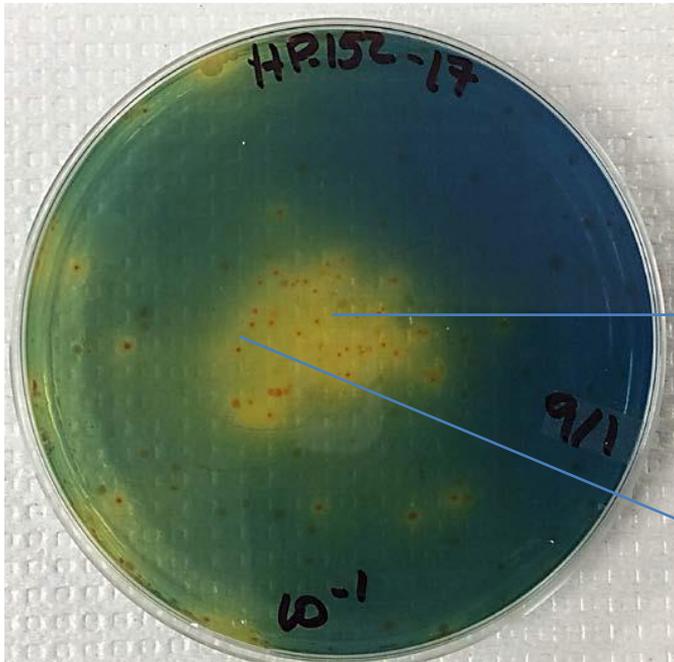
Next generation sequencing (NGS) of 16S rRNA amplicons used to characterize enrichment culture

14 organisms total, 2 taxa identified as potential sulfolane degraders based on increased relative abundance when incubated with sulfolane

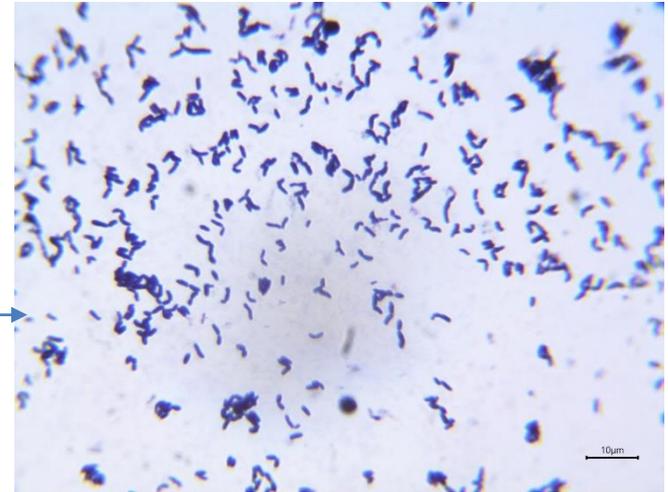
- *Comamonadaceae*: Family contains several sulfolane degraders (*Acidovorax*, *Variovorax*, *Rhodoferrax* spp.) also *Delftia* which is dominant in SC25
- *Rhodococcus*: versatile genus previously described to desulfinate other cyclic organosulfur compounds.



Stage 3: Isolation & Identification of a Putative Sulfolane Degradator



Microscopy
Gram stain



16S rRNA gene
sequencing

Named *Rhodococcus* sp. strain SC25

Selective media used to isolate colonies of sulfolane degraders

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhodococcus sp. (in: high G+C Gram-positive bacteria) strain zw82 16S ribosomal RNA gene, partial sequence	Rhodococcus sp. (in: high G+C Gram-positive bacteria)	2272	2272	100%	0.0	99.84%	1284	MH337995.1
Rhodococcus pingshengii CS98 DNA complete genome	Rhodococcus pingshengii	2272	11185	100%	0.0	99.84%	6240414	AP023172.1
Rhodococcus sp. (in: high G+C Gram-positive bacteria) strain K1 16S ribosomal RNA gene, partial sequence	Rhodococcus sp. (in: high G+C Gram-positive bacteria)	2272	2272	100%	0.0	99.84%	1398	MF359740.1

