Decades of Dedication

For more than 50 years, GTI has been a leader in providing expert research, development, and testing services for the natural gas industry. While GTI provides services for a wide variety of industries, its unique expertise has historically focused on the natural gas and energy industries.

Clients Have Questions, GTI Provides the Answers

GTI works closely with customers to clearly understand their needs, develop guidance and solutions, and deliver results quickly and confidentially. Results from GTI’s testing help clients answer many questions regarding environmental issues related to the energy industry. From advanced testing in microbiology and other sciences, to research and development for new applications, GTI offers solutions.

Genetic MIC Testing Capabilities

GTI offers solutions to many energy industry problems through comprehensive, state-of-the-art microbiological research and testing services by integrating our molecular genetics techniques to areas such as pipeline microbial corrosion prevention and treatment, microbial characterization, molecular biology, and anaerobic digestion studies. GTI has been providing microbiological testing for more than 25 years to enhance technologies, reduce costs, and minimize environmental impacts for the energy industry.

Background

Microbially-influenced corrosion (MIC) can degrade the integrity, safety, and reliability of pipeline operations and is one of leading causes of pipe failure. NACE International initiated a study that was conducted from 1999 to 2001 which determined that the direct corrosion costs associated with oil and gas production are estimated to be $1.4 billion, with $0.6 billion attributed to surface piping and facility costs, $0.5 billion to downhole tubing, and $0.3 billion to capital expenditures related to corrosion. Cost-effective MIC management requires the early detection of MIC problems, which can only be achieved by routine monitoring of the physical, chemical, and biological characteristics of pipeline systems.

Traditionally, corrosion-causing bacteria are detected and quantified with bacterial growth tests in different enrichment media. The traditional growth method (Most Probable Number test or
MPN test) often relies on liquid samples containing live bacteria, and takes up to 4 weeks to obtain the results. Unfortunately, as numerous researchers show, only 0.1% to 10% bacteria can actually grow in an artificial growth medium, and the significant portion of bacteria growing in the media are actually not the target bacteria. Therefore, the growth test is unable to provide an accurate detection and quantification of target bacteria in the samples.

With the shortcomings of traditional growth method in mind, GTI started the genetic MIC program about 10 years ago with the goal to develop quicker and more accurate detection and quantification methods for corrosion-causing microorganisms. We first determined the identities of major microorganism species in oil and gas pipelines with a focus on corrosion-related species using techniques such as PCR, TTGE, and DGGE, followed by cloning and sequencing. After reviewing the available techniques (traditional growth method, TTGE/DGGE, RSGP, FISH, regular PCR, gene chip, etc), we concluded that they were either too time-consuming, too expensive, or lacked quantitative precision. As a result, we focused on quantitative polymerase chain reaction (qPCR) methods to detect and quantify major groups of corrosion-related microorganisms.

Scope of Genetic MIC Testing Service

GTI now offers a comprehensive corrosion testing service that directly detects and quantifies (without prior growth) corrosion-causing microorganisms typically found in pipes, production wells, and other equipment used by the natural gas, petroleum, chemical, water, produced water and wastewater industries. Quantitative polymerase chain reaction (qPCR) is a technique that detects and quantifies specific genes of target bacteria, which are essential for the metabolism of corrosion causing bacteria in the samples. The method is far more accurate than traditional growth tests, with an accuracy of ±10%, and can analyze almost any type of samples (e.g., water, oil/water, solid, oil/solid, dry, old samples, etc.), whether or not the bacteria in the samples are still alive. GTI’s genetic tests can be completed in 24 to 48 hours depending on the number of samples and number of tests, compared to several weeks often required by traditional tests.

Currently GTI offers genetic detection and quantification of 8 types of microorganisms.

- Total bacteria
- Sulfate-reducing bacteria (SRB)
- Denitrifying bacteria (DNB)
- Total archaea
- Sulfate-reducing archaea (SRA)
- Acid-producing bacteria (APB) (acetic acid-producing bacteria and butyric acid–producing bacteria)
- Iron-oxidizing bacteria (IOB) (Leptothrix and Sphaerotilus, and Gallionella species)
- Methanogens

The number of total bacteria and archaea indicates the overall environmental conditions for the growth of microorganisms. Sulfate-reducing prokaryotes, including SRB and SRA, have been isolated from a wide range of oil-bearing subsurface environments and various pipelines, and are widely considered the most aggressive corrosion-causing microorganisms in various environments. In addition to SRB and SRA, APB and IOB are also considered aggressive
corrosion-causing bacteria. DNB and methanogens are frequently retrieved from pipeline samples, and also cause corrosion. In particular, the denitrifying bacteria need to be closely monitored due to the increasing use of nitrate in some industries as a means to reduce the activity of SRB.

