

MicrobeID™ Report

Project ID: OG182112

Prepared for:

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Sample Information

Sample Count: 4
Shipped From: 100100 River Don Lane
Middletown, TX, 79002

Shipped Date:05/01/18
Arrived Date:05/02/18

Sample and Project Overview: (Table 1)

4 samples were received at Ecolyse Labs on May 2, 2018. These consisted of 2 water and 2 solids samples.

TABLE 1. Sample Overview

Sample ID	Sample Label	Tests Performed
OG182112-001	Tank 1 Waters	1. MPN*, 2. qPCR, 3. Metagenomics,
OG182112-002	Tank 2 Waters	1. MPN*, 2. qPCR, 3. Metagenomics
OG182112-003	Tank 1 Solids	1. MPN*, 2. qPCR, 3. Metagenomics
OG182112-004	Tank 2 Solids	1. MPN*, 2. qPCR, 3. Metagenomics
*MPN with these medias: PRD (detects APB), MPB (detects SRB), NRB (detects Nirgrate Reducing Bacteria), IRB (detects Iron Reducing Bacteria)		

Description of Analysis Methods:

The microbial population of each sample will be analyzed by a maximum of the 3 following methods, each of which provides a different perspective:

1. **MPN analysis** following NACE corrosion industry standard methods, is used to quantify viable and culturable bacteria able to grow in five different growth media preparations
 - a. Quantifies cells/ml of SRB, IRB, NRB, APB, and GHB
 - b. Dependent on bacteria being alive and able to grow in the media
 - c. Viable cell count assay
2. **qPCR** analysis is a DNA based analysis that quantifies total microbes in a sample
 - a. Quantifies cells/ml of all microbes in a sample
 - b. Does not distinguish between living and dead cells
 - c. Does not give information on types of cells
 - d. Total cell count assay
3. **Amplicon Metagenomics** provides information on the types and relative abundance of bacteria and archaea in a sample
 - a. Provides a list of all bacteria in the sample
 - b. Does not distinguish between live and dead cells
 - c. Does not provide cells/ml quantification
 - d. Provides relative abundance
 - e. Is not dependent on growth in media
 - f. Detailed Population Structure analysis

Project Results Overview: Bacterial Quantification by MPN Growth and qPCR

MPN Analysis Methods Overview

- Samples were serial diluted and injected into indicator media, set up in triplicate
- Cultures were read weekly for 30 days, following NACE Standard TMO 194-2004
- Medias used were:
 - MPB (Growth of Sulfate Reducing Bacteria, SRB)
 - PRD (Growth of Acid Producing Bacteria, APB and GHB)
 - IRB (Growth of Iron Reducing Bacteria, IRB)
 - NRB (Growth of Nitrate Reducing Bacteria, NRB)
- Results are provided in Table 2.

qPCR Analysis Methods Overview

- DNA was isolated from each sample
- Samples were qPCR amplified using universal 16S (16S)
- Raw copy count was determined from qPCR results (CT Value).
- Microbes per ml results calculated from raw copy count and the volume of sample used for DNA isolation
- Results are provided in Table 2

TABLE 2. Bacterial Quantification by MPN and qPCR						
Sample ID	Sample Label	SRB	IRB	APB	NRB	qPCR
OG182112-001	Tank 1 Waters	4.20E+06	2.00E+05	4.20E+02	2.30E+02	1.30E+07
OG182112-002	Tank 2 Waters	9.30E+05	2.00E+03	4.20E+05	2.30E+05	1.42E+07
OG182112-003	Tank 1 Solids	2.30E+03	2.80E+07	9.20E+03	NG	1.57E+08
OG182112-004	Tank 2 Solids	9.30E+07	2.80E+03	9.20E+03	NG	1.57E+08

Values are expressed in viable cells per mL. Values > E+05 are yellow. Values of E+04 are green, NG = no growth, grey, indicates bacterial levels are less than detection threshold (0.5E+00 cells per ml).

