

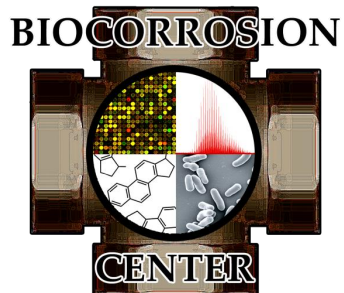
# The prevalence of thiosulfate-reducing fermentative bacteria in oil production facilities



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# Why Consider Thiosulfate Reducing Bacteria?



# Sulfidogenesis in the Pipeline Environment



**$\text{SO}_4^{2-}$**   
Sulfate

Dissimilatory Sulfate  
Reduction Pathway

**$\text{S}_2\text{O}_3^{2-}$**   
Thiosulfate

Multiple pathways

**$\text{HS}^-$**

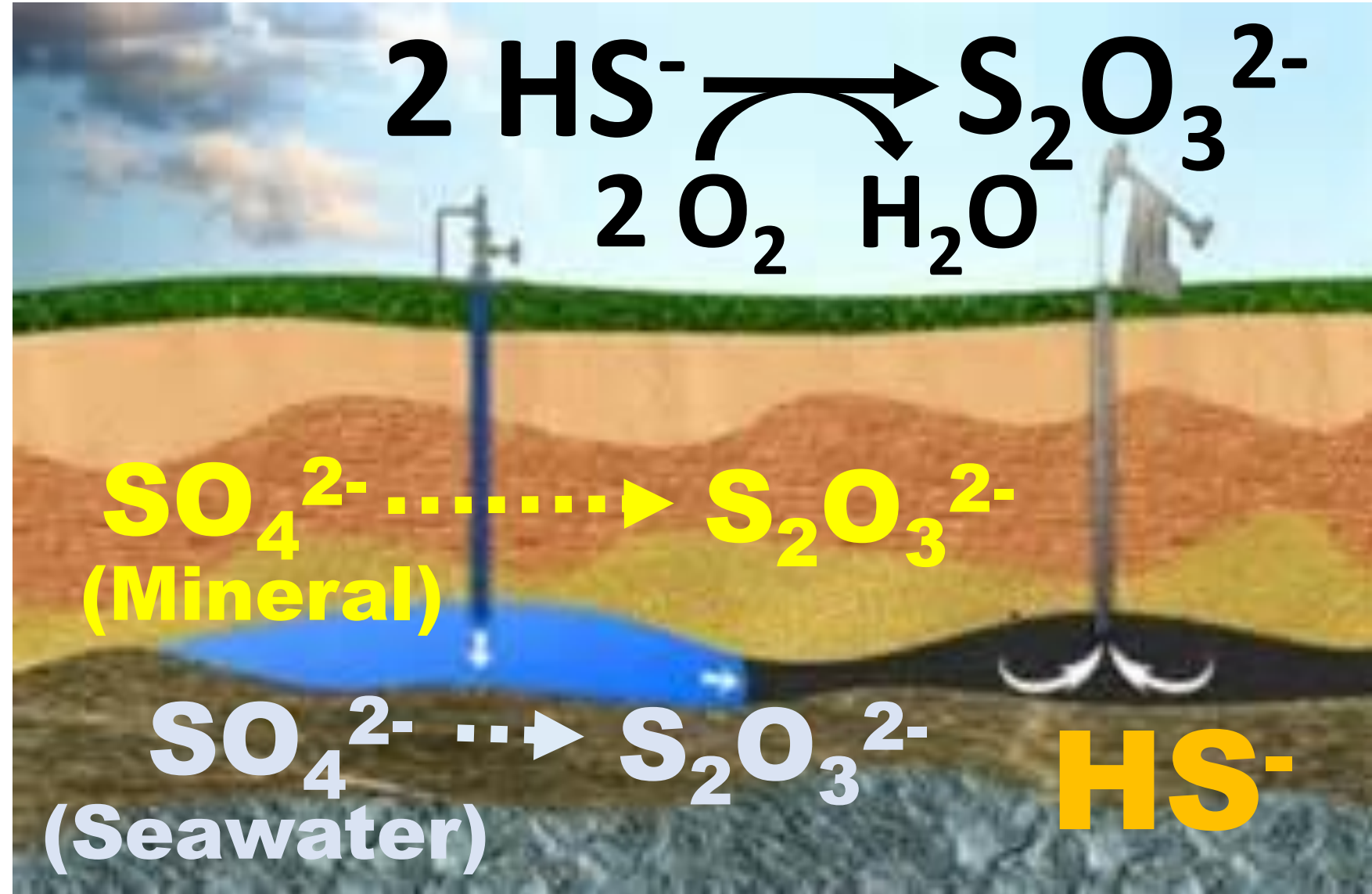
# Sources of Sulfate and Thiosulfate

## Sulfate

- Production waters
- Seawater
