Genomics 101:
How genomics can assist in MIC management

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geno-MIC
microbiologically influenced corrosion

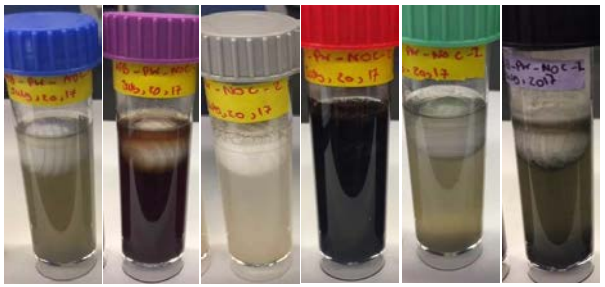
Microbiological Monitoring

- Environmental samples can be monitored for the presence/activity of microorganisms in several ways – **most common ways in O&G industry:**
  - Enzyme tests – e.g., ATP assay
  - 'Bug bottles' – e.g., BART tests, MPNs
    - SRB
    - APB
    - HAB
  - **Molecular microbiological methods (MMM)**

    = Genomics

Microbiological Monitoring

- ATP assay
- ‘Bug bottles’ – e.g., BART tests, MPNs
  - SRB
  - APB
  - HAB

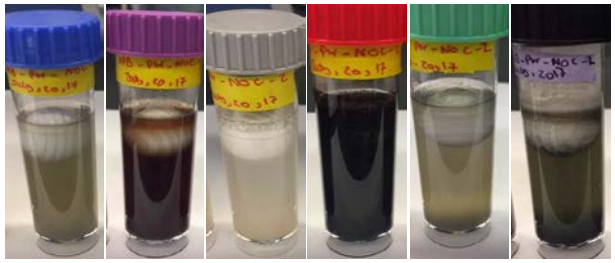


KPI – ‘number of bacteria’ for system monitoring

- ‘High’ numbers don’t necessarily mean MIC will be a problem
- ‘Low’ numbers don’t necessarily mean MIC won’t be a problem

Microbiological Monitoring

- ‘Bug bottles’ – e.g., BART tests, MPNs
  - SRB
  - APB
  - HAB
  - IRB, etc



Major Limitation:

- Growth based, **targets <1% of microbes in a sample**

  -e.g., captures only **some** SRB but not S^0 or thiosulfate-reducers

- Most microbes will not be captured in growth-based tests

Microbiological Monitoring

Molecular microbiological methods (MMM)

a.k.a. Genomics

- Newer approach, but now being widely used to identify microbes in O&G systems
- **Not growth based**

Based on DNA – life's blueprint!

Microbiological Monitoring

Genomics

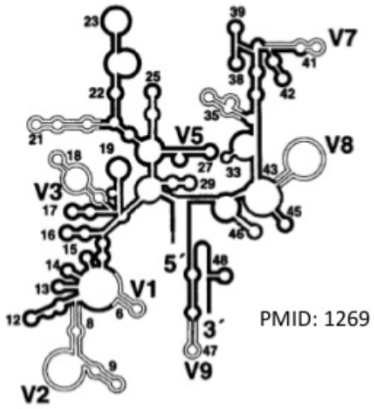
Based on DNA – life's blueprint!

- Uses genetic information contained in microbial cells to determine **what types of microbes and/or their potential metabolism (e.g., sulfide production)**
- Not based on growth therefore better at capturing the diveristy of microbes in a sample

Genomics as a Monitoring Tool – 3 ways

1. 16S rRNA gene survey – commonly-used method

- Biological molecule present in all microbial life (involved in protein synthesis) – conserved and variable regions



16S rRNA

- Extract DNA
- AMPLIFY using primers in a PCR reaction – make millions and millions of copies of a part of the 16S rRNA gene
- SEQUENCE on an instrument
- **LIST of microbial names –** not quantitative but get a 'relative abundance'