Results of MPN and qPCR Analysis Methods Overview

- All 4 samples contained bacterial levels >10⁷ cells per ml, by qPCR
- By MPN analysis, bacterial activity was detected in all 4 samples
- All 4 samples contained viable SRB, IRB, and APB strains.
- NRB were detected only in samples -001 and -002
- The very high levels of active SRB and IRB is of concern due to the potential of these organisms to cause or promote corrosion

Project Results Overview: Archaea and Bacterial Diversity Analysis

Genetic-Based Diversity Analysis-Method

- Total DNA is isolated from the samples.
- Bacterial and Archaeal diversity is determined by 16s MiSeq platform metagenomics.
- Following traits assigned to identified bacteria and archaea where possible:
 - **Sulfidogen**-includes all bacteria that can make sulfide or H₂S. This includes “true” SRB as well as other sulfidogens.
 - **SRB**-(sulfate-reducing bacteria) “true” SRB, utilize sulfate as respiratory electron acceptor
 - **APB**-(acid-producing bacteria) these make organic and/or inorganic acids.
 - **IRB**-(iron-reducing bacteria) many are strongly corrosive
 - **NRB**-(nitrate-reducing bacteria) many bacteria are nitrate reducers.
 - **NRSOB** (nitrate-reducing sulfur-oxidizing bacteria) promoted by nitrate injections.
 - **Biodeg**-biodegrading bacteria. These bacteria are capable of breaking down unusual substrates such as O&G hydrocarbons, petrochemicals, cellulose, toxic chemicals etc.
 - **Methanogen** – Organisms that produce methane as a metabolic byproduct in anoxic conditions. These include organisms that live on the surface, as well as deep subsurface extremophiles.

Genetic – Based Diversity Analysis – Overview Results

- DNA was isolated from four samples (Table 1).
- 63239 microorganisms were analyzed genetically.
- These were grouped into 68 different microbial types (OTU).
- 4 Archaeal OTU were present in the samples.
- The distribution of SRB, IRB, APB, Biodeg, and NRB is provided (Table 2).
- A list of the most abundant bacteria (greater than 1% of the population) with a trait of interest is provided (Table 3).
- A list of all sulfidogens, SRB, IRB, and methanogens is provided (Table 4)
- A complete list of all bacteria in the samples is provided (Table 5).

Table 2. Summary of Bacterial Diversity Using Genetic Analysis					
ID#	, -001	, -002	, -003	, -004	Total
Sample Label	Tank 1 Waters	Tank 2 Waters	Tank 1 Solids	Tank 2 Solids	
# Bacteria Tested	17874	15766	16972	12627	63239
# Unique Species	59	43	13	25	68
Metabolic Profile (% of the Population; # of Bacteria species)					
Metabolism	-001	-002	-003	-004	Total Taxa
APB	3.69 ; 5	0.634 ; 4	0.006 ; 1	18.66 ; 4	6
Biodeg	22.33 ; 8	14.11 ; 7	0.018 ; 1	0.055 ; 3	10
IRB	11.04 ; 5	4.04 ; 6	96.78 ; 2	3.02 ; 2	7
NRSOB	38.45 ; 2	36.08 ; 3	0.018 ; 1	0.008 ; 1	3
Methanogen	0.481 ; 2	0.051 ; 1	0.006 ; 1	31.88 ; 2	2
Sulfidogen	14.1 ; 9	4.69 ; 6	2.92 ; 3	44.56 ; 6	13
SRB	3.44 ; 4	1.75 ; 4	0.035 ; 1	2.99 ; 3	6

First number is % of the sample. Second number is the number of taxa (different organisms)

- A list of the dominant microbial species is provided in Table 3.
- These organisms are present in >1% of the population of at least 1 sample
- A characteristic trait of relevance of each organism is provided

