

New-Generation Microbial Testing Compares Favorably With Traditional Technologies in Identification of Bacterial Contamination of Oil and Gas Systems

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ABSTRACT

Ever-increasing demands are being placed on oil and gas production operations to become more efficient, cost-effective, and environmentally compliant. Greater emphasis is now being placed on the use of monitoring tools to measure system performance. Consequently, monitoring and controlling microbiological contamination in upstream oil and gas processes and facilities are common practice. Uncontrolled microbiological growth and activity can create severe operational, environmental, and safety issues, resulting from microbially induced influenced corrosion (MIC), solids production, and hydrogen sulfide (H₂S) generation. In addition, bacteria can degrade drilling and well stimulation fluids and enter the producing formation, and subsequently the surface production equipment and tanks, during the initial drilling, stimulation, and completion of a well. These problems can be managed by understanding where microorganisms exist in the systems, the quantity of microorganisms present, and the relative activity of the microbial populations. In short, the ability to more proactively and comprehensively monitor microorganisms gives an enhanced ability to control their proliferation and thus reduce their impact.

There are several microbiological monitoring tools in common use today, ranging from direct microscopic examination to culture-based counting techniques. Other industries have benefited from the use of various cutting-edge molecular monitoring tools, such as the Adenosine Triphosphate (ATP) measurement technique. Traditionally, the measurement of ATP in oilfield operations has been problematic due to various assay interferences prevalent in oilfield samples, including oil, suspended solids, and dissolved solids. The continued interest in the ATP assay, due to the rapid acquisition of results, has led to a new generation of methodology suitable for use in oilfield samples.

This paper summarizes results obtained from testing various surface, seawater, and produced water samples from oil and gas systems throughout North America. Microbiological characteristics of each sample were qualified and quantified using traditional techniques, microscopic examination, and culture testing, and then compared to the new-generation ATP analysis results. A high-level comparison of these methodologies is presented, and subsets of the data are presented for in-depth examination in a series of case studies. It was found that the 2nd Generation ATP test used in these studies compared favorably with findings from traditional microbial measurement tools, including epifluorescence microscopy and serial dilution-to-extinction culture tests, while providing the benefits of field readiness and immediate feedback.

INTRODUCTION

The proliferation of microorganisms is a significant concern in the production and distribution of oil and natural gas. Microbially induced corrosion, formation souring, equipment and reservoir fouling and drilling and stimulation fluid degradation are the focus of attention as they contribute to lifting and production costs for the producer. Once bacteria become established in oilfield systems, it is necessary to implement bacterial mitigation programs. These programs involve the use of mechanical and chemical treatments, including microbiocides (chemicals that kill microorganisms), biostats (chemicals that limit bacterial growth and/or activity) and chemical solvents used to clean up and dissolve solids. In order to determine if these treatments are adequately controlling the planktonic and sessile bacterial growth in the system, a number of methods have been developed and used over the years to enumerate the bacteria found in oilfield systems and evaluate the efficacy of bacterial control programs.

The current industry standard for detecting bacterial presence is the serial dilution to extinction technique using specialized bacterial culture media. This method uses various types of bacterial growth media designed to grow the aerobic and facultative anaerobic bacteria (including the acid-producing bacteria) and SRB, contained in oilfield waters. Since microorganisms are extremely sensitive to pH, total dissolved solids (TDS) concentrations, nutrient availability, and temperature, culture media are designed to closely emulate the indigenous bacteria's environment to fully support the growth of the microorganisms. Unfortunately, selected culture media and incubation conditions do not always accurately reproduce the bacteria's environmental conditions, resulting in an underestimation of the actual bacterial concentration. In fact, it is estimated that culture media based methodologies only recover 1% to 15% of the total population present in a given sample. While the incubation period for the aerobic and facultative anaerobic bacteria is fairly short with results available in 3 to 7 days, the incubation time period for the SRB is 14 to 28 days, which tends to limit its utility for optimizing bacterial mitigation programs. It is not uncommon that, during the time required for incubation, the problem has grown or has spread to other processes, resulting in increased costs from downtime, lost production, and higher material and manpower costs.

Epifluorescence microscopic analysis using the acridine orange (AO) stain is one of the most accurate and rapid bacteria enumeration techniques available. This technique does not rely on recreating environmental conditions conducive to growth, but rather involves isolation, staining and counting of the bacteria present in a sample. The technique allows for enumeration of the total bacteria population in a sample but does not distinguish between different classes of bacteria, such as sulfate reducing bacteria and acid producing bacteria. It also does not distinguish between living and dead organisms. The technique is a valuable tool for rapidly determining locations of contamination, as well as for verifying performance of other enumeration techniques. The analysis is considered a specialty technique that is not typically used by field service engineers as it requires specialized equipment and extensive training to differentiate bacteria from other components such as hydrocarbon, scale, and other solids in the samples.

In the oil and gas industry, molecular techniques are becoming more widely used due to their ability to provide a more comprehensive representation of the complexity and diversity of the microbial consortia present in oilfield samples. However, the various technologies have not advanced to the stage where they would be considered field

friendly and, therefore, would not be readily available for all potential users. The techniques require specialized training and equipment for the end-user.