Genomics as a Monitoring Tool – 16S

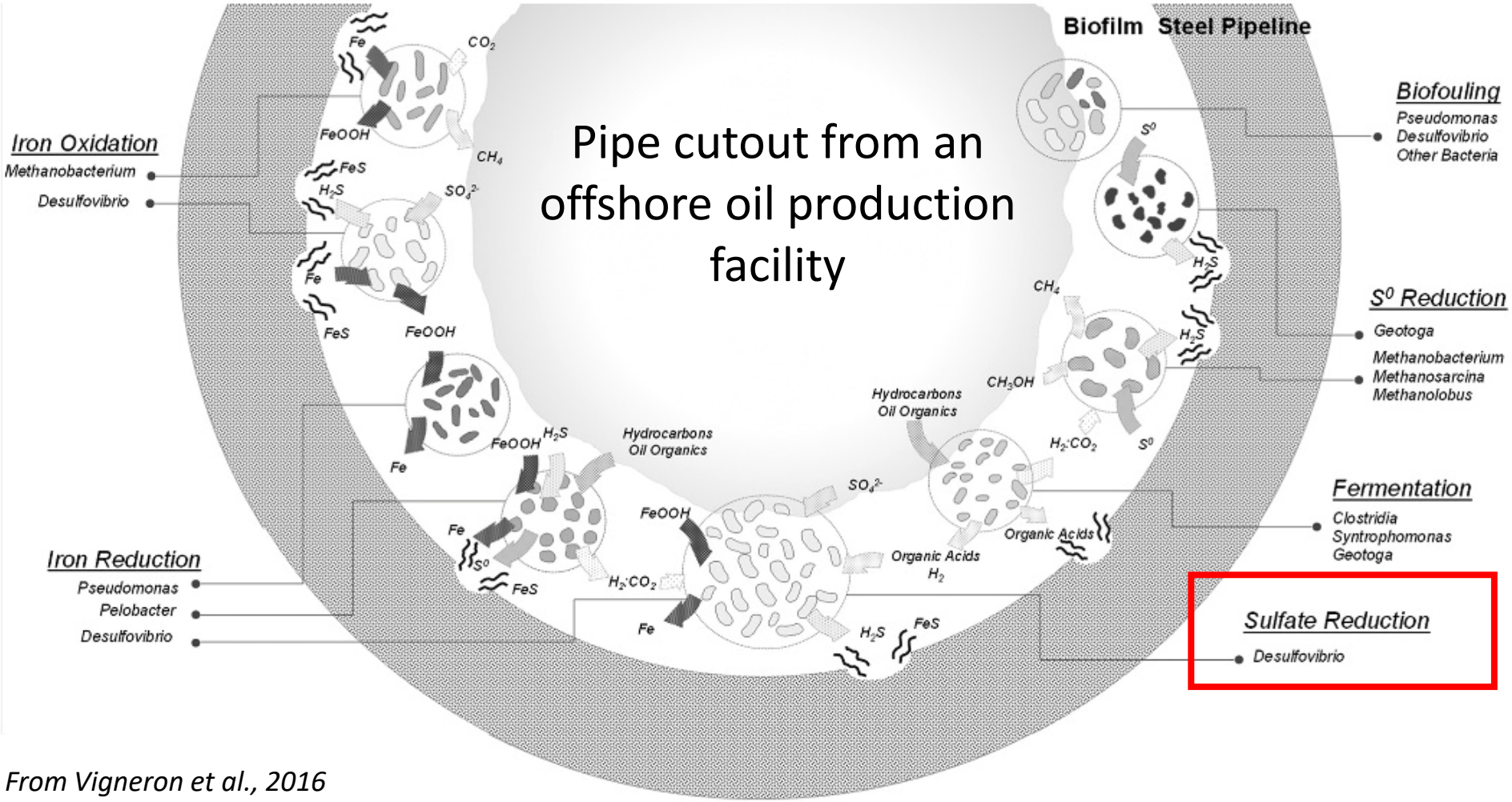
Relative abundance (% of total sequencing reads)