Table 3. List of the Dominant Microbial Species in Each Sample Samples					
ID#	, -001	, -002	, -003	, -004	ID#
Sample Label	Tank 1 Waters	Tank 2 Waters	Tank 1 Solids	Tank 2 Solids	Trait
<i>Acetobacterium sp</i>	0.18	0.05	0.01	18.40	APB
<i>Arcobacter sp</i>	37.18	35.91	0.02	0.01	NRSOB
<i>Citrobacter freundii</i>	0.03	0	2.88	41.11	Biofilm; MIC; TRB Sulfidogen
<i>Desulfomicrobium sp</i>	3.15	1.69	0	0.02	SRB Sulfidogen
<i>Desulfovibrio alaskensis</i>	0.23	0.01	0	2.26	SRB Sulfidogen
<i>Desulfuromonas acetoxidans</i>	10.37	2.44	0.01	0.02	IRB; Sulfidogen
<i>Marichromatium sp</i>	0.01	23.71	0.01	0.25	Sulfidogen
<i>Marinobacter hydrocarbonoclasticus</i>	15.57	0.01	0	0.02	Biodeg (HC); NRB
<i>Marinobacterium sp</i>	5.54	13.91	0.02	0	Biodeg (HC); NRB
<i>Methanothermobacter sp</i>	0.02	0	0.01	31.87	Archaea; Methanogen
<i>Paracoccus sp</i>	1.07	0	0	0	APB; NRB
<i>Pseudomonas sp</i>	0.08	1.94	0.19	0.01	Varies
<i>Shewanella algae</i>	0.24	0.95	96.78	2.99	IRB; MIC
<i>Sphingobacterium sp</i>	1.34	0.34	0	0	GHB
<i>Sulfurospirillum sp</i>	1.26	0.15	0	0	NRSOB
<i>Trichococcus sp</i>	2.03	0	0	0.01	APB
Unclassified	16.31	14.54	0.05	0.39	Unknown

Values are % Abundance. Samples are highlighted by abundances: >10%, yellow, >1%, green, 0%, grey

Trait abbreviations:

APB, Acid-Producing Bacteria; Biodeg (HC), Biodegradation of Hydrocarbons; GHB, General Heterotrophic Bacteria; IRB, Iron-Reducing Bacteria; MIC, Microbial-Influenced Corrosion; NRB, Nitrogen-Reducing Bacteria; NRSOB, Nitrogen-Reducing Sulfur-Oxidizing Bacteria; SRB, Sulfate-Reducing Bacteria; TRB, Thiosulfate-Reducing Bacteria.

Corrosion Associated Organisms

A list of all sulfidogens, IRB, and methanogens is provide in Table 4
 These types of organisms are often corrosion-associated

Table 4. Sulfidogens, Iron Reducing Bacteria, and Methanogens					
SAMPLE	, -001	, -002	, -003	, -004	Metabolism Lookup
Species	Tank 1 Waters	Tank 2 Waters	Tank 1 Solids	Tank 2 Solids	Taxa, Physiology, and Metabolism;
<i>Citrobacter freundii</i>	0.034	0	2.881	41.11	Gammaproteobacteria; Aerobe; Sulfidogen; NRB; MIC; Biofilm;
<i>Deferribacter sp</i>	0	0.044	0	0	Deferribacteres; Sulfidogen; SuRB; IRB; NRB; Thermophile; Anaerobe; Oilfield;
<i>Desulfobacter vibrioformis</i>	0.011	0	0	0	Deltaproteobacteria; Sulfidogen; SRB; Anaerobe;
<i>Desulfobotulus sapovorans</i>	0.028	0	0	0	Deltaproteobacteria; Sulfidogen; SRB; Anaerobe;
<i>Desulfomicrobium sp</i>	3.15	1.687	0	0.016	Deltaproteobacteria; Sulfidogen; SRB; Anaerobe;
<i>Desulfovibrio alaskensis</i>	0.229	0.006	0	2.257	Deltaproteobacteria; Sulfidogen; SRB; oilfield; Anaerobe;
<i>Desulfovibrio longus</i>	0.045	0	0	0	Deltaproteobacteria; Sulfidogen; SRB; Anaerobe;
<i>Desulfuromonas acetoxidans</i>	10.367	2.436	0.006	0.024	Deltaproteobacteria; Sulfidogen; SuRB; IRB; Anaerobe;
<i>Desulfuromonas sp</i>	0.157	0	0	0	Deltaproteobacteria; Sulfidogen; SuRB; IRB; Anaerobe;
<i>Desulfuromusa ferrireducens</i>	0	0.501	0	0	Deltaproteobacteria; Sulfidogen; SuRB; IRB; Anaerobe;
<i>Dethiosulfatibacter sp</i>	0.084	0	0	0	NC10; Sulfidogen; SuRB; TRB; Spore;
<i>Geobacter sp</i>	0.045	0.07	0	0	Deltaproteobacteria; IRB; BioDeg; BioDeg HC; Metal Reduction; Biofilm; Microbial Fuel Cell; Anaerobe;
<i>Geotoga petraea</i>	0.006	0.063	0	0	NC10; Sulfidogen; SuRB; Thermophile;
<i>Kosmotoga sp</i>	0.011	0	0	0	Thermotogae; Anaerobe; Thermophile; Sulfidogen; TRB; Oilfield; Produced Water;
<i>Methanlobus sp</i>	0.464	0.051	0	0.008	Archaea; Methyloolithotroph; Methanogen; Anaerobe; Oilfield;
<i>Methanothermobacter sp</i>	0.017	0	0.006	31.868	Archaea; Methanogen; Anaerobe; Thermophile;
<i>Pelobacter sp</i>	0.235	0.038	0	0	Deltaproteobacteria; IRB; Anaerobe;
<i>Shewanella algae</i>	0.235	0.951	96.777	2.994	Gammaproteobacteria; IRB; Sulfidogen; TRB; Facultative Anaerobe;
<i>Thermoanaerobacter Brockii</i>	0	0	0	0.428	Firmicutes; Clostridia; Ethanologenic; Anaerobe; Thermophile; Sulfidogen; TRB; Firm;
<i>Thermodesulfobacterium commune</i>	0	0.013	0.035	0.721	Thermodesulfobacteria; Sulfidogen; SRB; Thermophile; Anaerobe;