The measurement of Adenosine Triphosphate (ATP) has been used for decades as a means to rapidly quantify total living microorganisms in a variety of food hygiene and industrial water applications. ATP is the primary energy carrier molecule in all forms of life. In early protocols, ATP was extracted from cells by boiling the sample in a buffer. The extracted ATP was then reacted with the enzyme-substrate complex Luciferin-Luciferase. In this reaction, the Luciferase enzyme catalyzes the ATP-mediated oxidation of Luciferin. In the process, ATP is cleaved to yield Adenosine Monophosphate (AMP) and pyrophosphate, concurrently releasing a photon of light. The amount of light generated is proportional to the ATP concentration, which is proportional to the amount of total biomass in a sample. In this way, it presents a user-friendly, field-ready means to quantify total microbial concentration in a given sample.

Unfortunately, these early ATP measurement protocols were unsuitable for use in oilfield environments because of a variety of interferences, including dissolved and suspended solids, hydrocarbon contamination, and residual biocides or other production chemicals. All of these interferences can contribute to the inhibition of the ATP-Luciferase assay and/or the ability for light to penetrate to the photodetector of the luminometer. Since these early days, numerous improvements have been made to the ATP test protocol and it has gained widespread commercial and industrial use. However, many of the interferences characteristic of oilfield samples had remained a problem. This paper documents the use of a second-generation ATP method which has been adapted to mitigate the traditional interferences associated with oilfield samples. The second-generation technology can be used for fresh, produced and seawater samples, fluids containing hydrocarbons and sessile samples from corrosion coupons, deposits, and surface swabs. Since this method provides an on-the-spot indication of total living biomass, it can be used to seek out contamination 'hot spots,' implement one or more corrective measures, and verify efficacy in a far shorter period than via traditional techniques alone.

This paper documents several case histories comparing the second-generation ATP technology with bacterial culture media and microscopic enumeration.

EXPERIMENTAL PROCEDURE

Water Sampling Process

For in-field analyses, fresh seawater and produced water samples were collected from sampling points in 200-ml nominal capacity glass bottles. Each sample point was flushed thoroughly before sample collection. All bottles were rinsed with sample fluids before collection of the sample used for analyses. Sample bottles were filled to overflowing and capped tightly to maintain sample integrity.

Sessile Sampling Process

Sessile samples were obtained from corrosion coupons that had been in the system for 30 days to allow time for a stable biofilm to develop. Two different methodologies were used to collect sessile coupon samples. The first set of sessile samples was obtained by

removing the coupon from the system and swabbing one side of the 0.5- by 1.5-inch coupon surface with a sterile cotton swab. The coupon solids and the cotton swab tip were placed into a dilution blank containing 10 ml of 0.2-micron filter-sterilized system water. Each sample was then shaken for 2 to 3 minutes, allowing the solids to disperse into the system water, creating a slurry. Phenol red dextrose culture media, SRB culture media, and ATP analysis were completed on these samples. The second set of sessile samples were created by completely immersing the coupon in the ATP lysing solution (UltraLyse™ 7) and only ATP results were run.

Serial Dilution Culture Methods

Water samples were prepared for semi-quantitative enumeration of viable GHB (general heterotrophic bacteria which includes aerobic and acid-producing bacteria) and SRB using the serial dilution method^{2,3}. Modified Phenol Red Dextrose Medium was used for GHB enumeration and a Baker Hughes proprietary modified Postgate medium referred to as West Texas SRB medium was used to enumerate the SRB population size (C&S Laboratories, Inc., Broken Arrow, OK). Each medium was adjusted to match the salinity of the fresh or produced water. The culture media bottles were incubated for 7 days (GHB) or 28 days (SRB) at system temperature.

Acridine Orange Epifluorescence Microscopy

Samples were enumerated for total numbers of bacteria by epifluorescence microscopy. This is one of the most accurate methods of enumeration and is used as a benchmark for comparison with other techniques. However, microscopy cannot distinguish if the bacteria are alive or dead, nor distinguish between GHB and SRB. Samples were filtered through black, 25-mm-diameter, 0.2-micron, polycarbonate filters. The bacteria trapped on the filters were stained with acridine orange, a chemical that binds to nucleic acids contained within all bacterial cells. The filters were mounted on microscope slides and analyzed using epifluorescence microscopy at 1000x magnification on a Nikon Eclipse microscope equipped with a fluorescence epi-illuminator. For each sample, the number of bacteria was determined by averaging the number of bacteria counted over numerous microscopic fields and then calculating bacteria/ml of sample based on the amount of sample filtered, the size of the filter, and the size of the microscopic fields.

Second-Generation ATP Measurement

Samples were enumerated for total living microorganisms via second-generation ATP measurement (LuminUltra Technologies Ltd., Fredericton, NB, Canada). Adenosine Triphosphate (ATP) is a molecule that stores energy for chemical reactions within all living organisms. When ATP is combined with the enzyme luciferase (isolated from fireflies), light is produced. The amount of light is directly proportional to the amount of ATP and thus energy present in the sample. This is an accurate method for estimating the total size of the microbial population in a sample. The test does not distinguish between the different types of bacteria, such as APB and SRB.

