Applying molecular microbiological methods to understand and prevent microbiologically influenced corrosion

Date:3 October 2017

Prepared by: Krista L. Heidersbach, Ph.D., PE

How Bacteria Cause Corrosion?

- Varies significantly from metal to metal & type of bacteria
  - Slime producing microbes cause differential aeration cells / under deposit corrosion
    - Fungi, Algae, Protozoa, Bacteria
    - Provide microclimate for sulfate reducing bacteria
  - Aerobic bacteria may oxidize iron
  - Aerobic acid producing bacteria produce organic acids
  - Sulfate reducing bacteria produce H_2S and/or sulfuric acid
  - Depolarization of the cathode by hydrogen-utilizing bacteria

Consequences of Bacteria in Shale Production

- Corrosion of pipelines
- Corrosion of facilities
- Souring of reservoirs
- Plugging of filters

Corrosion Rates from MIC

RO Water Piping

- 6” Sch 10S 304/304L Line (0.148”)
- Hydrotest water: potable water stored in an open air tank
- Water filled: 3 weeks
- Operated: 3 weeks
- Corrosion Rate:
  - Assume 6 weeks: 1286 mpy

Utility water Piping

- 4” 304L stainless steel, 0.213” thick
- Leaked after several months
- Hydrotest water: chlorinated potable water
- Corrosion Rates:
  - Assume 3 months: 852 mpy
  - Assume 6 months: 426 mpy

Pipeline

- 18” carbon steel seamless pipeline, 0.312” thick
- Hydrotest water: Untreated creek water
- Water filled: several months
- Drying: via gel pig
- Sat empty: 2 months
- In service: 3 years
- Corrosion Rates:
  - Assume 3.5 years: 90 mpy
  - Assume 5 years: 62 mpy

Take away: Stainless Steels are much more rapidly affected than carbon steel

Primary Standard Associated with Bacterial Testing

NACE TM 0194 -2014 - Field Monitoring of Bacterial Growth in Oil and Gas Systems

- Sampling Procedures for Planktonic Bacteria
- Assessment and Sampling of Sessile Bacteria
- Culture Techniques
- Evaluation of Chemicals for Control of Bacteria
- Non-Media-Based Field Methods
- Select Appendixes
  - Alternative Methods for Assessing Bacterial Populations
  - Growth Media Formulations

Basics of Bacteria Detection

Media Based

- Serial Dilution – MIC kit tests

Non-Media Based

- ATP - Adenosine triphosphate
- APS - Adenosine 5 Phosphosulfate reductase
- Hydrogenase Measurement