Table 5. Project OG182112 Complete List of Identified Organisms Most abundant bacteria (defined as present in at least 1% of one sample) are given, along with the percent abundance in that sample and a characteristic trait of relevance. Samples are highlighted by abundances (yellow, green, white, gray).

Species	-001 Tank 1 Waters	-002 Tank 2 Waters	-003 Tank 1 Solids	-004 Tank 2 Solids	Metabolism;
<i>Acetobacterium sp</i>	0.179	0.051	0.006	18.397	Firmicutes; Clostridia; Acetogen; APB; Acetic Acid; Anaerobe;
<i>Acholeplasma morum</i>	0.006	0.057	0	0	Tenericutes; GHB; Facultative Anaerobe;
<i>Acinetobacter sp</i>	0.011	0	0	0	Gammaproteobacteria; BioDeg; BioDeg HC; Aerobe; Soil;
<i>Alkalibacter sp</i>	0.095	0.006	0	0	Firmicutes; Clostridia; BioDeg; Alkaliphile;
<i>Allochromatium vinosum</i>	0.117	0.203	0	0.071	Gammaproteobacteria;
<i>Anaerovorax sp</i>	0.034	0	0	0	Firmicutes; Clostridia; Anaerobe;
<i>Arcobacter nitrofigilis</i>	0	0.025	0	0	Epsilonproteobacteria; NRB; NRSOB; SOB; Nitrate Injections;
<i>Arcobacter sp</i>	37.182	35.913	0.018	0.008	Epsilonproteobacteria; NRB; NRSOB; SOB; Nitrate Injections;
<i>Bacteroides graminisolvens</i>	0.006	0.063	0	0	Bacteroidetes; Ferm; BioDeg; Anaerobe;
<i>Bacteroides luti</i>	0.062	0	0	0	Bacteroidetes; Ferm; BioDeg; Anaerobe;
<i>Bacteroides sp</i>	0.106	0	0	0	Bacteroidetes; Ferm; BioDeg; Anaerobe;
<i>Burkholderia zhejiangensis</i>	0.196	0	0.006	0	Betaproteobacteria; BioDeg; Aerobe; Soil;
<i>Citrobacter freundii</i>	0.034	0	2.881	41.11	Gammaproteobacteria; Aerobe; Sulfidogen; NRB; MIC; Biofilm;
<i>Clostridium amygdalinum</i>	0	0	0	0.024	Firmicutes; Clostridia; Anaerobe; Spore; Benzaldehyde-Converting bacteria; BioDeg;
<i>Clostridium sp</i>	0.201	0.901	0	0.016	Firmicutes; Clostridia; Anaerobe; Spore; Ferm; Divers; APB; Sulfidogen; TRB; SuRB;
<i>Cytophaga sp</i>	0.134	0.006	0	0	Bacteroidetes; BioDeg; BioDeg Cellulose; Aerobe; Soil;
<i>Deferribacter sp</i>	0	0.044	0	0	Deferribacteres; Sulfidogen; SuRB; IRB; NRB; Thermophile; Anaerobe; Oilfield;
<i>Desulfobacter vibrioformis</i>	0.011	0	0	0	Deltaproteobacteria; Sulfidogen; SRB; Anaerobe;
<i>Desulfobotulus sapovorans</i>	0.028	0	0	0	Deltaproteobacteria; Sulfidogen; SRB; Anaerobe;
<i>Desulfomicrobium sp</i>	3.15	1.687	0	0.016	Deltaproteobacteria; Sulfidogen; SRB; Anaerobe;
<i>Desulfovibrio alaskensis</i>	0.229	0.006	0	2.257	Deltaproteobacteria; Sulfidogen; SRB; oilfield; Anaerobe;
<i>Desulfovibrio longus</i>	0.045	0	0	0	Deltaproteobacteria; Sulfidogen; SRB; Anaerobe;
<i>Desulfuromonas acetoxidans</i>	10.367	2.436	0.006	0.024	Deltaproteobacteria; Sulfidogen; SuRB; IRB; Anaerobe;