- **Interconversion to other sulfur anions**

## Thiosulfate

- Production waters
- Seawater
- **Oxidation of sulfides**

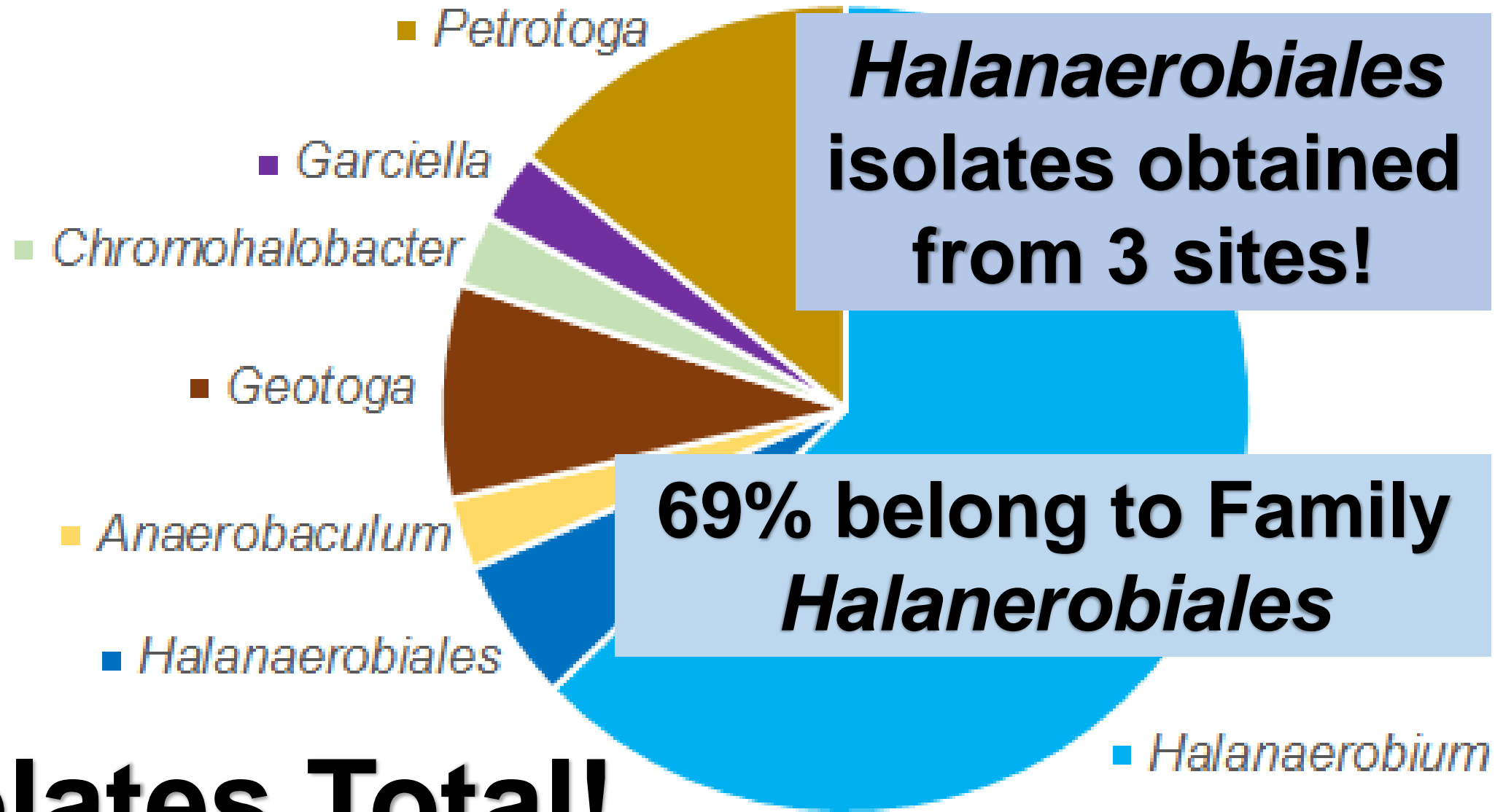


# Materials

Sample	[Cl <sup>-</sup> ] (Molar)	Temperature
Angola production waters	1.5	49°C
European production waters	2.5	37°C
Middle East Field production waters	2.5	49°C
Alaskan North Slope PIG Envelope	0.34	49°C

**High Temperature, High Salt Production Waters**

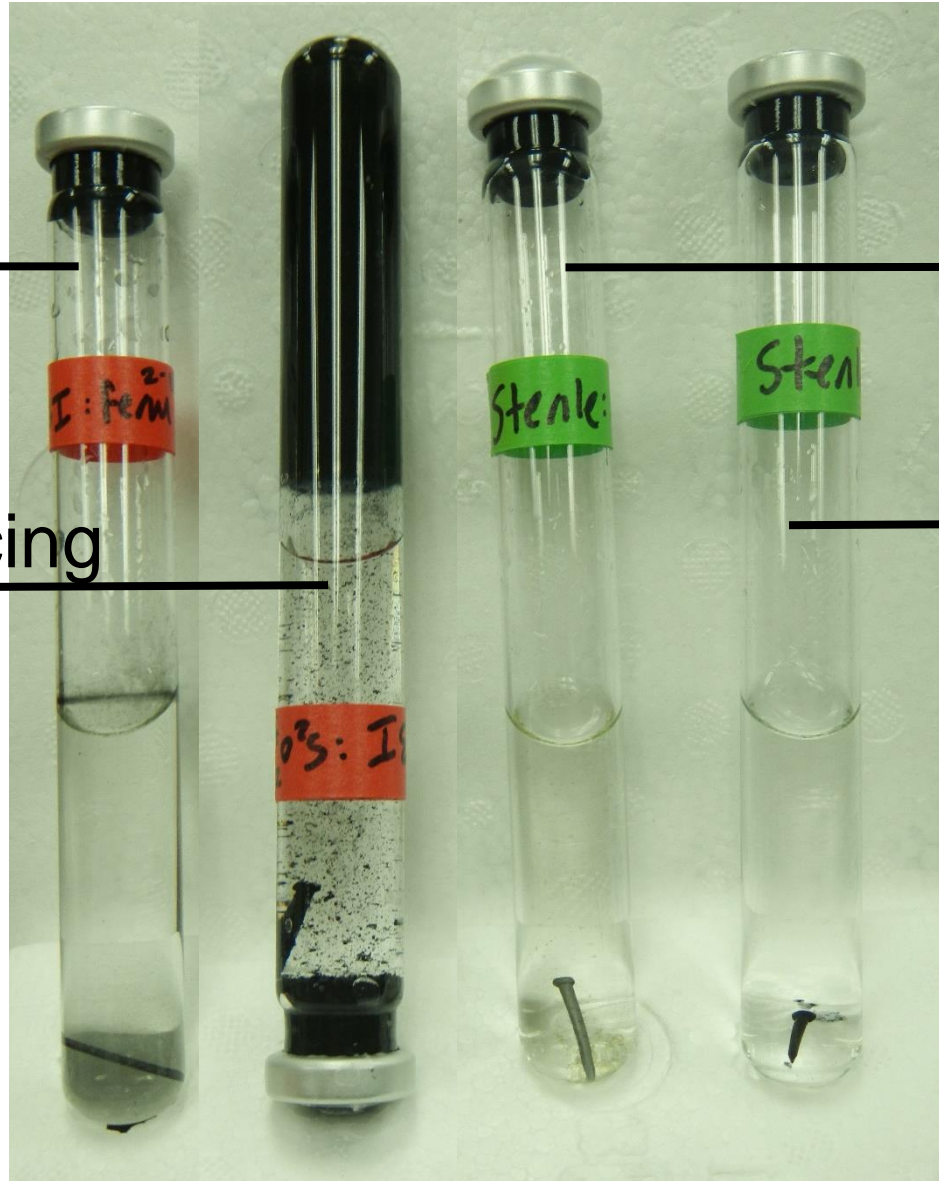
# Isolation Results



**35 Isolates Total!**

# Screening Isolates for Thiosulfate Reduction

Fermentative  
(No  $S_2O_3$ )