Stage 4: *Rhodococcus* qPCR Test Development

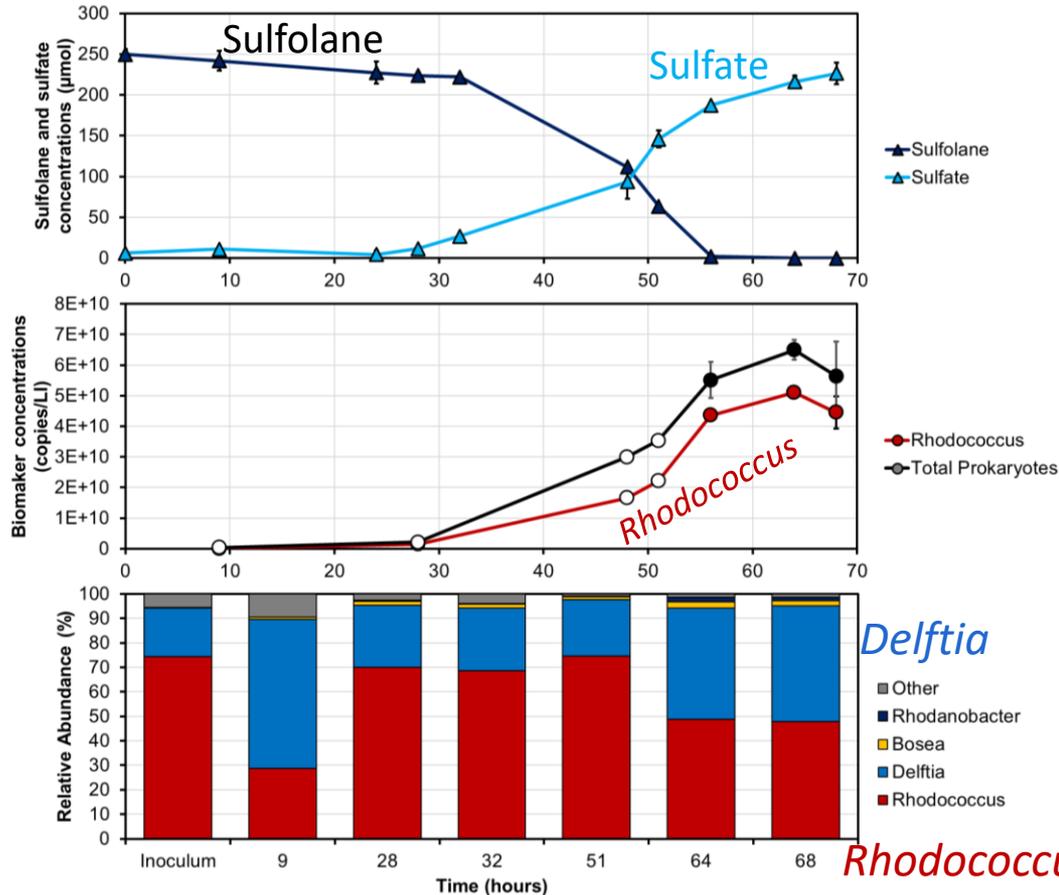
- 16S rRNA gene sequence from colony used to design PCR primers to track *Rhodococcus* SC25