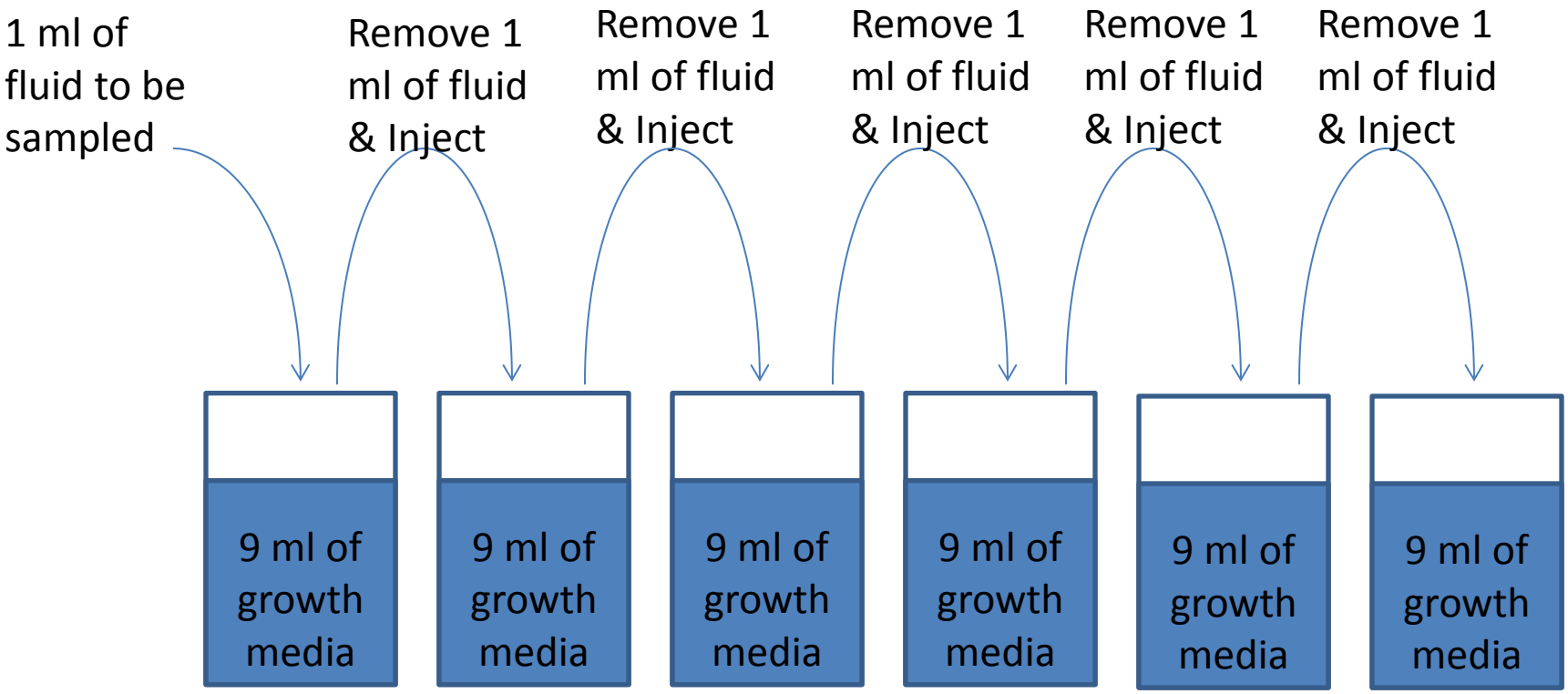
Molecular Microbiological Methods (MMM)

- FISH - fluorescent *in situ* hybridization
- qPCR – Quantitative real-time polymerase chain reaction
- DNA Sequencing by PCR-DGGE or TGGE - Polymerase chain reaction denaturing gradient gel electrophoresis or thermal gradient gel electrophoresis
- Next Generation Sequencing (NGS)

Serial Dilution

- Need living bacteria
- 30 day wait for results
- Everyone in oil field knows what serial dilution is except the new people
  - API Bug Bottles
    - RP 38 – Biological Analysis of Subsurface Injection Waters
    - Written 1975
    - Withdrawn 1982?
  - NACE
    - TM 0194 -2014 Field Monitoring of Bacterial Growth in Oil and Gas Systems
    - TM0106-2006 Detection, Testing, and Evaluation of Microbiologically Influenced Corrosion (MIC) on External Surfaces of Buried Pipelines

Serial Dilution



Growth Media will change color to signal bacteria viability

Serial Dilution

Sulfate Reducing Bacteria

Small piece of steel in SRB bottles which reacts with H_2S to form FeS

Acid Producing Bacteria

Phenol red changes to orange or yellow depending on the pH

GHB bottles will turn cloudy if bacteria are present

Culturing

- Salinity should match process
- Temperature should match process (+/- 5 C)
- Bottles should be inoculated on site / in the field
- If not inoculated on site, sample should be refrigerated if more than 48 hours to transport from the field
- SRB incubate for 28 days
- Other bacteria incubate for 14 days
- For SRB testing, should a vial turn black within 2 hours of sampling, it is assumed that sulfides in the sample caused the color change

Serial Dilution – Simplistic Estimate of Population

# of Bottles Turned	Range of Viable Bacteria per mL of Sample
1	1 to 10
2	10 to 100
3	100 to 1000
4	1,000 to 10,000
5	10,000 to 100,000
6	100,000 to 1,000,000

