Aegis Tech Line

Aegis Chemical Solutions

Technical Newsletter

Volume 03, November 2017

BACTERIA IN OIL & GAS PRODUCTION

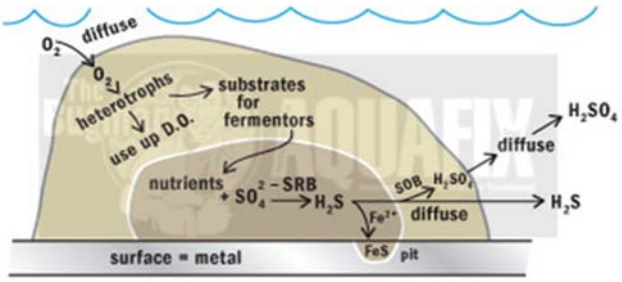
Bacteria is of concern in oil & gas production and transportation systems because of the tremendous damage that can be caused by its by-products. This newsletter will deal with describing and profiling bacteria, and the next issue will deal with the prevention and mitigation alternatives used in the oilfield with respect to bacteria.

TYPES OF BACTERIA OF CONCERN IN THE OILFIELD

There are several different types of bacteria that are of concern in the oilfield. Depending on the type or types of bacteria that are present or might develop in the system, different techniques are used to prevent or mitigate them. It is therefore essential to identify the bacteria.

Sulfate-Reducing Bacteria (SRB) – Anaerobic

Significant emphasis is placed on sulfate reducing bacteria (SRB) because of their clear links to the production of hydrogen sulfide, which is corrosive to steel. SRB were thought to be able to cause corrosion as a single species, possibly growing within a tubercle at the site. It is now recognized that SRB form a part of a microbial community (sometimes referred to as a "consortium"). Within these consortia, SRB can function deep within biofilms under both anaerobic (no oxygen) and aerobic (contains oxygen) conditions. Generally, the biofilms are formed within tubercles, encrustations, and slimes. Since SRB are deeper down in these growths, they may not be recovered in water samples taken from flow over the growths.



- Heterotroph is organism that uses carbon from oil, sugars, fats, etc. and not from carbon dioxide.
- Fermenters are typically acid producing bacteria (APB)

Acid-Producing Bacteria (APB) – Aerobic

Acid-producing bacteria (APB) have been recognized as a possible major cause of corrosion, mainly because their fermentative activities will cause the pH (particularly in the biofilms) to drop into the acid range. This is due to production of fatty acids and acetic acid.

Iron-Oxidizing Bacteria (IOB) – Aerobic

Iron-Oxidizing Bacteria (IOB) are those that convert iron from the ferrous [Fe^{+2}] to the ferric [Fe^{+3}] state and produce ferric hydroxide [$Fe(OH)_3$]. Ferric hydroxide is a highly insoluble by-product that will damage formations and plug pipes and equipment. IOBs also produce corrosion, but they are considered harmful mainly because they cover SRB colonies and protect those colonies from mitigation using bactericides.

BACTERIA GROWTH CONDITIONS

It is important to understand the conditions in which bacteria grow and reproduce to determine how to deal with them in oilfield systems.

- **Temperature:** Estimated from 14°F to 210°F. Optimum temperature range for SRBs is 77°F to 120°F.
- **pH**: Estimated from 0 to 10.5. However, the optimum pH range is 5 to 9.
- **Total Dissolved Solids (TDS):** Bacteria prefer fresh water but can grow in brines. Sea water has a TDS of over 35,000 mgs/L and easily supports bacteria growth. SRB have been reported in brines with a TDS >100,000 mgs/L, but the growth rate is reduced at higher TDS.
- **Dissolved Oxygen:** Aerobic bacteria require oxygen to grow. Anaerobic bacteria grow best in the absence of oxygen. Facultative bacteria grow in either the presence or absence of oxygen.

Aegis Tech Line

Aegis Chemical Solutions

Technical Newsletter

Volume 03, November 2017

PLANKTONIC AND SESSILE BACTERIA

Planktonic bacteria: those organisms that live in the water column and are incapable of swimming against a current.

Sessile bacteria: those organisms associated with biofilm development and corrosion related issues.

Sessile bacteria are protected in antagonistic environments (such as produced waters) by growing as colonies encased in an extracellular matrix of carbohydrates (exopolysaccharide).

- According to NACE International: "attached microbes (sessile bacteria) are normally the most important biological component of the bacterial ecology of an oilfield system".

Methods used for measuring planktonic bacteria are of limited use for assaying sessile species. Therefore, it must be considered that both bacteria will exist in all aspects of water related applications of the oilfield where conditions are favorable for bacterial growth and reproduction. There must be protocols listed for assessing both.