#Taxonomy (Class; Order; Family; Genus)	9FW 08/15	10TW 08/15	9FW 01/15	10TW 01/15	4PW 01/15
Gammaproteobacteria;Alteromonadales; Alteromonadaceae;Marinobacter;	**2.7**	**1.0**	**8.1**	**1.4**	**1.8**
Clostridia; Halanaerobiales; Halanaerobiaceae; Halanaerobium;	**31.1**	**29.3**	**31.1**	**66.5**	**36.0**
Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae; Thiomicrospira;	0.1	0.2	0.0	0.0	0.0
Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Modicisalibacter;	**28.0**	**35.8**	0.0	0.0	0.0
Deltaproteobacteria; Desulfovibrionales; Desulfohalobiaceae; Desulfovermiculus;	**1.0**	**1.2**	**1.0**	**6.4**	**40.5**
Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Halomonas;	**1.4**	0.9	0.0	0.9	0.0
Methanomicrobia; Methanosarcinales; Methanosarcinaceae; Methanohalophilus;	**16.4**	**16.0**	**5.1**	**3.2**	**2.9**
Gammaproteobacteria;	0.0	0.0	0.4	0.3	0.0
Betaproteobacteria; Burkholderiales; Burkholderiaceae; Ralstonia;	0.0	0.0	**25.6**	0.1	0.0
Methanococci; Methanococcales; Methanococcaceae; Methanothermococcus;	**1.2**	**1.3**	0.0	**1.4**	**1.4**
Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Chromohalobacter;	**9.8**	**5.5**	0.0	0.0	**1.3**

From An et al., 2017

Genomics as a Monitoring Tool – 16S

‘List of microorganisms’ – infer metabolism!

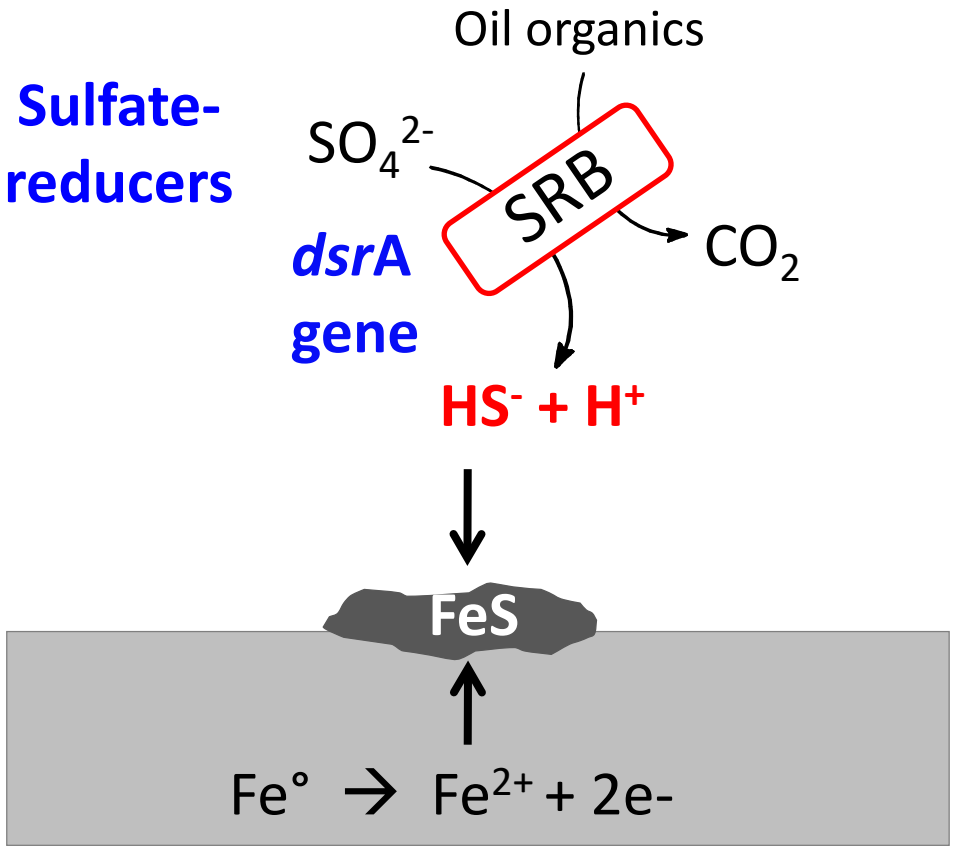


From Vigneron et al., 2016

Genomics as a Monitoring Tool – 3 ways

2. 'Functional' gene analysis – commonly-used method

- **Targets gene specific to a certain kind of microbe or metabolism**
- **Can quantify these to obtain numbers of specific microbes**
  - qPCR
  - **Primers specific to genes** for sulfate-reducers, methanogens, etc.
  - Total *Bacteria*, *Archaea*