<i>Desulfuromonas sp</i>	0.157	0	0	0	Deltaproteobacteria; Sulfidogen; SuRB; IRB; Anaerobe;
<i>Desulfuromusa ferrireducens</i>	0	0.501	0	0	Deltaproteobacteria; Sulfidogen; SuRB; IRB; Anaerobe;
<i>Dethiosulfatibacter sp</i>	0.084	0	0	0	NC10; Sulfidogen; SuRB; TRB; Spore;
<i>Draconibacterium orientale</i>	0.274	0.076	0	0	Bacteroidetes; Unknown;
<i>Dysgonomonas alginatilytica</i>	0.028	0	0	0	Bacteroidetes; Ferm; BioDeg; Facultative Anaerobe;
<i>Dysgonomonas sp</i>	0.05	0.121	0	0	Bacteroidetes; Ferm; BioDeg; Facultative Anaerobe;
<i>Ercella succinigenes</i>	0.369	0.019	0	0	Firmicutes; Clostridia; Sulfidogen; SuRB; Ferm; Anaerobe;
<i>Eubacterium sp</i>	0.039	0.545	0	0.008	Firmicutes; Clostridia; Acetogen; APB; Acetic Acid; Ethanol; Butyrate; SynGas; Anaerobe;
<i>Flavobacterium sp</i>	0.017	0	0	0	Bacteroidetes; Aerobe; Water; BioDeg; BioDeg HC;
<i>Geobacter sp</i>	0.045	0.07	0	0	Deltaproteobacteria; IRB; BioDeg; BioDeg HC; Metal Reduction; Biofilm; Microbial Fuel Cell; Anaerobe;
<i>Geotoga petraea</i>	0.006	0.063	0	0	NC10; Sulfidogen; SuRB; Thermophile;
<i>Idiomarina sp</i>	0.028	0.013	0	0	Gammaproteobacteria; Aerobe; GHB;
<i>Kosmotoga sp</i>	0.011	0	0	0	Thermotogae; Anaerobe; Thermophile; Sulfidogen; TRB; Oilfield; Produced Water;
<i>Leptospira sp</i>	0.112	0.006	0	0	NC10; GHB; Aerobe;
<i>Mangrovibacterium diazotrophicum</i>	0.839	0	0	0	Bacteroidetes; NiF;
<i>Marichromatium sp</i>	0.011	23.709	0.006	0.253	Gammaproteobacteria; SuRB;
<i>Marinilabilia salmonicolor</i>	0.045	0.108	0	0	Bacteroidetes; Ferm; BioDeg; NRB;
<i>Marinobacter hydrocarbonoclasticus</i>	15.565	0.006	0	0.016	Gammaproteobacteria; BioDeg; BioDeg HC; NRB; Aerobe;
<i>Marinobacterium litorale</i>	0	0.032	0	0	Gammaproteobacteria; BioDeg; GHB; Aerobe;
<i>Marinobacterium sp</i>	5.539	13.91	0.018	0	Gammaproteobacteria; BioDeg; GHB; Aerobe;
<i>Methanolobus sp</i>	0.464	0.051	0	0.008	Archaea; Methylotroph; Methanogen; Anaerobe; Oilfield;
<i>Methanothermobacter sp</i>	0.017	0	0.006	31.868	Archaea; Methanogen; Anaerobe; Thermophile;
<i>Moorella sp</i>	0	0.019	0	0.246	Firmicutes; Clostridia; Sulfidogen; TRB; Thermophile; Spore; Ferm; Anaerobe; NRB; Acetogen; APB;
<i>Moorella stamsii</i>	0	0	0	0.261	Firmicutes; Clostridia; Sulfidogen; TRB; Thermophile; Spore; Ferm; Anaerobe; NRB; Acetogen; APB;
<i>Owenweeksia sp</i>	0.011	0	0	0	Bacteroidetes;
<i>Paludibacter sp</i>	0.011	0.634	0.006	0	Bacteroidetes; Ferm;
<i>Paracoccus sp</i>	1.069	0	0	0	Alphaproteobacteria; NRB; Facultative Anaerobe; BioDeg; APB; Mitochondrial Ancestor;