BACTERIA RELATED OILFIELD PROBLEMS

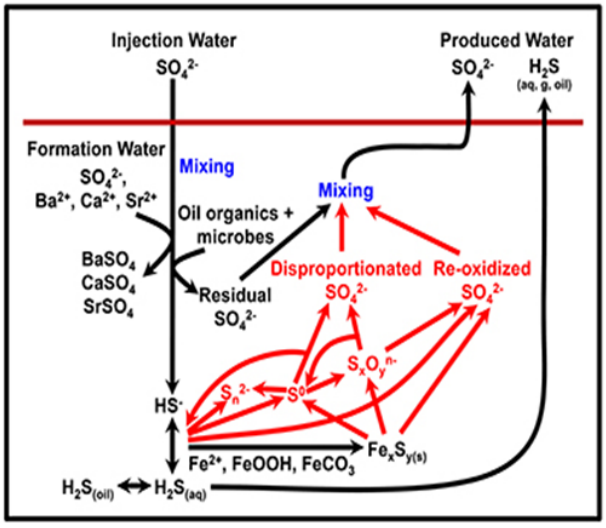
There are several oilfield problems that are associated or attributable to bacteria:

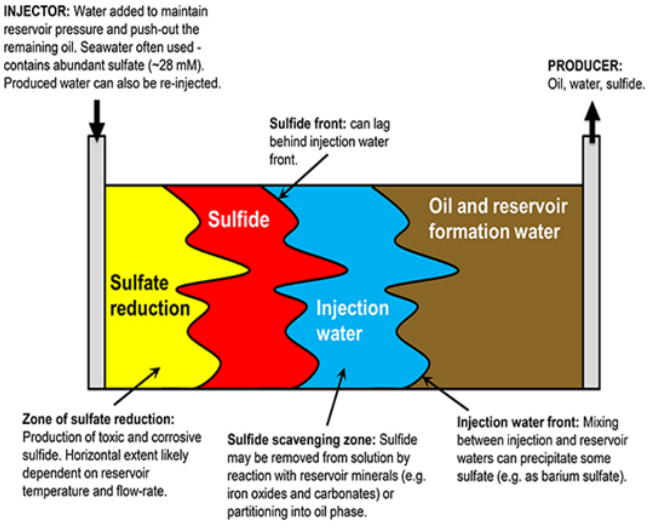
- Biogenic souring (H_2S) particularly of wells or formations
- Microbial Induced Corrosion (MIC)
- Plugging
- Emulsion Problems

Reservoir Souring – Fracking, Drilling or Pressure Maintenance Using Bacteria Contaminated Fluids

Microbiologically influenced corrosion is a concern for the oil and gas industry, especially in applications which involve injection of external water sources (e.g. hydraulic fracturing or fracking). Fracking processes involve injection of large volumes of water, often sourced from surface ponds, streams, rivers or lakes. Studies have shown that these sources are typically contaminated with bacteria. Without prevention methods or techniques, this contamination is subsequently transferred to the fractured well and reservoir. Fracturing fluids often contain polyacrylamide or sugar-based polymers that can serve as an energy (food) source for the injected bacteria. These conditions put downhole and surface equipment at risk of microbiologically influenced corrosion.

When water is used as a method to maintain reservoir pressure (commonly called "water flooding") and "sweep" the oil to the producing wells, this is also a potential source of bacterial introduction to the reservoir. This is illustrated below.





Aegis Tech Line

Aegis Chemical Solutions

Technical Newsletter

Volume 03, November 2017

Microbial Induced Corrosion (MIC)

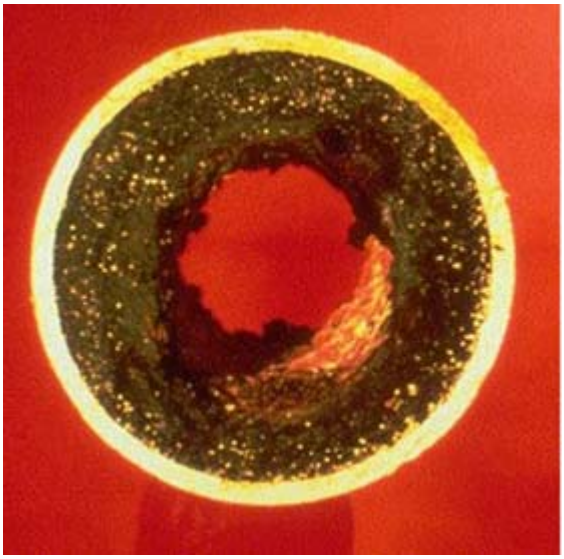
The photographs below illustrate MIC. MIC is often characterized by isolated pitting with sharper edges than sulfide corrosion. The pits are often layered or concentric as one can see in the cleaned pipe below.