Genomics as a Monitoring Tool – qPCR

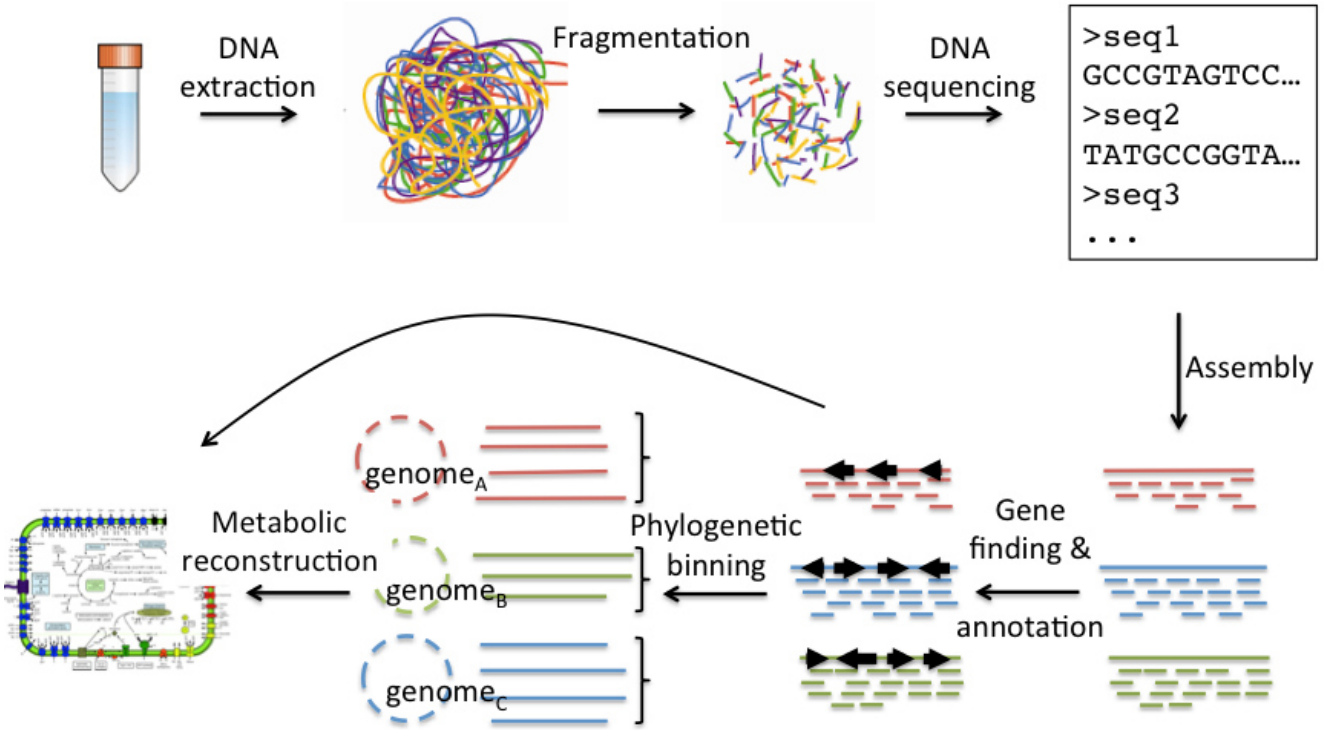
Analysis	Location[a]			
qPCR	Eider Production Manifold	Otter Production Pipeline	Inlet to Crude Oil Coalescer V-1100	Crude Oil Coalescer PW outlet V-1100
qPCR (gene abundance per cm^2)[c]				
Sessile samples from corrosion coupons				
Total bacteria	$< 4.0 \times 10^2$	4.6×10^5	$< 4.0 \times 10^2$	2.9×10^3
SRB	$< 4.0 \times 10^2$	2.9×10^4	$< 4.0 \times 10^2$	3.0×10^3
SRA	$< 4.0 \times 10^2$	1.9×10^4	$< 4.0 \times 10^2$	1.1×10^4
Methanogens	1.4×10^5	4.2×10^{11}	5.0×10^5	4.2×10^5
MPN (cells per cm^2)[b]				
Sessile samples from corrosion coupons				
mSRB (30 °C)	5.9×10^0	3.3×10^1	0.5×10^0	0.5×10^0