<i>Pelobacter sp</i>	0.235	0.038	0	0	Deltaproteobacteria; IRB; Anaerobe;
<i>Proteiniphilum sp</i>	0.336	0.349	0	0.024	Bacteroidetes; Ferm; APB, BloGas; Sludge; Facultative Anaerobe
<i>Pseudomonas sp</i>	0.084	1.935	0.189	0.008	Gammaproteobacteria; Aerobe; Varies;
<i>Psychromonas ingrahamii</i>	0.006	0.006	0	0	Gammaproteobacteria; Anaerobe;
<i>Schwartzia sp</i>	0.073	0.006	0	0	Firmicutes; Negativicutes;
<i>Shewanella algae</i>	0.235	0.951	96.777	2.994	Gammaproteobacteria; IRB; Sulfidogen; TRB; Facultative Anaerobe;
<i>Sphingobacterium sp</i>	1.337	0.336	0	0	Bacteroidetes; GHB; BioDeg; Aerobe;
<i>Spirochaeta sp</i>	0.022	0	0	0	Spirochaetes; Ferm; H ₂ ; H ₂ S resistant; Sulfidogen; TRB; Anaerobe; Halophile; Thermophile;
<i>Sulfurospirillum sp</i>	1.264	0.146	0	0	Epsilonproteobacteria; NRB; SOB; NRSOB; Oilfield; Facultative Anaerobe; Sulfidogen;
<i>Sunxiuqinia faeciviva</i>	0.067	0	0	0	Bacteroidetes; GHB;
<i>Synergistes sp</i>	0.034	0.228	0	0.832	Synergistetes; Ferm; Oilfield; 600 m deep oilfield;
<i>Thermoanaerobacter brockii</i>	0	0	0	0.428	Firmicutes; Clostridia; Ethanologenic; Anaerobe; Thermophile; Sulfidogen; TRB; Ferm;
<i>Thermodesulfobacterium commune</i>	0	0.013	0.035	0.721	Thermodesulfobacteria; Sulfidogen; SRB; Thermophile; Anaerobe;
<i>Thiobaca trueperi</i>	0.006	0.127	0	0	Gammaproteobacteria; Phototroph; Purple Sulfur Bacteria; Anaerobe;
<i>Treponema zuelzerae</i>	0.05	0	0	0	Spirochaetes;
<i>Trichococcus sp</i>	2.031	0	0	0.008	Firmicutes; Lactobacillales; APB; Ferm; Filamentous; Activated Sludge; Wastewater; WWTP; Facultative Anaerobe;
Unclassified	16.309	14.544	0.047	0.388	NC10;
<i>Vibrio metschnikovii</i>	0.929	0.038	0	0.016	Gammaproteobacteria; Facultative Anaerobe; GHB; Alkaliphile; BioDeg;

Values are % Abundance. Samples are highlighted by abundances: >10%, yellow, >1%, green, 0%, grey

Trait abbreviations: APB, Acid-Producing Bacteria; Biodeg (HC), Biodegradation of Hydrocarbons; GHB, General Heterotrophic Bacteria; IRB, Iron-Reducing Bacterial; MIC, Microbial-Influenced Corrosion; NRB, Nitrogen-Reducing Bacteria; NRSOB, Nitrogen-Reducing Sulfur-Oxidizing Bacteria; SRB, Sulfate-Reducing Bacteria; TRB, Thiosulfate-Reducing Bacteria.