MIC is most severe in stagnant or low velocity conditions, usually <5 feet/second liquid velocity. At low velocity, solids in produced fluids will settle and/or collect. Bacteria will grow under the solids producing severe isolated pitting.

Plugging

The photographs below illustrate two types of plugging problems that are the result of bacteria in oilfield systems:

Biomass Plugging of Filters



FeS Accumulation in Pipe

Aegis Tech Line

Aegis Chemical Solutions

Technical Newsletter

Volume 03, November 2017

Emulsion Problems

Bacterial slime and iron sulfide accumulates at the oil-water interface in vessels producing pads in tanks or emulsions. The bacterial slime and iron sulfide become coated with oil (oil-wet) and fall into the water layer, forming a reverse (oil-in-water emulsion). This can result in high oil carryover.

Oil-Wet Bacterial Slime and/or Iron Sulfide

BACTERIA MONITORING

Planktonic Bacteria

There are several methods used to identify and quantify the planktonic bacteria content in an oilfield water sample including:

- American Petroleum Institute Method API RP 38 which is similar in part to NACE Standard TMO194-94: *Field Monitoring of Bacterial Growth in Oilfield Systems* is widely used in the oilfield for assessing bacteria contamination. These are known as the "bug-bottle method" or serial dilution. Standard bug bottles may not work in systems where produced brine salinity is significantly different from culture media in the vials. The absence of a positive indication of bacteria by "bug bottles" does not indicate their absence in the system.
- Epifluorescence Microscopy
- ATP Analysis

Sessile Bacteria

Sessile bacteria are identified using several techniques including:

- Bio probes (Originally called Robbins device)
- Corrosion coupons (deposits removed, dispersed in distilled water, then serially diluted).

SERIAL DILUTION PROCEDURE

Line up desired number of serum bottles containing 9 mls. of growth medium. Inject 1 ml. of the field water sample into the first bottle and shake well. Withdraw 1 ml. of solution from the first bottle with a disposable syringe and inject it into the second bottle. Shake the bottle well. Withdraw 1 ml. of solution from the second bottle with a disposable syringe and inject it into the third bottle. Shake the bottle well. Continue procedure until all bottles are inoculated. Incubate bottles for 28 days at a temperature within 90°F of system temperature. Usually this may not be practical so it is common to use 100°F. Check bottles periodically to see if any are showing growth.

TABLE 5.1

Extinction Dilution Technique

1 mL Water Sample	Bottle No.	Dilution Factor	Number of Bacteria in Orfginal 1 mL Water Sample
1 mL	1	1/10	1-10
1 mL	2	1/100	10-100
1 mL	3	1/1000	100-1000
1 mL	4	$1/10^4$	1000-10 000
1 mL	5	$1/10^5$	10 000-100 000
1 mL	6	$1/10^6$	100 000-1 000 000
1 mL	7	$1/10^7$	1 000 000-10 000 000

Each Bottle Contains 9 mL of Growth Medium

Aegis Tech Line

Aegis Chemical Solutions

Technical Newsletter

Volume 03, November 2017

Anaerobic bacterial growth media, such as modified Postgate's B (MPB) and API-RP38 (API) for the detection of Sulfate Reducing Bacteria (SRB) is extremely sensitive to oxygen exposure. Biotechnology Solutions anaerobic bacterial growth media is manufactured inside custom built anaerobic chambers to ensure the highest quality bacterial growth media products.

If the fluids injected into the vial contain high levels of dissolved sulfide, the first one to two vials in the dilution series may turn black. This is a false positive. If vials precipitate iron sulfide immediately, or within an hour, these vials should be recorded as false positives for SRB activity.

Sulfate Reducing Bacterial (SRB) Serial Dilution

Acid producing bacteria (APB) will grow in the media and facilitate a color change from orange-yellow to a strong yellow coloration. General Heterotrophic Bacteria (GHB) will grow in this media and will not produce a color change. GHB can be identified in the vials by visually identifying turbidity or biomass.

Acid Producing Bacteria (APB) Serial Dilution

ATP (ADENOSINE TRIPHOSPHATE) OVERVIEW

ATP (adenosine triphosphate) is present in the cells of all living organisms. ATP is destroyed within 20 seconds of cell death through release of an ATP-destroying enzyme. The amount of ATP per cell is a linear function of cell volume (i.e., twice the cell volume results in twice as much ATP). ATP gives a very quick indication of bacteria levels and does not require 28 days of incubation. However, this technique does not distinguish the type of bacteria. ATP also requires a photometer which may not be conducive to field analysis.