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GGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT  
ATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA  
AACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGT  
GCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTGTCCGGAATTACTGGGCGTAAAGAGTTCGT  
AGGCGGTTTGTCGCGTCGTTTGTAAAACCAGCAG
```

- Test Prime (Silva) indicated PCR primers also match numerous other *Rhodococcus* (and a few *Nocardia*) so not completely specific for sulfolane degrader
- Identification of sulfolane degradation functional gene(s) would provide superior qPCR test target(s)



Time Course Experiments SC25 Culture



Conclusions:

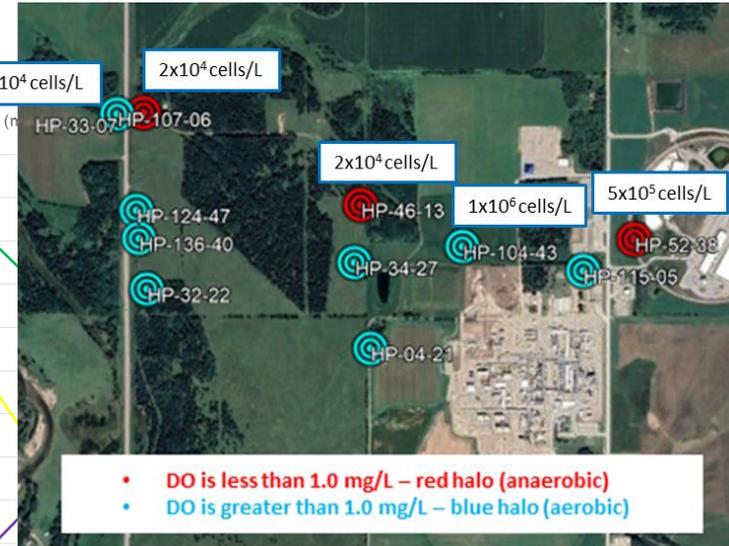
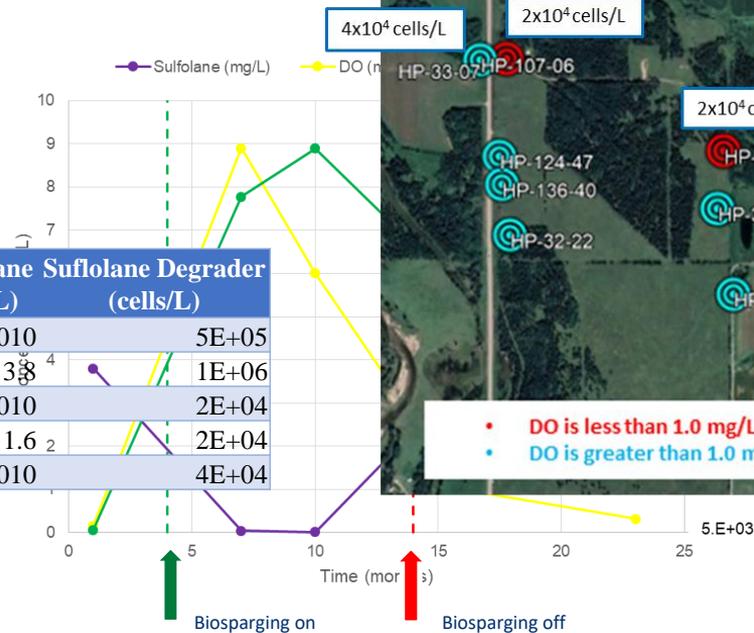
- Sulfolane Half-Life ~ 3.5 hours
- *Rhodococcus* SC25 (qPCR) increased during sulfolane degradation and declined after sulfolane exhausted
- *Rhodococcus* dominant (NGS) *Delftia* increase after sulfolane was degraded—growing on sulfolane breakdown products?

Rhodococcus qPCR Field Validation



- qPCR method was developed for *Rhodococcus* sp. present at the site and “ground-truthing” was completed
- qPCR method was applied to 10 site samples
 - 5 time-series samples in one well within the biosparge area
 - 5 samples down the plume length
 - *Rhodococcus* positively correlated with high sulfolane/high DO

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





Stage 5: Biomarker Discovery Genomics and Proteomics



Genome Sequencing of *Rhodococcus* SC25



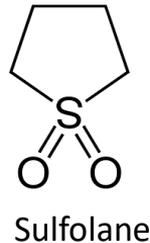
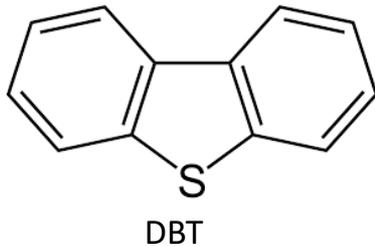
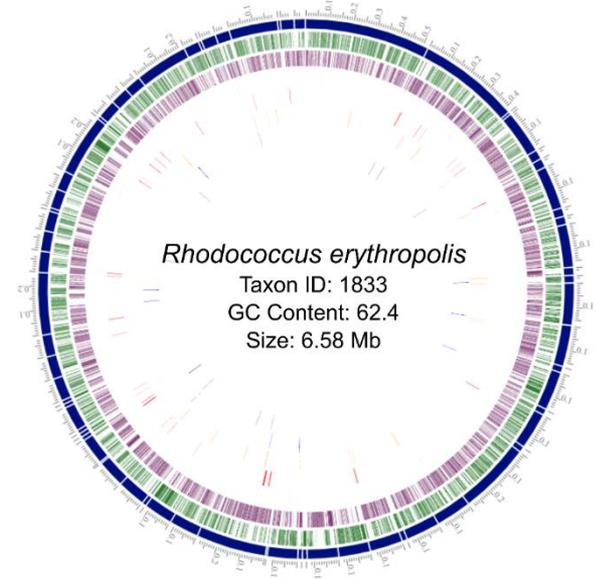