Conclusions of Bacterial Population Testing

- 4 samples were received for testing
- These originated from produced waters and pipe solids from oil pipelines
- Each of the 4 samples contained abundant SRB, IRB, and methanogens
- These organisms are considered problematic
- Data indicates these samples contain organisms capable of influencing corrosion

- **Notes on Taxonomic and Metabolic Assignment**

Organisms are referred to by the identity of the most closely matched organism in the database. However, this does not indicate 100% identity. In most cases, the most closely matched organisms are referred to as “uncultured organism” and as such there is no physiological or metabolic information for them. Organisms that fall below the cutoff for taxonomic assignment are listed as unclassified. Due to the unusual source of samples, a large number of organisms in the samples may be unclassified. This indicates that they are novel organisms that have not been described in the scientific literature.

Metabolic assignments are inferred by the metabolic characteristics of the most closely related organism for which experimental data has been provided. Some metabolic groupings are overlapping and non-exclusive, e.g. many fermentative organisms generate organic acids or are capable of sulfidogenesis under some conditions. An overview of select metabolisms is provided in Appendix B.

APPENDIX B. Overview of Select Metabolic Processes

APB: Acid-Producing Bacteria

Acid-producing bacteria are of specific interest to the oilfield community as acid production directly and aggressively promotes corrosion. Several metabolic pathways result in the production of acids, including fermentation pathways that generate organic acids such as lactic acid and acetic acid, as well as those that generate inorganic acids such as sulfuric acid as a byproduct of the oxidation of inorganic sulfur compound. It should be noted that not all fermentative pathways result in acidification of the surrounding environment. The identification of bacteria as acid producing does not necessarily indicate acidification of bulk fluids.

Biodeg: Biodegradation

Some bacterial genera and species have the capacity to utilize “atypical” or “unusual” substrates as carbon sources. These bacteria are loosely referred to as Biodeg, for “Biodegradation”. The definition used here for “atypical or unusual substrates” with reference to bacterial metabolism includes compounds that most bacteria cannot utilize as a food source. Unusual compounds Biodeg organisms might be able to “eat” include disinfectants, antibiotics, xenobiotics and detergents. Some degrade long chain polymers of sugars and carbohydrates, such as those found in cell wall materials. Others are able to degrade hydrocarbons. Hydrocarbons, including alkanes, alkenes, aromatic hydrocarbons, and waxes, are found naturally in great variety in crude oil and other petroleum compounds. Due to their structural diversity, most bacteria lack the capacity to utilize petroleum hydrocarbons as food sources. Each type of hydrocarbon-degrading microorganism is likely to be capable of metabolizing a few specific types of hydrocarbons.

IRB: Iron-Reducing Bacteria, Fe(III)RB

In the absence of oxygen, many microbes can use Fe(III) as an electron acceptor, reducing it to Fe(II). Iron reduction has been observed under both acidophilic and neutrophilic conditions. Two common iron-reducing genera are *Shewanella* and *Geobacter*. In addition to IRB activity, *Shewanella* species produce chelators that solubilize Fe(III) oxides (Lovley et al, 2004). *Shewanella* are capable of growing in corrosive biofilms where they have been shown to remove the protective H₂ film layer that normally protects iron surfaces from corrosion under anoxic conditions. IRB should not be confused with iron oxidizing bacteria, which are aerobes responsible for a rust brown staining and slimy growth in surface waters.

NRB: Nitrate Reducing Bacteria

NRB are able to reduce nitrates to nitrites, nitrous oxide, or nitrogen under anaerobic conditions in a process termed denitrification. Most are heterotrophic facultative anaerobic bacteria including such common bacteria as *Paracoccus*, *Pseudomonas*, *Alcaligenes*, and *Bradyrhizobium*. A few bacteria use

such reduction processes as hydrogen acceptor reactions and hence as a source of energy; in this case the end product is ammonia. Denitrification is a normal part of nitrogen cycling and not all NRB are of concern to O&G infrastructure.