In addition to genetic MIC tests (qPCR), GTI offers to differentiate the live and dead bacteria in the samples. Coupled with qPCR, Live/Dead Differentiation helps determine the effect of biocide application. We can also perform the traditional MPN testing if preferred.

**Advantages of Genetic MIC Testing**

GTI is the pioneer in applying molecular technology to MIC detection, and the first one to use qPCR techniques, starting back in 2002. GTI’s qPCR technology is well recognized via about a dozen publications and presentations. GTI also has a lead in the number of qPCR assays specifically tailored to major MIC-related microbes. The table below compares GTI’s qPCR technology and traditional MPN tests for detection and quantification of various microorganisms.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>qPCR</th>
<th>Traditional growth method (MPN test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mechanism</strong></td>
<td>Detect and quantify specific genes of target bacteria</td>
<td>Grow in selective medium, score positive/ negative growth based on indicators (color, turbidity, etc), and enumerate statistically</td>
</tr>
<tr>
<td><strong>Quantitative?</strong></td>
<td>Yes*</td>
<td>Semi-quantitative**</td>
</tr>
<tr>
<td><strong>Time to obtain result</strong></td>
<td>A few hours per bacterial type</td>
<td>1-4 weeks***</td>
</tr>
<tr>
<td><strong>Sample type</strong></td>
<td>Any types</td>
<td>Only if bacteria are still alive</td>
</tr>
<tr>
<td>liquid</td>
<td>Yes</td>
<td>Yes, if fresh</td>
</tr>
<tr>
<td>solid</td>
<td>Yes</td>
<td>Yes, if fresh and moist</td>
</tr>
<tr>
<td>biofilm</td>
<td>Yes</td>
<td>Yes, if fresh and moist</td>
</tr>
<tr>
<td>dry</td>
<td>Yes</td>
<td>No (generally bacteria are already dead)</td>
</tr>
<tr>
<td>old</td>
<td>Yes</td>
<td>No (generally bacteria are already dead)</td>
</tr>
<tr>
<td>colored</td>
<td>Yes</td>
<td>No (interfere with some growth indicators)</td>
</tr>
<tr>
<td><strong>Sampling procedure</strong></td>
<td>Simple and same for both aerobic and anaerobic bacteria</td>
<td>Difficult to achieve anaerobic condition in the field for anaerobic bacteria</td>
</tr>
<tr>
<td><strong>Microbial type</strong></td>
<td>with qPCR</td>
<td>with growth</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Denitrifiers (DNB)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sulfate-reducing bacteria (SRB)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Acid-producing bacteria (APB)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Iron-oxidizing bacteria (IOB)</td>
<td>Yes</td>
<td>Extremely difficult</td>
</tr>
<tr>
<td>Total archaea</td>
<td>Yes</td>
<td>Extremely difficult</td>
</tr>
<tr>
<td>Sulfate-reducing archaea</td>
<td>Yes</td>
<td>Yes?</td>
</tr>
<tr>
<td>Methanogens</td>
<td>Yes</td>
<td>Extremely difficult</td>
</tr>
</tbody>
</table>

*: In the range of ± 10% of actual number of bacteria in the sample.

**: - 90% to 99.9% bacteria in an environmental sample cannot grow in an artificial growth medium
- The vast majority of bacteria growing in an artificial growth media are not the target bacteria
- Bacteria profile from growth medium is not representative of profile in the original sample

***: SRB needs a 4-week incubation to read the results
Price List for MIC Related Testing

1. Sample preparation (required analysis)  $145 __X___
2. Total Bacteria (required analysis)  $145 __X___
3. Total Sulfate-reducing bacteria (SRB)  $145 ______
4. Total Denitrifying bacteria (DNB)  $145 ______
5. Total Acid-producing bacteria (APB) (2 analyses)  $265 ______
6. Total Iron-oxidizing bacteria (IOB) (2 analyses)  $265 ______
7. Total Methanogens  $145 ______
8. Total Archaea  $145 ______
9. Total Sulfate-Reducing Archaea (SRA)  $285 ______
10. MPN Growth Test (both aerobic and anerobic)  $250 ______
11. Live/Dead Analysis  $115 ______

Environmental Chemistry Laboratory

GTI employs numerous other environmental forensic tools to address contamination and corrosion issues for industry customers. Common analytical analyses include volatile fatty acids, oil extraction and simulated distillation, X-ray diffraction to determine corrosion by-products, and elemental distribution.