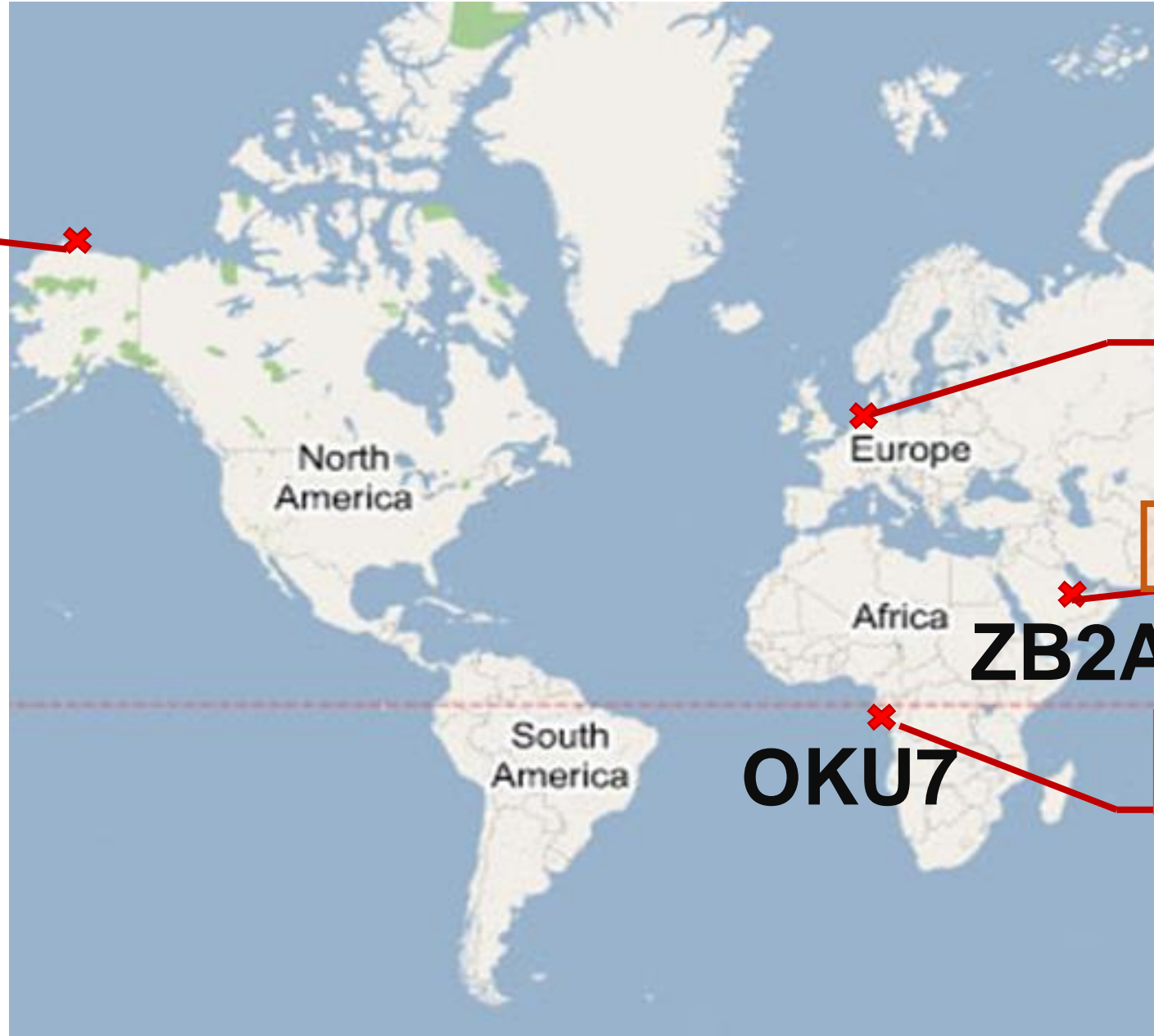
Sterile Fermentative  
(No  $S_2O_3$ )

Thiosulfate-reducing  
(10 mM  $S_2O_3$ )

Sterile Thiosulfate-reducing  
(10 mM  $S_2O_3$ )

Confirmation of  
thiosulfate reduction with  
terminal electron  
acceptor assay.