The metagenome of SC25 culture was sequenced and used as DNA “blueprint” for proteomics analysis

- 146,184 genes were identified
- 13 draft genomes were reconstructed (1 genome = 1 species)

Rhodococcus. genome was closely related to *R. erythropolis* known to degrade crude oil and dibenzothiophene (DBT) and able to grow at low temperatures (4°C)

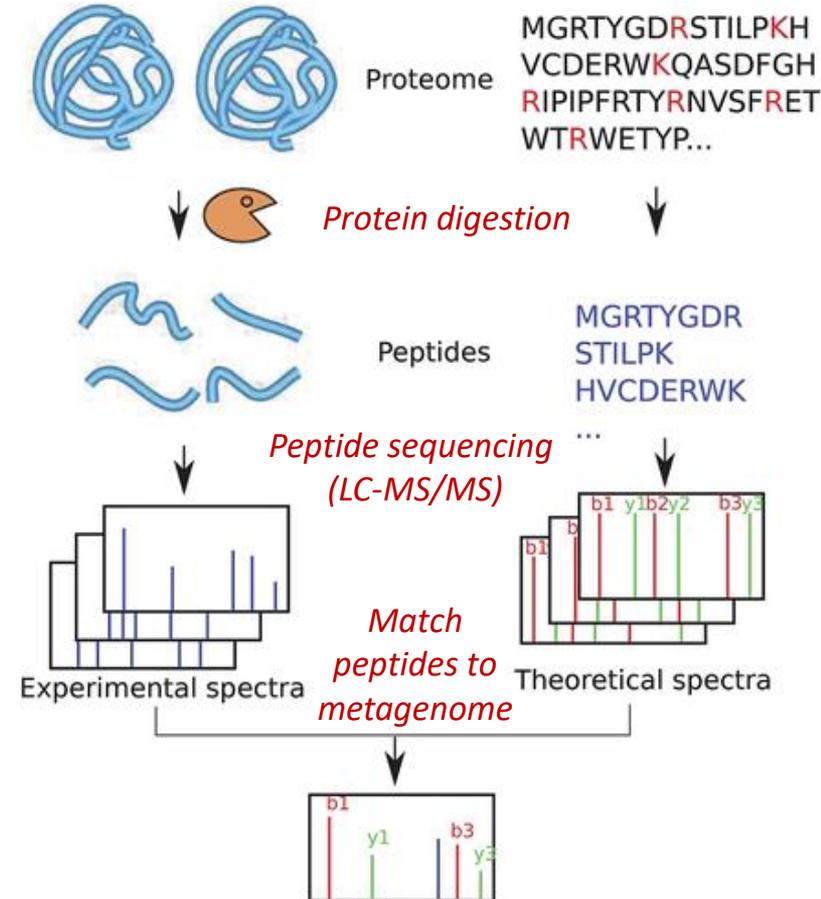
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Proteomics Summary

- Four rounds of proteomics conducted on SC25 culture to identify functional genes & corresponding enzymes involved in sulfolane biodegradation
- 697 unique proteins were identified (0.5% of total putative proteome)



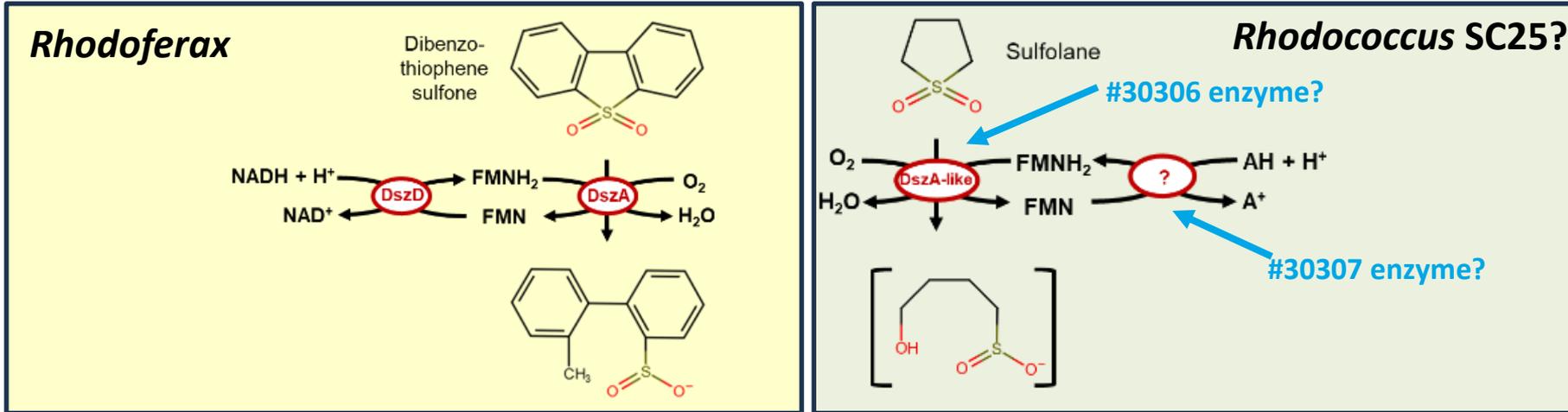
- Proteins matching a putative *Rhodococcus* gene cluster of unknown function were repeatedly identified in SC25 proteomes



- Functional analyses identified a putative monooxygenase (#30306) gene in this gene cluster

Sulfolane Monooxygenase: a Novel Biomarker

- Gene #30306 encodes a protein most structurally similar to dibenzothiophene sulfone monooxygenase (DszA), in *Rhodoferax* which is an enzyme suspected to also degrade sulfolane



- Gene #30306 most likely codes for “sulfolane monooxygenase”, the 1st enzyme in the sulfolane degradation pathway.
- PCR primers targeting Gene #30306 were developed to better track sulfolane degradation activity

Sulfolane Monooxygenase qPCR Testing of Site Samples

Sample ID	Sampling Date	Sulfolane Monooxygenase (SMO) Copies/L	% SMO	<i>Rhodococcus</i> 16SrRNA Gene Copies/L (SAR)	% SMO/SAR	Comment
HP-151-17	3-Mar-21	1.43E+04	0.13%	1.00E+07	0.1%	Near but Downgradient of Biosparging Zone
HP-151-17	8-Jun-22	ND	NA	Not Tested	NA	
HP-151-17	22-Nov-23	1.20E+05	0.01%	Not Tested	NA	