A subcategory of NRB is the **NRSOB**: Nitrate-Reducing Sulfur-Oxidizing Bacteria are a specific subgroup of NRB whose levels are increased in reservoirs following nitrate injections (Gittel et al 2009; Grigoryan et al, 2008; Hubert and Voordouw, 2007). Growth of NRSOB suppresses the activity of SRB, and thus reducing sulfidogenesis. Some Epsilonproteobacteria can also oxidize petroleum sulfur compounds and utilize nitrate as an electron acceptor for growth, and thus may be considered hydrocarbon degrading. Massive dominance of related Epsilonproteobacteria has been observed in other petroleum samples, for example in formation waters from a Canadian oil sands reservoir containing severely biodegraded oil. (Kodama, Y and Kazuya Watanabe, 2003; Hubert et al, 2011). Sulfurospirillum are nitrate-reducing, sulfur oxidizing bacteria (NRSOB) members of the class Epsilonproteobacteria and are sometimes referred to as “Campylobacter” in older publications. The way in which nitrate addition can affect the SRB population involves several pathways. First, nitrate is a thermodynamically more favorable electron acceptor than sulfate, thus NRB have a competitive advantage. To emphasize the complexity of the metabolism in oilfield samples, it should be noted that under some conditions, these bacteria are also sulfidogens capable of reducing sulfur and thus producing H₂S (Finster K et al, 1997).

Sulfidogenesis: (e.g. SRB, TRB, SuRB)

The metabolic pathways of most interest to the oilfield community are those that generate significant levels of hydrogen sulfide (H₂S). In addition to inorganic processes, biogenic processes can generate significant levels of hydrogen sulfide, primarily through the action of sulfidogenic bacteria. Bacteria that evolve hydrogen sulfide are commonly referred to as “sulfidogens”. Sulfate-reducing bacteria (SRB) are particularly aggressive at sulfide production and are the group of bacteria most commonly implicated oil field biogenic sulfide production (Barton et al, 2009). Hydrogen sulfide formation by sulfate-reducing bacteria (SRB) under strict anaerobic circumstances is a common problem in sediments, sewer systems, oil reservoirs and anaerobic effluents (Holmer & Storkholm, 2001; McComas et al., 2001). The emission of H₂S into the atmosphere of sewer systems does not only imply odor nuisances and possible health risks. It also induces the biological production of sulfuric acid in the aerobic zones, causing severe corrosion of the inner surface of concrete sewer structures (Sand, 1987; Vincke et al., 2002). Hence, preventive or curative actions are needed.

While SRB are traditionally associated with O&G system sulfide generation, sulfur- and thiosulfate-reducing bacteria (SuRB and TRB, respectively) can also generate significant levels of H₂S and contribute to corrosion and souring (Hulecki JC et al, 2009, Magot et al 1997, Agrawal et al, 2010). Compared to SRB, the TRB are harder to classify taxonomically, as they are members of bacterial genera that can include non-tSRB members. Examples of sulfidogenic TRB commonly found in oilfield samples include *Halanaerobium congolense*, as well as some *Thermoanaerobacter*, and *Spirochaeta*. Additionally, many common enteric bacteria are sulfidogenic, including *Citrobacter* and *Salmonella*.

Thermophiles:

A thermophile is an organism that can survive and often thrives in environments having relatively high temperatures ranging between 45 and 122 °C.

Methanogens:

Methanogens are Archaea that produce methane as a metabolic byproduct in anoxic conditions. Methanogens are found in different environments including wetlands (marsh gas), animal digestive tracts (methane production of cattle and in farts), and anaerobic digester sludges of wastewater treatment systems. Some methanogens are extremophiles and can be found in hot springs, submarine hydrothermal vents as well as in the “solid” rock of the Earth’s crust,

kilometers below the surface. Methanogens are associated with MIC. Hydrogenotrophic methanogens are believed to cause metal corrosion through cathodic depolarization, whereas the acetotrophic methanogens grow syntrophically with corrosion-causing SRB

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