# Sulfide Producing Isolates



*Anaerobaculum* TR

**TR:**  
Confirmation of thiosulfate reduction using TEA assay

**SP:**  
Sulfide production when amended with thiosulfate

*Halanaerobium* SP

*Halanaerobium* SP / TR

ZB2A

*Halanaerobiales* SP / TR

OKU7

*Petrotoga* SP

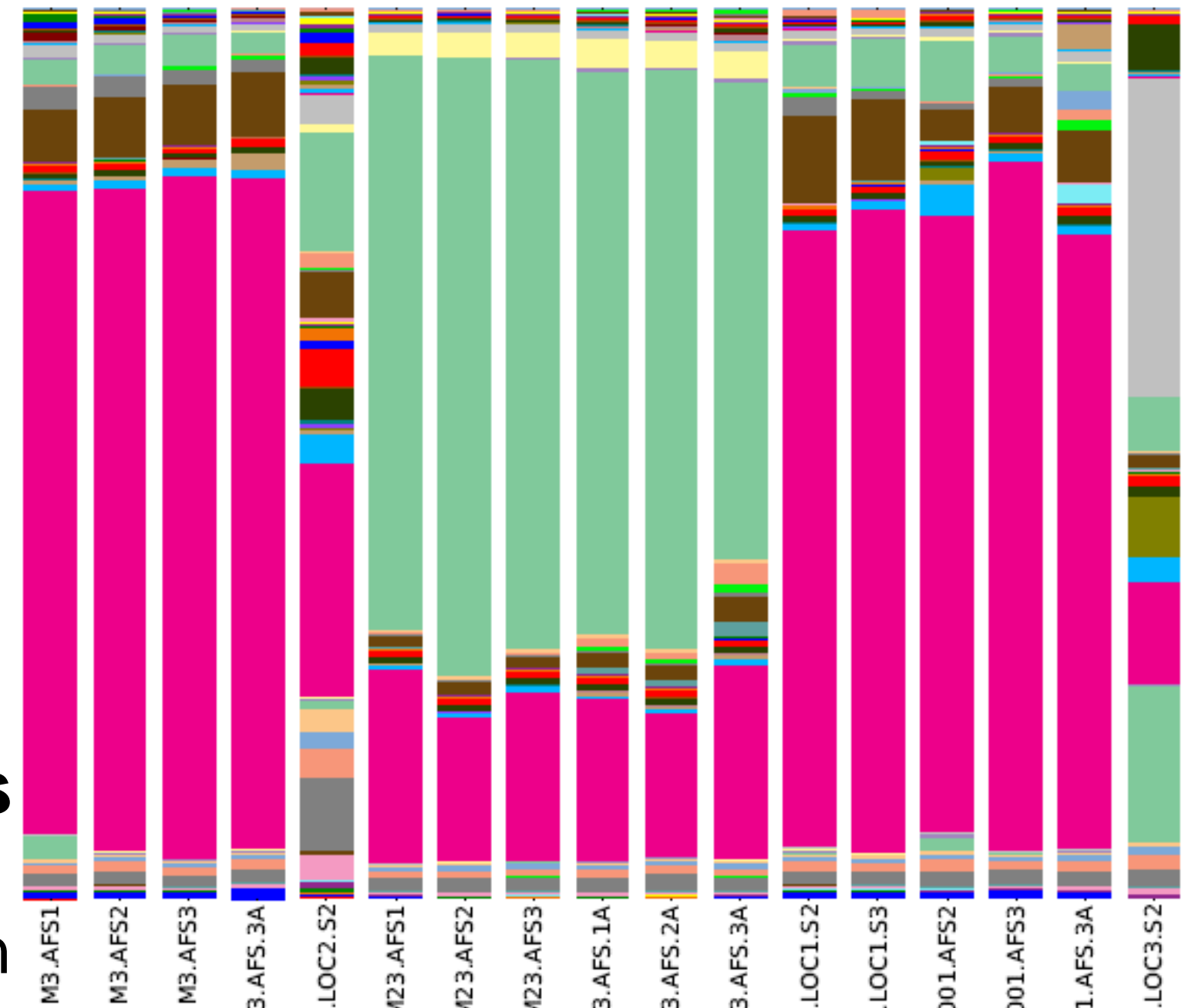


# Molecular Techniques

Middle East Field

*Halanaerobium!*

Anaerobic fermenters common to high salt, high temperature oil production waters.



Middle East Field 16S Results (OU Biocorrosion Center)