HP-152-17	3-Mar-21	2.04E+05	1.16%	2.00E+07	1.0%	Wells Within the Biosparging Zone
HP-152-17	8-Jun-22	6.15E+05	0.38%	Not Tested	NA	
HP-152-17	22-Nov-23	3.67E+05	0.09%	Not Tested	NA	
HP-102-65	24-Jun-24	9.79E+03	0.01%	5.91E+04	16.6%	Wells Closer to Source Zones
HP-54-21	29-May-24	2.55E+02	NA	1.87E+05	0.1%	
HP-53-08	24-Jun-24	8.97E+04	0.94%	9.20E+04	97.5%	
HP-66-30	24-Jun-24	4.06E+06	3.68%	5.06E+06	80.2%	
HP-51-09	24-Jun-24	1.10E+06	4.47%	1.08E+06	101.2%	
HP-33-07	27-May-24	ND	NA	ND	NA	Wells Closer to the Plume Fringe
HP-159-30	27-May-24	ND	NA	ND	NA	
HP-67-56	24-Jun-24	ND	NA	ND	NA	
HP-67-30	24-Jun-24	3.91E+03	NA	6.74E+06	0.1%	
HP-107-23	24-Jun-24	ND	NA	ND	NA	
HP-136-40	29-May-24	1.35E+03	NA	3.50E+04	3.8%	
HP-113-16	29-May-24	3.54E+01	NA	5.10E+05	0.0%	
HP-75-11	3-Mar-21	3.66E+05	2.54%	1.00E+07	3.7%	Wells Upgradient of Biosparging Zone
HP-75-11	12-Oct-22	5.99E+05	0.11%	5.00E+08	0.1%	
HP-75-11	22-Nov-23	8.70E+05	0.05%	Not Tested	NA	
PW-02-17	27-May-24	6.32E+03	0.01%	3.88E+05	1.6%	

Conclusions



Biosparging successfully demonstrated to decrease sulfolane concentrations, in groundwater. Was it aerobic biodegradation?

- A novel aerobic sulfolane degrader *Rhodococcus* strain SC25 was identified in groundwater that is not closely related to known sulfolane degraders
 - Was correlated with biodegradation of sulfolane in the lab and field using qPCR
 - A putative sulfolane monooxygenase has been identified
 - Additional lines of evidence that sulfolane is biodegrading in situ
- Molecular biological tools (e.g., qPCR) can improve conceptual site models, performance monitoring, and optimization of remediation technologies

Questions?
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