From Skovhus et al. 2017

Genomics as a Monitoring Tool – 3 ways

3. **Metagenomics** – less commonly used

- **All** DNA sequenced in a sample
  - **All** the genetic potential of all microorganisms



Genomics as a Monitoring Tool – metagenomics

All sulfate-reducers

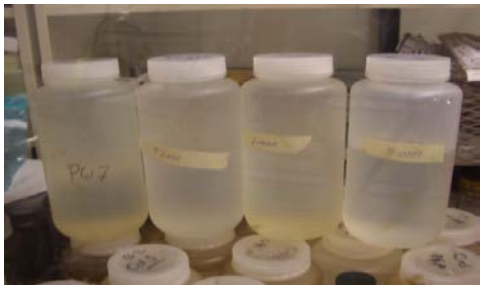
All sulfate-reducing genes

	Sample	sulfate adenylyl-transferase	APS reductase	dsrA	dsrB	dsrC	dsrD	dsrK
Desulfonauticus sp.	I2	+	+	+	+		+	
Archaeoglobus fulgidus (partial)		+	+	+	+			
Thermodesulfobacterium commune		+	+	+	+	+	+	+
Archaeoglobus fulgidus	K2	+	+	+	+			
Thermodesulfobacterium commune		+	+	+	+	+	+	+
Moorella-like genome				+	+	+		+
Clostridiales bacterium*	K3		+	+	+	+		
Thermodesulfobacterium commune		+	+	+	+	+	+	+
Desulfotomaculum sp.	SB1					+		
Desulfotomaculum sp.	SB2					+		

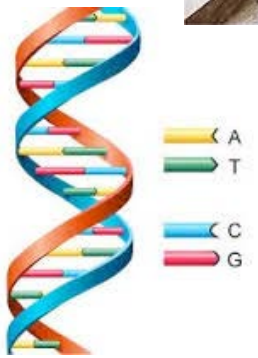
From Hu et al. 2016

Genomics as a Monitoring Tool

Field Samples



Extract DNA



Amplicon sequencing

-16S rRNA gene

-Identifies all microbes

-Other 'functional' genes

-Identifies specific microbes/functions

Metagenomic Sequencing

-All DNA sequenced

-Complete genetic potential

Genomics as a Monitoring Tool

- **Sampling & sample preservation very important!**

Surface solids samples – where the key microbes are

- Very important to measure physical & chemical parameters
- Know operating conditions!

*Genomics data must be interpreted in context of a given operation and associated chemistry!

Microbiologically-influenced corrosion = microbiology + corrosion

*holds true for data from all microbial tests

Genomics in MIC Management

Corrosion Management Process Step	Benefits of applying Genomics/MMM
MIC Threat Assessment	• Characterize baseline microbiological conditions • Look for associations between biofilm, biofilm composition and corrosion • Relate biofilm characteristics with operating conditions
MIC Mitigation Selection	• Evaluating the effect of candidate mitigation measures on corrosion and biofilm • Aid in selection of biocides or anti-microbial chemicals
MIC Barrier Monitoring	• Monitor long term and short term effects of mitigation on biofilm • Monitor changes in chemical effectiveness • Watch for shifts in microbiological populations

A ‘Genomics’ Case Study

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2011, p. 6908–6917 Vol. 77, No. 19
0099-2240/11/$12.00 doi:10.1128/AEM.05891-11

Effect of Sodium Bisulfite Injection on the Microbial Community Composition in a Brackish-Water-Transporting Pipeline▽†