# Genus *Halanaerobium*

Family *Halanaerobiales*

Phylum *Firmicutes*

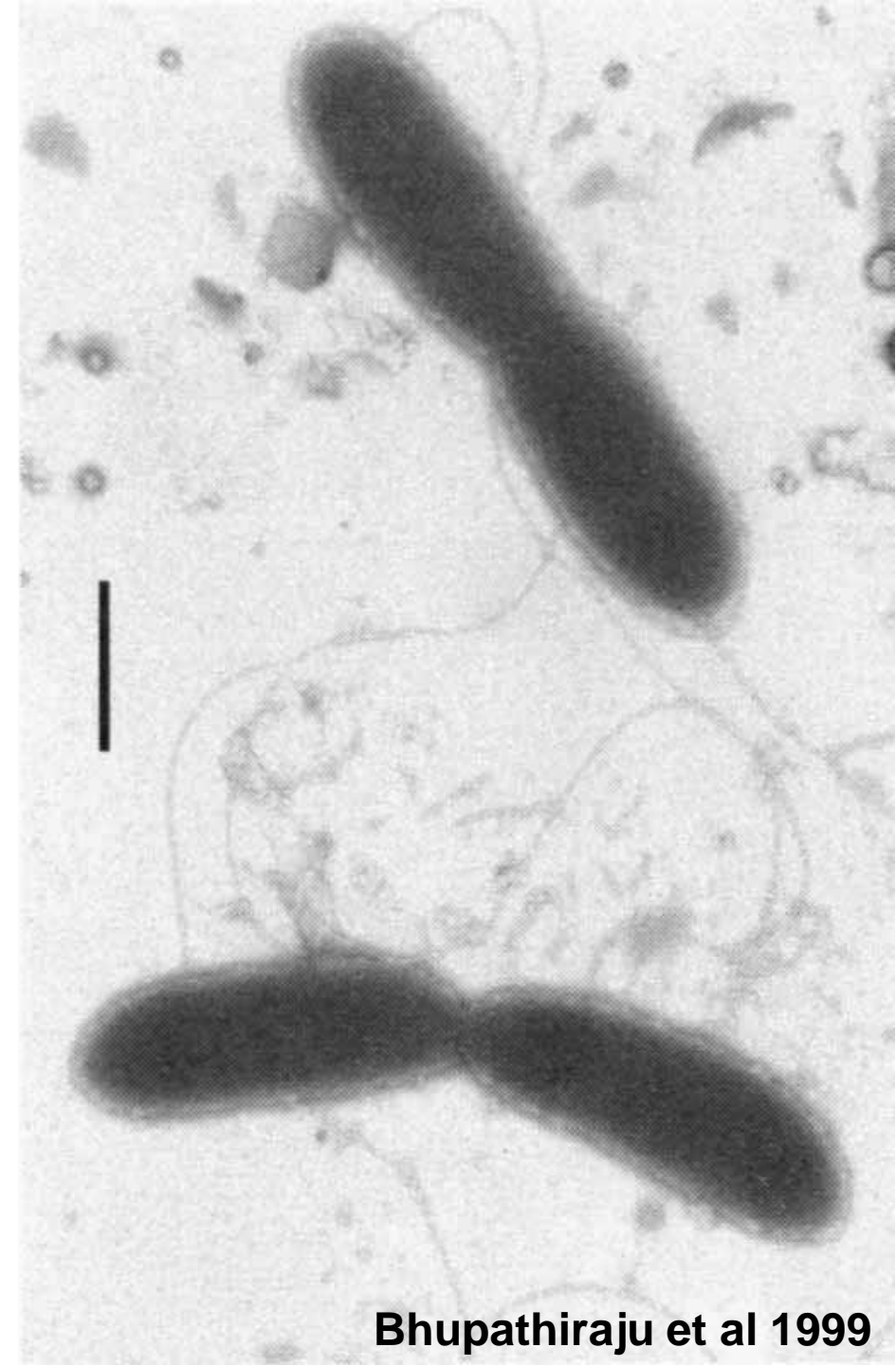
Obligate halophiles

Isolated from saline production waters,  
brine lakes, and microbial mats

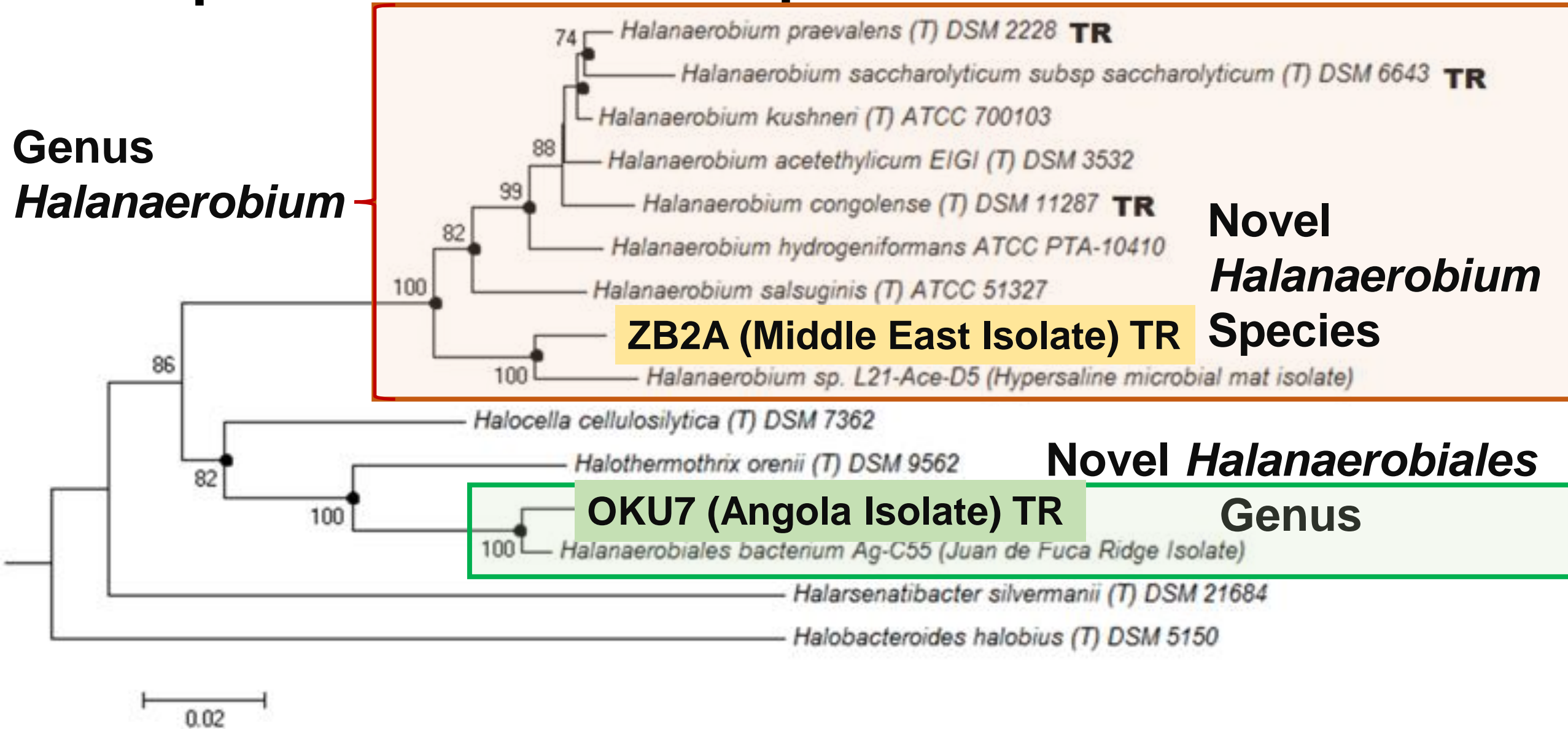
Fermentative metabolism

Do not reduce sulfate!