Serial Dilution – Statistical Estimates

# of Bottles Turned	Dilution of Sample	Estimated Range of Bacteria per mL	# of Bottles Turned	Dilution of Sample	Estimated Range of Bacteria per mL	# of Bottles Turned	Dilution of Sample	Estimated Range of Bacteria per mL
Single Sample			Duplicate Samples			Five Replicate Samples		
1	1:10	1 to 145	1	1:10	1 to 66	1	1:10	1 to 33
2	1:100	7 to 1,450	2	1:100	15 to 660	2	1:100	33 to 330
3	1:1000	69 to 14,500	3	1:1000	150 to 6,600	3	1:1000	330 to 3,300
4	1:10,000	690 to 145,000	4	1:10,000	1,500 to 6,6000	4	1:10,000	3,300 to 33,000
5	1:100,000	6,900 to 1,450,000	5	1:100,000	15,000 to 660,000	5	1:100,000	33,000 to 330,000
6	1:1,000,000	69,000 to 145,000,000	6	1:1,000,000	150,000 to 6,600,000	6	1:1,000,000	330,000 to 3,300,000

Problems with Serial Dilution

- Contamination
- Samples degrade over time
- Takes 28 days for reliable SRB results
- Culturing should occur at temperature of process
- Culturing should occur at salinity of process

- "While great advances have been made in cultivation techniques, it is now accepted that only 0.001-15% of the viable bacteria are culturable by these classical microbiological methods[1]."
  - Corrosion 08652 *Molecular Identification of MIC Bacteria from Scale and Produced Water: Similarities and Differences*, Jan Larsen et al

Non-Media Based Field Methods

- General Population
  - ATP Photometry
- Sulfate Reducing
  - APS Reductase Measurement
  - Hydrogenase Measurement

ATP - Adenosine triphosphate

- “ATP is a molecule found in and around living cells, and as such it gives a direct measure of biological concentration and health. ATP is quantified by measuring the light produced through its reaction with the naturally occurring firefly enzyme luciferase using a luminometer. The amount of light produced is directly proportional to the amount of ATP present in the sample.”
  - https://en.wikipedia.org/wiki/ATP_test

ATP Photometry

- Produced by all living cells – degrades quickly upon death
- Can be measured with light
- Does not distinguish between types of bacteria
- Does not distinguish between other living cells
- No direct conversion of ATP concentrations to microbial concentrations*
- Used for trending, measuring biocide efficacy

APS - Adenosine 5 Phosphosulfate Reductase Measurement

- SRB's possess a unique enzyme, APS - Adenosine 5 Phosphosulfate reductase measurement
- No growth media
- Independent of salinity, temperature, and redox condition
- Color change proportional to concentration of APS-reductase present
- Testing takes minutes
- Brand names: Rapid Chek, Quick Chek

Hydrogenase Measurement

- Bacteria which use hydrogen as part of their metabolism produce a hydrogenase enzyme
- Quantifies the activity of the hydrogen utilizing bacteria
- No growth media
- Performed on filters or sessile samples
- Independent of salinity, temperature, and redox condition
- Color change proportional to concentration of hydrogenase present
- Testing takes hours
- Testing is non-specific – will reflect the general population active hydrogen utilizing bacteria, not specific species

Quantitative Fluorescent *In Situ* Hybridization (qFISH)

- Stain cells of interest with fluorescent dye
  - Stains may target specific groups
- Perform epifluorescence microscopy at 1000X
- Count the bacteria
- Detects active bacteria, not inactive or dead
  - DAPI – a generic stain that stains all organisms present (bacteria & Archaea)

Quantitative Polymérase Chain Reaction (qPCR)