ATP Test Procedure

A water sample is filtered through a 0.45-micron filter that removes bacteria from the water and retains them on the filter paper. This concentrates the bacteria on the filter. The ATP must then be extracted from the cells prior to its destruction by enzymes. Usually the filter is placed in a boiling solution of a buffer that destroys the enzymes and bursts the bacteria cell membrane releasing ATP into solution. Luciferin/luciferase is then added to the solution. This reacts with ATP and emits light which can be detected by a photometer. The amount of light emitted is proportional the concentration of ATP. A calibration curve is used to determine cells/ml.

Aegis Tech Line

Aegis Chemical Solutions

Technical Newsletter

Volume 03, November 2017

BIO PROBE OR ROBBINS DEVICE FOR MEASURING SESSILE BACTERIA

This technique uses a flush mounted probe with mild steel studs that is inserted into the system or a side-stream of the system. After a given exposure to system fluids, the studs are removed and analyzed. Each stud is placed in a sterile medium in a test tube and thoroughly mixed. A portion of the medium is then serially diluted using APB and SRB serial dilution bottles, or it is analyzed microscopically. Alternatively, the studs may be scraped with a sterile scalpel into the sterile media. Each stud can also be analyzed by epifluorescence microscopy directly. In addition, ATP testing may also be used.

Bio-probes

Bio probes are not widely used, primarily because rectangular corrosion coupons can serve the same purpose as the mild steel studs in the bio probe. Corrosion coupons are widely used in oil and gas production and can serve a dual purpose: corrosion rate determination, and sessile bacteria detection. The coupons are treated in the same way the steel studs are treated (described above). After scraping the coupon into a sterile medium, the coupon can be submitted to the lab for standard corrosion rate analysis. The sterile medium containing the scraped solids from the coupon are analyzed with serial dilution or the other techniques described earlier.

Aegis Tech Line

Aegis Chemical Solutions

Technical Newsletter

Volume 03, November 2017

BIOCIDES

The strict definition of 'biocide' is: it will kill living cells. Whether it can successfully “kill” bacteria depends upon several variables not least of which is the dose (i.e. the concentration of biocide) and the time it is in contact with microorganisms.

Most biocides can also be regarded as biostatic. At concentrations lower than that required to kill, the biocide inhibits cell growth, while it is present. Once the chemical is removed, the bacteria will continue to grow again. At doses lower than biostatic the biocide can even become a source of nutrition and therefore encourage growth.

In some cases, it may be necessary to run a “time-kill” test to evaluate the effectiveness of a given biocide. A time-kill test is usually run on location with fresh, field brine. A properly run test will yield information on type of biocide, dosage and contact time.

AEGIS FACILITIES LOCATIONS

Corporate Headquarters *Houston* 4560 Kendrick Plaza Dr., #190 Houston, TX 77032 Tel: (855) 532-2033	**Permian Basin** *Midland* 2200 Market St. Midland, TX 79703 Tel: (432) 695-6285	**Permian Basin** *Abilene* 4657 FM 707 Abilene, TX 79606
Permian Basin *Breckenridge* 566 FM 2231 Breckenridge, TX 76424	**Permian Basin** *Forsan* 300 1st St. Forsan, TX 79720	**Permian Basin** *Lubbock* 13622 Hwy 87 S. Slaton, TX 79423 Tel: (806) 329-4609
Permian Basin *Monahans* 1332 PR Thrasher Ln. Monahans, TX 79756	**East Texas** *Kilgore* 4524 State Hwy 42 N. Kilgore, TX 75662 Tel: (903) 218-0058	**East Texas** *Raywood* P.O. Box 219 Raywood, TX 77582 Tel: (409) 550-5211
East Texas *Teague* 931 W. Hwy 84 Teague, TX 75860 Tel: (903) 218-4795 x1058	**South Texas** *Freer* 5743 Hwy 44 Freer, TX 78357 Tel: (361) 226-3103	**South Texas** *Asherton* 664 Hwy 190 Asherton, TX 78827
South Texas *Cuero* 424 U.S. Hwy 87 S. Cuero, TX 77954	**South Texas** *Jourdanton* 8229 S. State Hwy 16 Jourdanton, TX 78026 Tel: (830) 265-5595	**South Texas** *San Antonio* 311 N. Frank Luke Dr., #103 San Antonio, TX 78226
North Louisiana *Haughton* 140 Sligo Industrial Dr. Haughton, LA 71037 Tel: (318) 261-8333 x1056	**South Louisiana** *Elizabeth* 3552 Highway 10 Elizabeth, LA 70638 Tel: (318) 634-5252	**Mid-Con** *Ratliff City* P.O. Box 98, 1500 Old Hwy 7 Ratliff City, OK 73481 Tel: (580) 856-2015
Mid-Con *El Reno* 801 N. Willie L. Minor, #B-1 El Reno, OK 73036	**Mid-Con** *Hennessey* 1117 S. Main St. Hennessey, OK 73742	