**3 species can reduce thiosulfate**



# Phylogenetic Relationship of Isolates with Respect to Cultivated Representatives



# ZB2A: Novel *Halanaerobium* Species

Temperature: 22-50°C, **50°C**

Salinity: 5-30%, **15%**

pH: 6.0-9.0, **6.5**

Can reduce thiosulfate  
and elemental sulfur



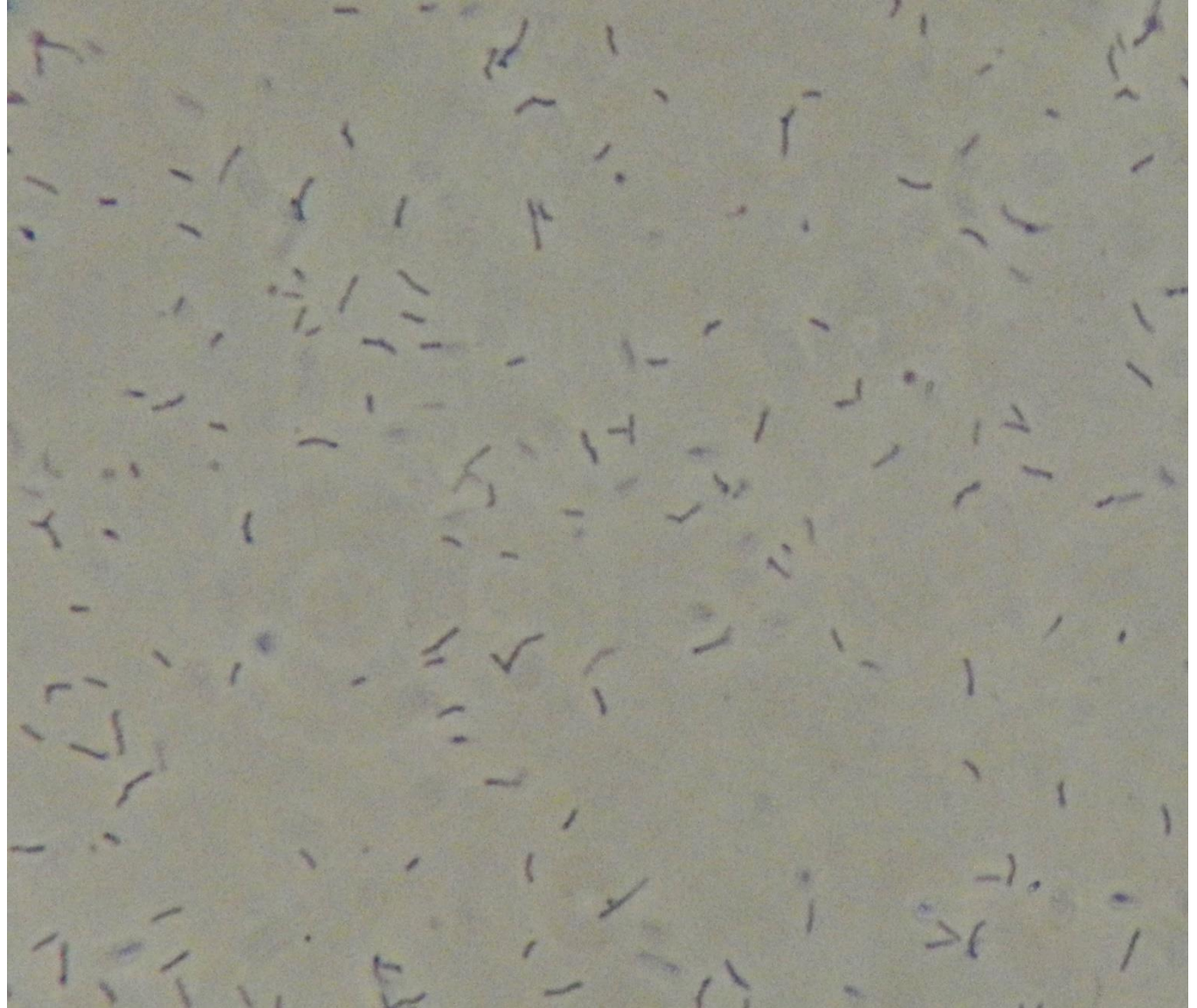
# OKU7: Novel *Halanaerobiales* Genus

Temperature: 37-60°C, **45°C**

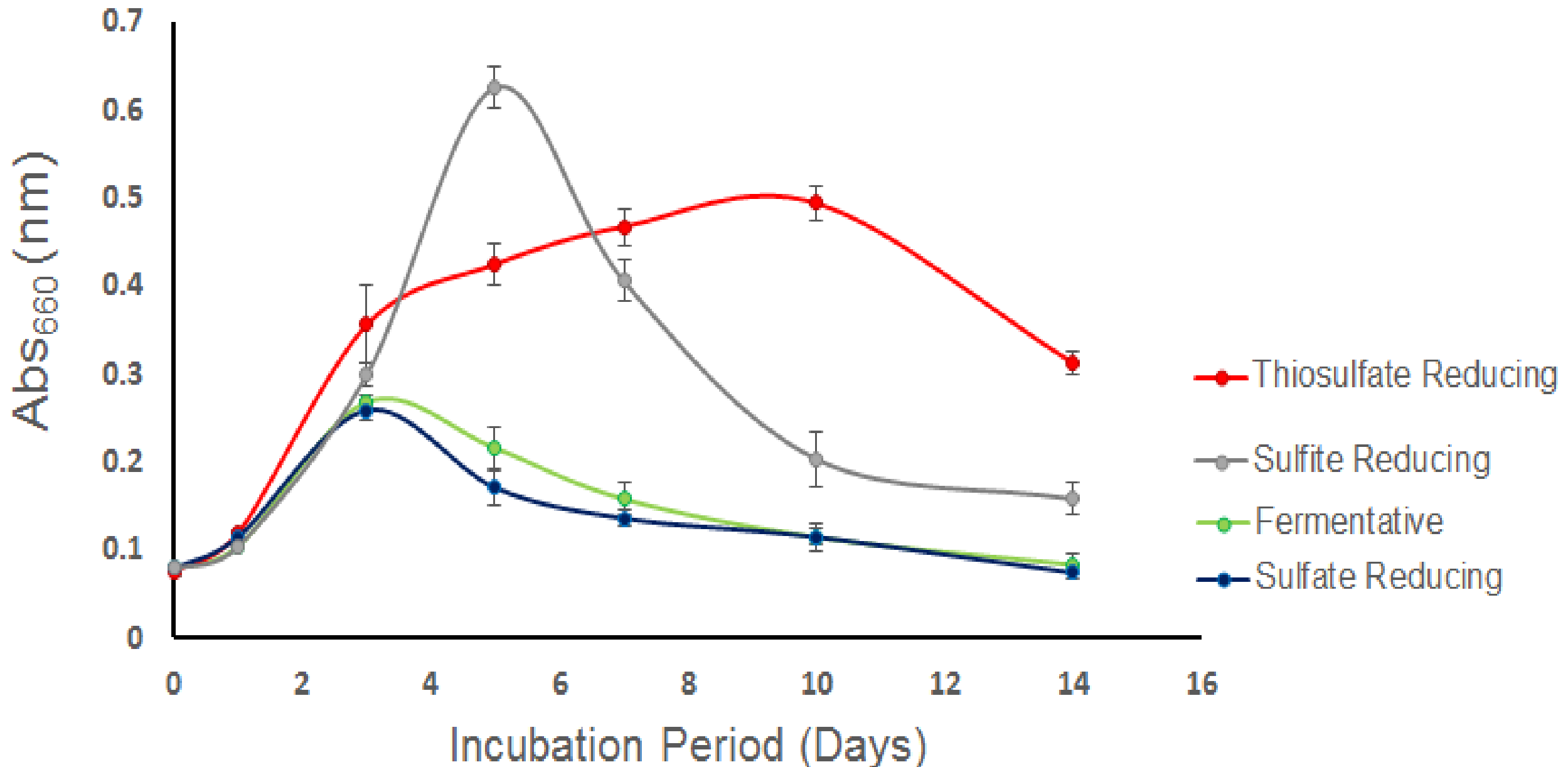
Salinity: 1-9%, **7%**

pH: 6.0-7.5, **6.5**

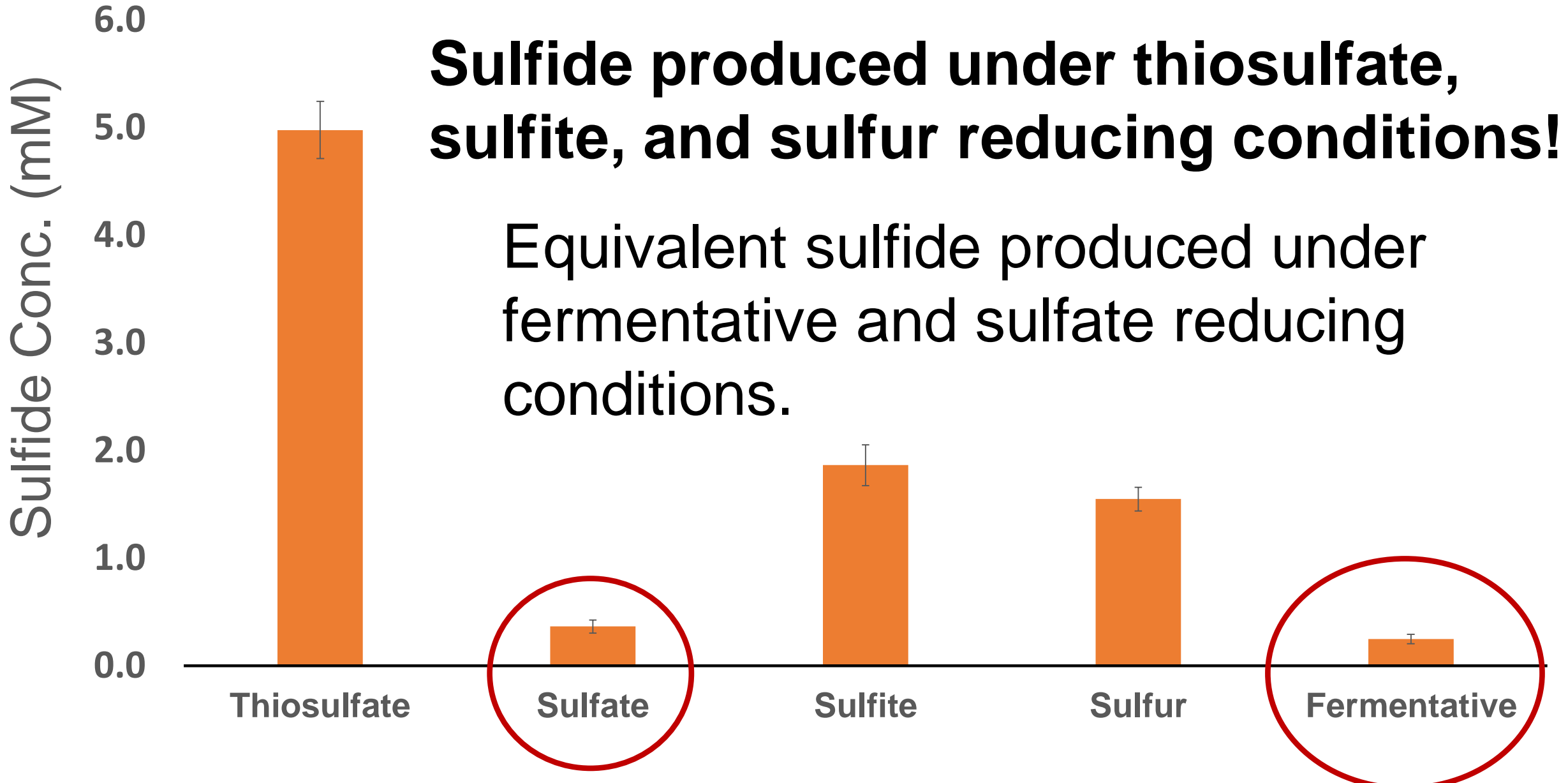
Can reduce  
thiosulfate, sulfite,  
and elemental sulfur