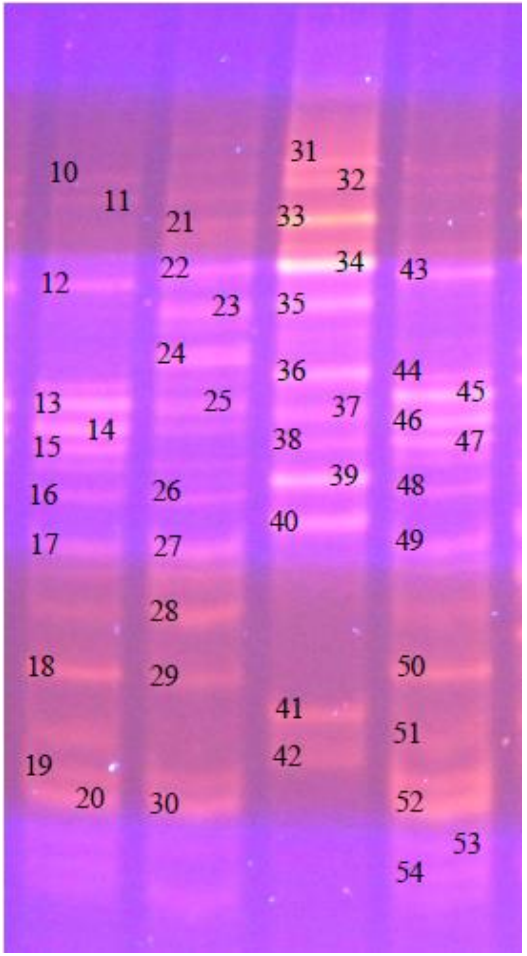
- The polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
  - https://en.wikipedia.org/wiki/Polymerase_chain_reaction

Quantitative Polymerase Chain Reaction (qPCR)

- Amplifies specific gene sequences from target organisms
- Genetic material is extracted from a sample
- Quantify the copies of a specific gene
- Does not distinguish between live, inactive or dead cells
- Quantify the total amount of bacteria and subspecies against a known sample
- Cost $350 / sample at Company A + disposal + reporting
  - Total Bacteria, SRB, APB, Archaea
- Cost $900 / sample at Company B
  - SRB, APB, Methanogens, IRB, Metal Oxidizing, SOB, Denitrification, Nitrification, Nitrogen Fixing, Slime Producing, Total Bacteria

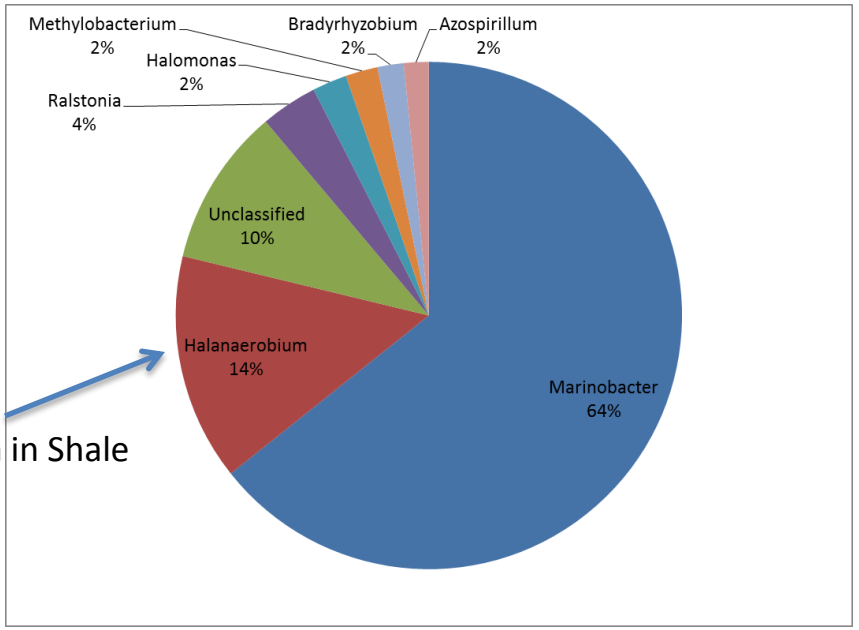
PCR-DGGE - Polymerase chain reaction denaturing gradient gel electrophoresis

- PCR-DGGE - PCR is used to replicate a gene fragment (usually) 16S rRNA
- Denaturing gel (DGGE) or temperature (TGGE) causes gene fragments to spread, creating a unique fingerprint
- https://en.wikipedia.org/wiki/Community_Fingerprinting



DNA Sequencing

- "DNA sequencing of bacterial 16S rRNA gene fragments"
  - Fingerprint generated via PCR-DGGE or PCR-TGGE
  - Compared to a standard database to identify specific genus and species of bacteria present
- Cost –~$4000, or $1000/sample with a minimum of 5 samples
- Good for determining effect of biocide treatments
  - Halanaerobium – resistant to gluteraldehyde