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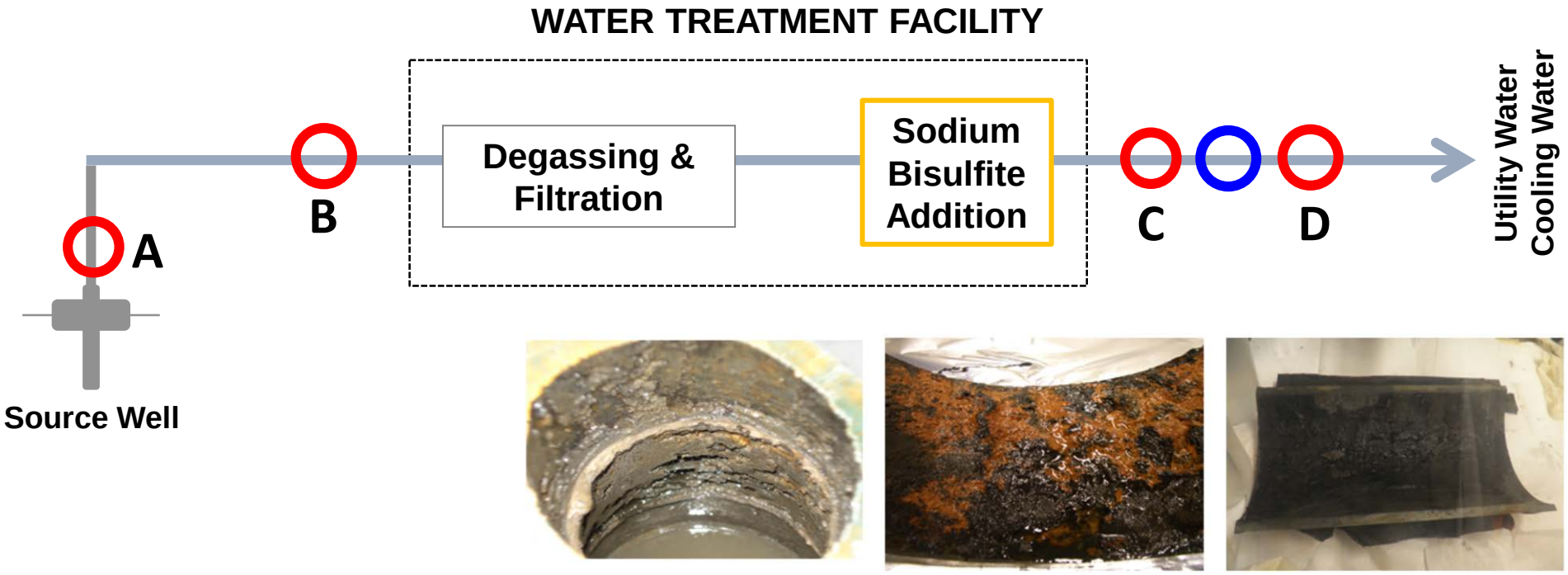
Effect of Bisulfite on Biocorrosion