# Growth of OKU7 in the Presence of Sulfur Anions



# OKU7: Sulfidogenesis from Sulfur Compounds



# Role of *Halanaerobium* in Biocorrosion

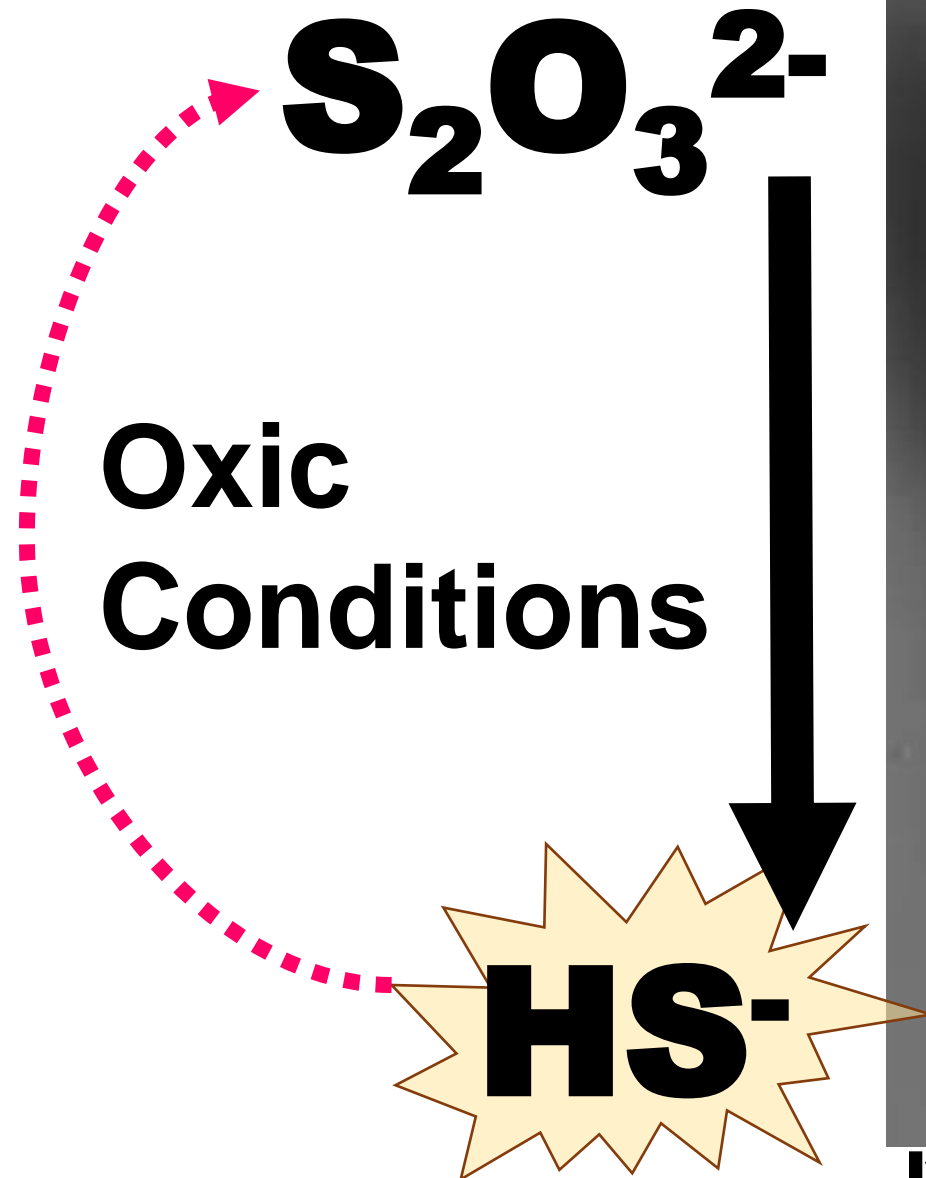
- Corrosive fermentation products

**CO<sub>2</sub> and Acetate**

**Volatile fatty acids**

- Sulfide produced from thiosulfate reduction

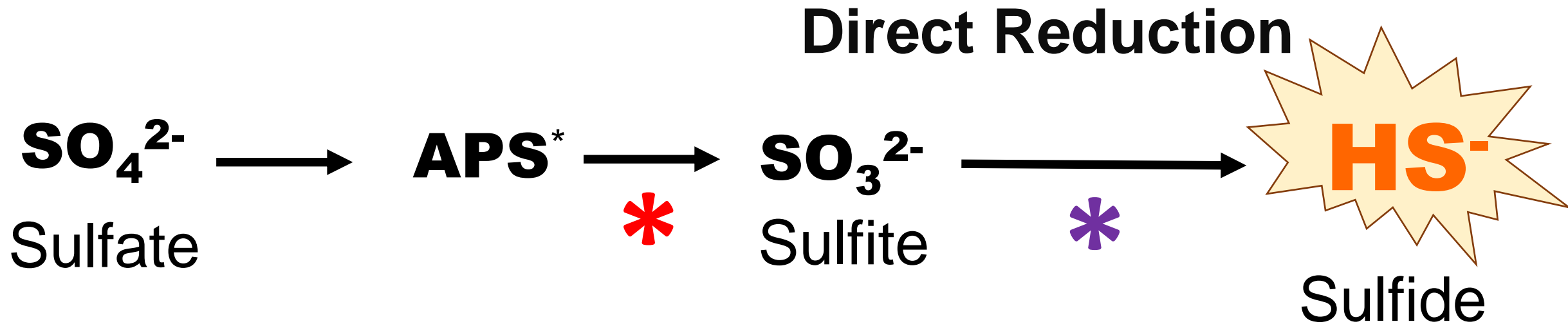
- Thiosulfate can be regenerated abiotically.



Ivanova et al 2011



# Target Genes for Sulfate Reduction

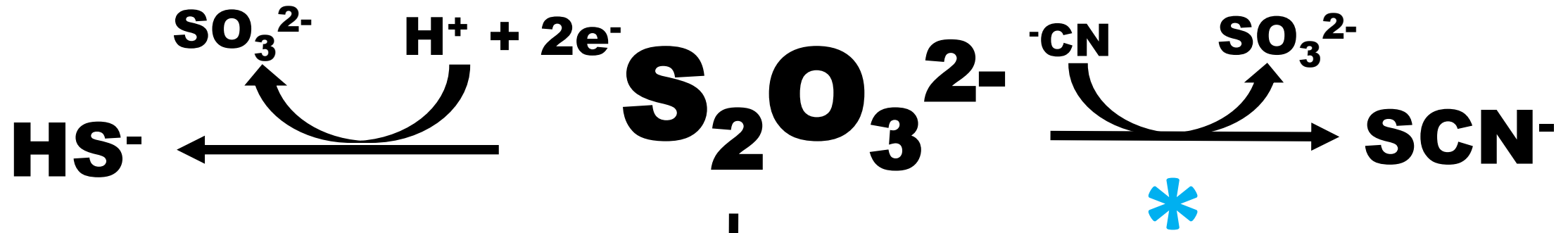


## SRB Target Genes:

- \*** = APS (Adenosine-5'-phosphosulfate) Reductase
- \*** = DSR (Dissimilatory sulfite reductase)

# Potential Target Genes for Thiosulfate Reduction

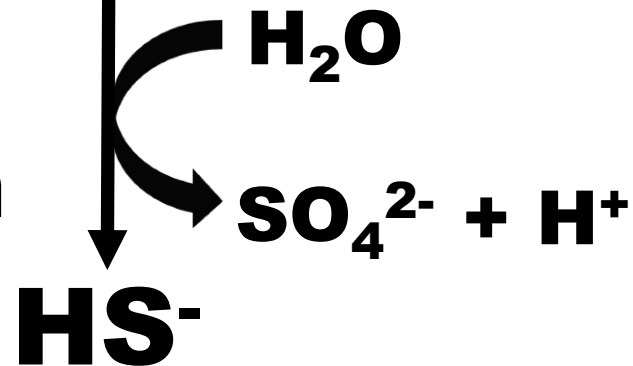
Thiosulfate reductase



Rhodanese-like proteins

Thiosulfate

Disproportionation



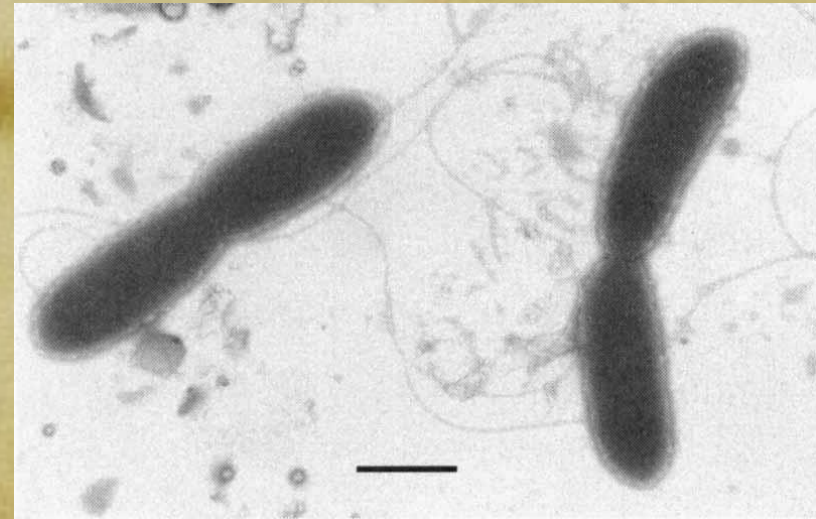
# Take home message

- SRB are not the only outlaws!
- TRB > SRB in some pipelines.
- TRB specific detection is needed.
- How do TRB produce sulfide?
  - Connect enzymatic mechanisms to gene targets

**WANTED**

**DEAD OR ALIVE**

*Halanaerobium*



**Guilty of sulfide production!  
Suspected of corrosive crimes!**

# Acknowledgements

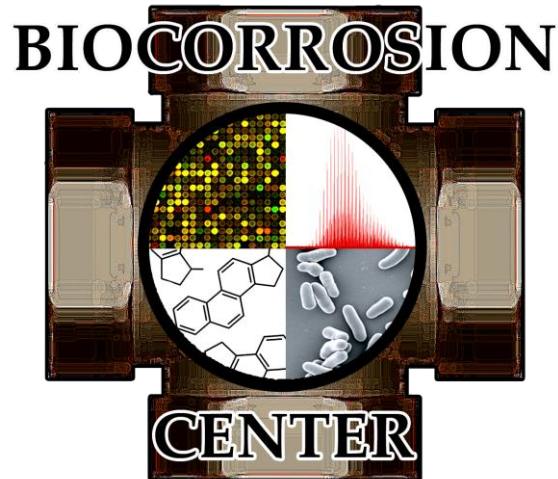
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**Funded by  
OU Biocorrosion  
Center**

## Special Thanks:

Dr. Neil Wofford

Dr. Athenia Oldham

Dr. Deniz Aktas

Dr. Tiffany Lenhart

Chris Marks

Renxing Liang

Katy Brown

Vince Sandifer

**Thank you for your time.**



**I welcome your questions and comments!**