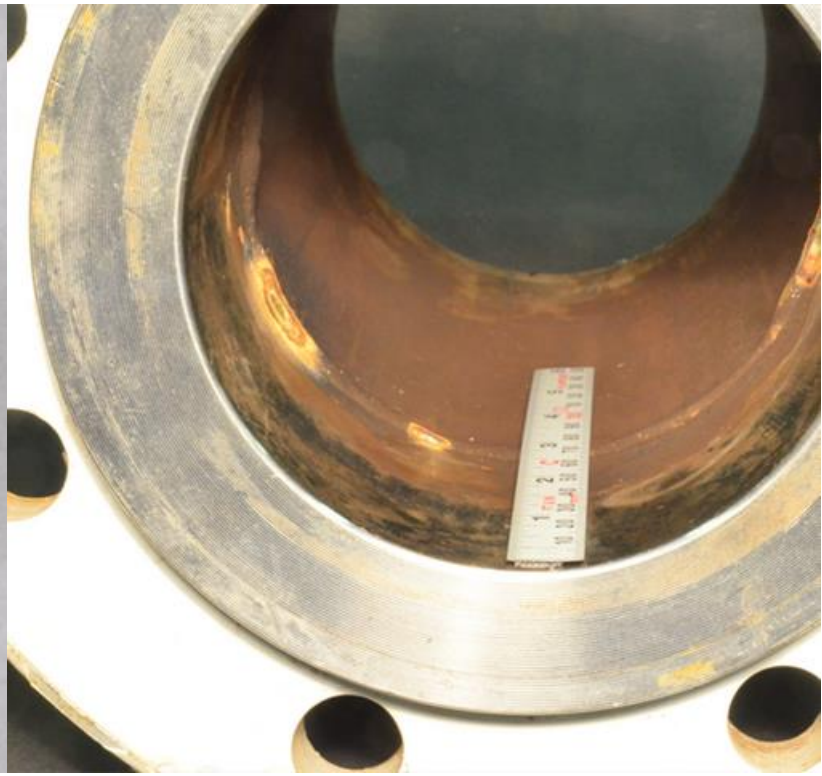


Field Failure Example #1

- Land based facility in Central US
- Water comes from
  - City potable water
  - Well A
  - Well B – Known to have oil contamination
  - Blended to keep chloride concentration around 200 ppm
- 316 Piping Failed
  - New water plant, started up in July 2015 operates at 60 F
  - 20+ Leaks
  - April 2016 sample sent for failure analysis
  - Hypochlorite dosage 1 ppm target
    - Known to be as low as 0.5 ppm or as high as 4 ppm