Chemical Fingerprinting

Through a methodology called “Chemical Fingerprinting,” GTI is able to identify source contamination of trace-level organic compounds by using three types of forensic techniques to identify chemical compounds of interest.

- Gas Chromatography/Flame Ionization Detection (GC/FID), which produces a unique chemical fingerprint depending on the type of organic contaminant(s) in the sample.
- Gas Chromatography/Mass Spectrometry (GC/MS), which can sensitively quantify the concentrations of the organic contaminants present within the sample. GC/MS data is also used to create polyaromatic hydrocarbon (PAH) profiles to discriminate between pyrogenic and petrogenic sources. Select PAH ratios can be used as indicators for source identification.
- Gas Chromatography/Isotopic Ratio Mass Spectrometry (GC/IRMS), which measures stable isotope ratios of carbon to discriminate between different sources of the carbon-
containing contaminants. This technique becomes extremely useful when the concentrations of the contaminants are at a low levels (where GC/FID and GC/MS cannot provide conclusive results), as well as when samples have undergone environmental weathering.

**Analytical Chemistry Laboratory**

For more than 40 years, GTI’s analytical chemistry laboratory has provided GTI and energy-related industries a wide range of customized support services unavailable in most commercial laboratories. Specific areas of expertise include analytical chemistry services, certified calibration gases, and gas quality measurement.

Gas analysis and other analytical services are conducted in laboratories and facilities that include the latest developments in analytical instrumentation for chemical analysis and physical testing. GTI’s staff of analytical chemists and technicians provides comprehensive services for fuel analysis, gas quality assessment, and materials characterizations. State-of-the-art instrumentation includes multiple gas and liquid chromatographs, thermal analyzers, physical testing equipment, and various spectrometers, including atomic (AA and ICP), FTIR, and a scanning electron microscope (SEM) with EDX capabilities. Also available are instruments that employ coupled technologies, such as gas chromatography and mass spectroscopy (GC-MS), or gas chromatography and atomic spectroscopy (GC-AED).

**Analytical Method Development**

GTI’s analytical laboratories have complete facilities for sample preparation, fuel analysis, compositional and trace analysis, and characterization of a variety of materials related to the natural gas industry. Our experts have contributed to the establishment of numerous ASTM standard methods of analysis, and are very experienced in developing analytical methods for unique applications.

**Laboratories & Facilities**

It is the consistent policy of GTI that good laboratory practice and quality assurance/quality control (QA/QC) procedures are implemented to comply with client and contractual obligations. Its management policies, objectives, principles, organizational responsibilities and standard operating procedures were developed with the goal of providing quality control from receipt of samples at the laboratory to generation of final reports. Its highly qualified staff uses state-of-the-art and well-maintained equipment and follows recognized standard tests such as NACE, ASTM, EPA and GPA methods whenever possible. Standards traceable to NIST are used for instrument calibration if they are available. A networked laboratory information management system (LIMS) is employed to enable rapid and precise sample status tracking, accurate accounting and data reporting, and vital controls of quality and cost.
Brief List of Laboratory Instrumentation Related to Corrosion and Environmental Activities

- Epifluorescent/Light Microscope
- Quantitative polymerase chain reaction thermocycler used to amplify a desired portion of nucleic acids and determine nucleotide sequence and size of DNA/RNA.
- Gel electrophoresis to confirm PCR products
- Aerobic and anaerobic growth chambers used to culture and isolate microorganisms
- Gas chromatography using a multitude of detectors (flame ionization, thermal conductivity, flame photometric, photoionization, chemiluminescence) that allows for the analysis of gaseous and liquid samples for major component and trace level organic species and pollutants.
- GC/MS-Gas chromatography/mass spectrometry to quantify PAHs, MAHs, alkyl-PAHs, petroleum markers, and identification of unusual or unknown species
- GC/AED-Gas chromatography/atomic emission spectroscopy to quantify v
- Pyrolysis-GC/MS to identify the complex macromolecular matrix in water, soil, and sediment samples
- GC/IRMS-Gas chromatography/isotope ratio mass spectrometry to determine compound-specific stable isotope ratios
- Toxicity analyzer to measure toxicity of a substrate to Vibrio fischeri microorganisms.
- ICP and mercury analyzer
- FTIR and Raman spectroscopy

Gas Technology Institute

1700 South Mount Prospect Road
Des Plaines, Illinois 60018-0501
847-768-0500; FAX: 847-768-0501
www.gastechnology.org