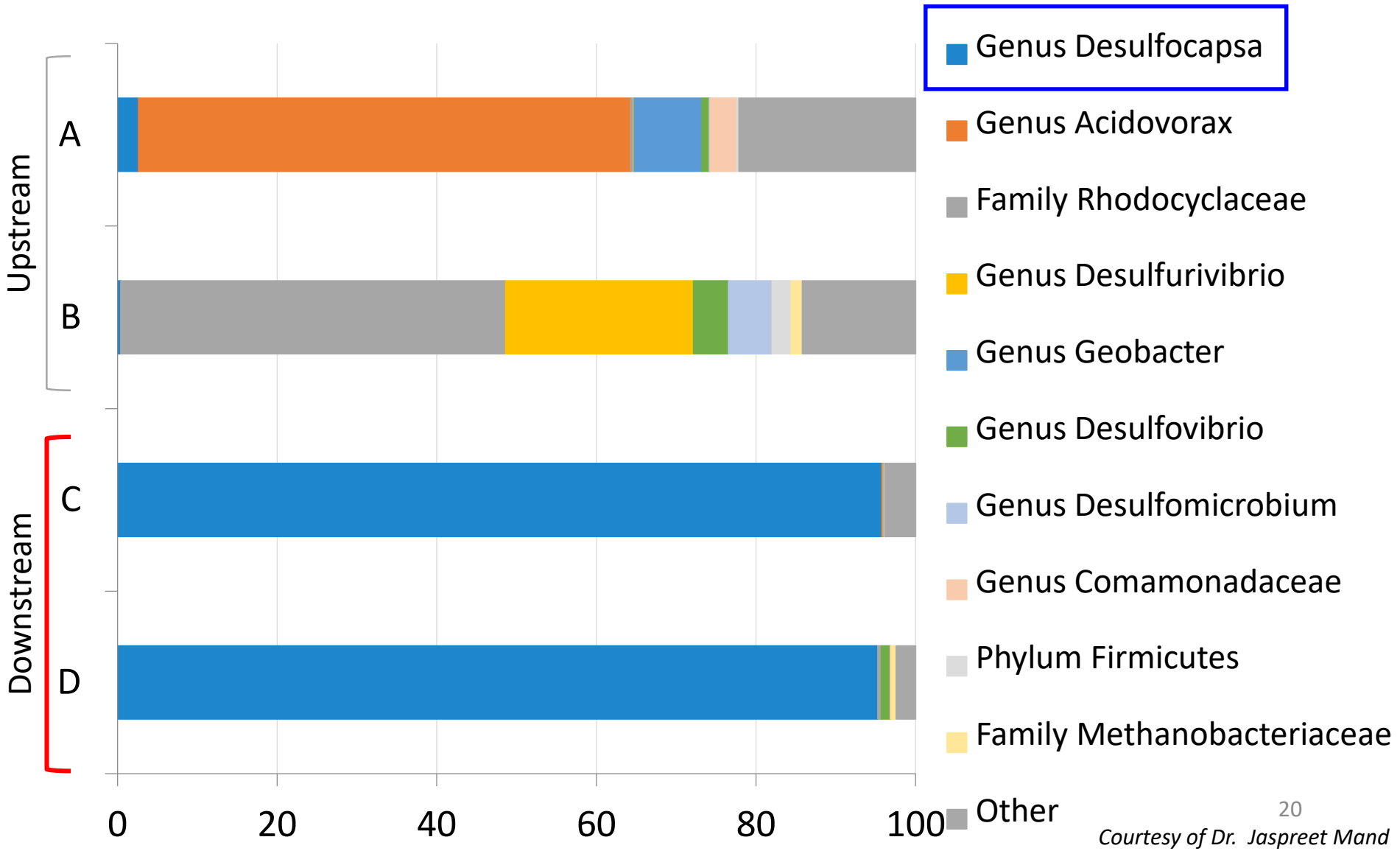
‘Who is there?’ 16S rRNA gene sequencing…



- Water Samples
  - Undetectable sulfate
  - Low sulfide concentrations
- Pipeline Sample
  - **Elemental sulfur deposits**
  - **Iron sulfide deposits**

Courtesy of Dr. Jaspreet Mand

Microbial Community Composition



Courtesy of Dr. Jaspreet Mand

Sulfite and MIC

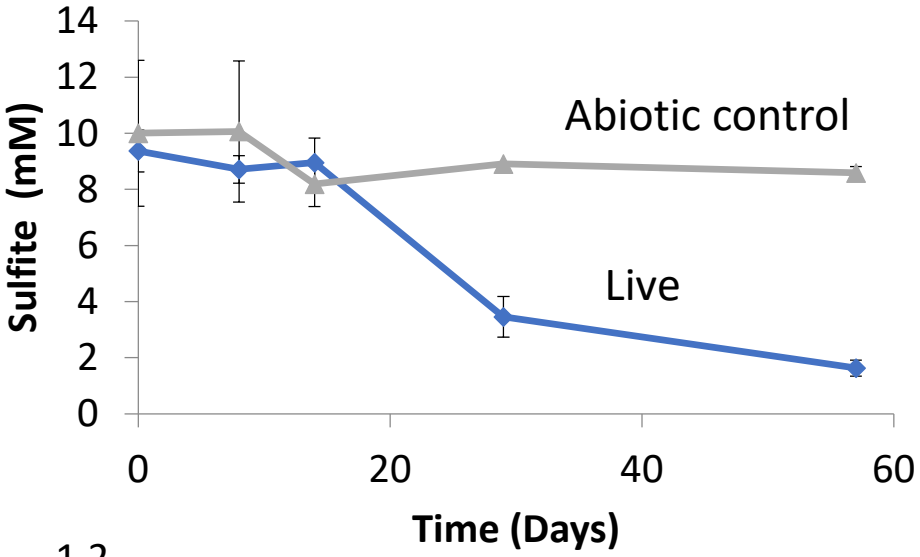
- It was assumed that sulfur, found downstream of bisulfite addition, was due to oxygen ingress