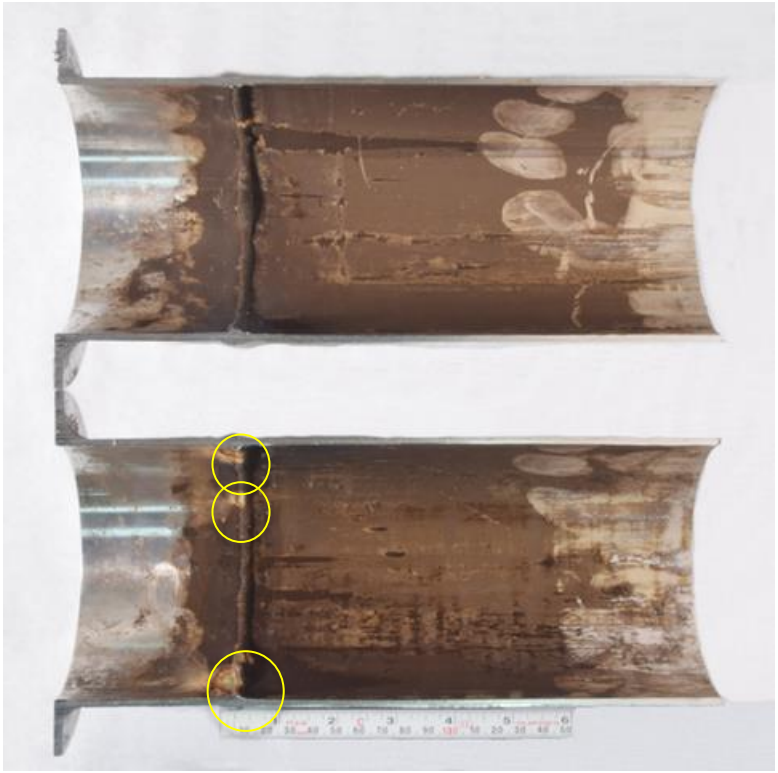
Field Failure Example #1

Sample Received at SES



Field Failure Example #1

Interior View

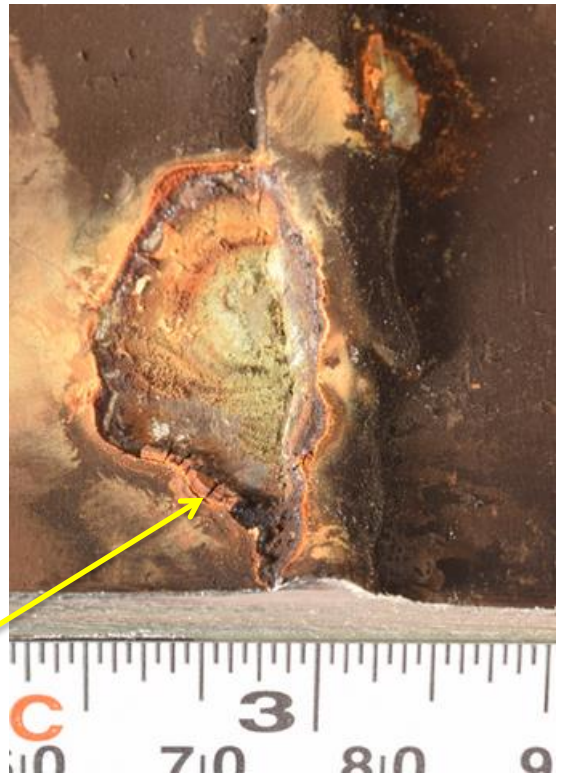


Three pits identified

Exterior View

Significant Iron Contamination

Field Failure Example #1



Classic Tubercle Crust

Field Failure Example #1

Metallographic Sample Through Pit #1

Field Failure Example #1

qPCR Results

Sample	Total Bacteria	SRB	APB	Archaea
Non-corroded area	3.7×10^4	5.7×10^2	2.0×10^1	$< 10^1$
Undercut pit sample	3.1×10^5	7.5×10^3	1.1×10^2	$< 10^1$

Hypo-chlorite is not enough

Field Failure Example #1

DNA Results

Genus	% Abundance	Species Detected	
Marinobacter	54.2	sediminum, santoriensis, aquaeoli	Iron Oxidizing
Halanaerobium	12.2	saccharolyticum, praevalens	Anaerobic (no oxygen)
Unclassified	8.5		
Ralstonia	3.06	insidiosa	
Halomonas	1.86	fontilapidosi	
Methylobacterium	1.73		
Bradyrhyzobium	1.41		
Azospirillum	1.34		

Comments on Example #1

- Reputable firm designed and built the facility
- Hypo-chloride rates, salinity and temperatures matched published laboratory data (metal manufacturer) aimed at justifying the use of 316 under these conditions
  - Samples in study were pickled and passivated
- Heat tint, iron contamination and bacteria all contributed to the problem

Prevention

- Recognize the risk
  - Check for a written water standard
    - Does it address MIC, souring?
  - Plan for chemical treatment
  - Understand the benefits of filtration
- Understand source water & your chemical treatment
  - Focus is often on chloride content, not bacteria
  - Potable water is clean, but may need treatment
  - Pond water, bay water & seawater are generally not clean
    - Definitely need treatment
  - Understand if biocide is affected by oxygen
    - THPS, Glutaraldehyde will react with oxygen
  - Disposal of treated water / exposure of personnel

Open Discussion
Questions, comments?

Krista L. Heidersbach, PhD, PE
Sr. Staff Consultant
Stress Engineering Services, Inc.
13800 Westfair East Drive, Houston, TX 77041
Phone: (281) 955-2900
Mobile: (713) 647-1326
krista.heidersbach@stress.com
www.stress.com