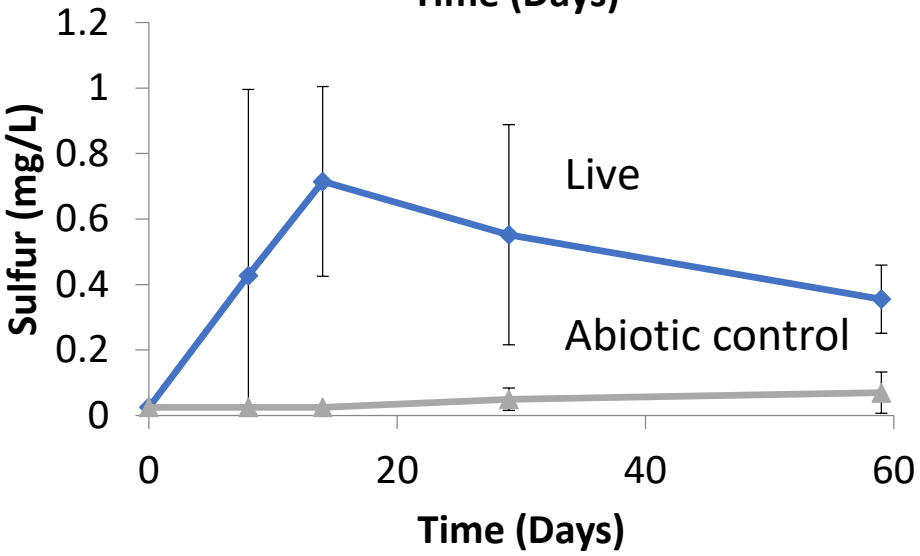
- However, sulfur may be result of bisulfite metabolism (disproportionation) by *Desulfocapsa*:

$$3HSO_3^- \rightarrow \mathbf{S^0} + 2\ \mathbf{SO_4^{2-}} + H_2O + H^+$$

- Resulting byproducts may be used by other microorganisms in the system

Microbial Enrichment on Sulfite





- Sulfite degradation is seen in incubations with the field sample relative to the abiotic control
- Elemental sulfur is produced

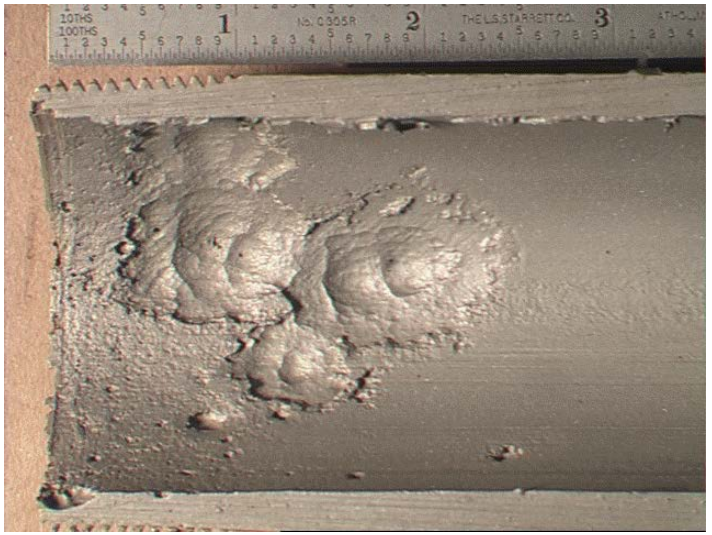
- Genomics helped to pinpoint the problem
- Altered KPI for bisulfite treatment

Courtesy of Dr. Jaspreet Mand

Genomics in MIC Management

- **Genomics is a powerful microbiological monitoring tool that captures broadest diversity in a field sample**
- contributes a ‘piece of the corrosion puzzle’ to help guide corrosion management and mitigation
  - monitoring programs (KPI)
  - effectiveness of treatments
  - failure analysis
  - risk management

Thank you!
Questions?



R. Eckert, Materials Performance