To assess the ATP activity in an aqueous (water) sample, an aliquot of sample was filtered through glass microfiber filters, trapping the biomass in the filter. The microorganisms trapped on the filter were then treated with a lysing agent (UltraLyse 7) to extract the ATP from the cells and preserve it for analysis. The ATP extract was diluted with a cleansing solution and a 100-microliter volume of the diluted extract was

added to a reagent containing the luciferase enzyme (Luminase™). This preparation was immediately placed in a luminometer and the amount of light produced by the reaction was recorded as relative light units (RLU).

The method described above is available commercially as Quench-Gone™ Aqueous (QGA™). Similar methods are used herein for testing organic fluids (Quench-Gone Organic Modified, or QGO-M™) and deposits (Deposit & Surface Analysis, or DSA™).

The RLU of the sample is compared to the RLU produced from a known standard of ATP (UltraCheck™ 1) to account for the current activity of the luciferase enzyme and multiplied by the ratio of volumes used in the preparation of the sample calculating the picograms of cellular ATP per mL. Using a conversion factor that estimates the average concentration of ATP in an *E. coli* bacterium and assuming that most oilfield bacteria will have similar size and activity to an *E. coli* bacterium, the pg ATP/mL data can then be converted and reported as number of bacteria per mL of sample. This test can provide an assessment of the total living bacterial population within 5 minutes of sample collection down to a detection limit of 100 living bacteria per mL using standard procedures.

RESULTS

Case History #1: Bucking the Cliché “Good and Quickly Seldom Meet”: Comparison of Second-Generation ATP With Traditional Industry Standard Methodology for Eagle Ford Shale Fracturing Process Survey

An Eagle Ford Shale operator requested that Baker Hughes evaluate their fracturing process to determine if their current biocide program was providing control over the bacteria in their fracturing water sources. A survey was performed of the fracturing process and production wells on flowback. The results of this survey are shown to demonstrate the strong correlation between culture media, microscopy and the second-generation ATP assay (Table 1).

Table 1. Eagle Ford Shale Survey Results: Comparisons of Culture Media, ATP and Microscopy

Sample ID	Culture Media		ATP Analysis		Microscopy
	APB/mL	SRB/mL	pg/mL	Bacteria/mL	Total Bacteria/mL
Fracturing Source Waters					
Water Well #1	< 10	< 10	BDL	BDL	< 10 ³
Water Well #2	= 10 ⁴	< 10	56	5.6 x 10 ⁴	2.3 x 10 ⁴
Frac Pond #1	= 10 ⁴	10 ³	117	1.2 x 10 ⁵	4.1 x 10 ⁵
Frac Pond #2	= 10 ⁴	= 10 ⁴	1175	1.2 x 10 ⁶	4.5 x 10 ⁶
Frac Pond #3	10 ⁵	< 10	54.2	5.4 x 10 ⁴	4.2 x 10 ⁵
Frac Pond #4	10 ⁵	< 10	335	3.4 x 10 ⁵	5.3 x 10 ⁵
Wellhead					
Wellhead #1	10 ³	10 ²	3.1	3.1 X 10 ³	3.5 X 10 ³
Wellhead #2	10 ³	10 ³	22	2.1 x 10 ⁴	2.4 x 10 ³
Wellhead #3	10 ³	< 10	0.5	5.0 x 10 ²	1.2 x 10 ³
Wellhead #4	10 ³	10 ³	40	4.0 x 10 ⁴	6.2 x 10 ³
Wellhead #5	= 10 ⁴	10	95	9.5 x 10 ⁴	1.1 x 10 ⁵
Wellhead #6	10 ²	< 10	23	2.3 x 10 ⁴	3.1 x 10 ³
Production Equipment					
Separator	= 10 ⁴	< 10	90	9.0 x 10 ⁴	2.0 x 10 ⁵
Produced Water Tank	= 10 ⁴	< 10	58.6	5.9 x 10 ⁴	3.5 x 10 ⁴

BDL = below detectable limits of assay (<10³ bacteria/mL)

The survey results show that all of the water sources used for fracturing with the exception of water well #1 showed bacteria concentrations in excess of the typical industry specification of = 10³ bacteria/mL. In most cases, the predominant bacterial population was acid-producing bacteria. Each of the wellhead samples showed viable bacterial populations; however, only wellhead #5 showed bacterial concentrations in excess of the targeted specification of = 10³ bacteria/mL. The production equipment showed a one-log increase over the average population observed in the wellhead samples.

Case History #2: Time Is Money! Haynesville Shale Drilling Mud Survey

A Haynesville Shale operator was experiencing issues with casing failures at two of their drilling sites and requested technical support to determine if bacteria were contributing to the failures. A microbiological survey was performed on the drilling process to assess the microbial activity. Fresh water samples were collected from the pump withdrawing water from the drilling pit and from the water storage tank. Mud samples were taken from the inlet and outlet of the drilling process. Samples were processed using traditional culture media, microscopy, and the ATP assay (Table 2).

Table 2 Haynesville Shale Drilling Mud Survey Results

Location	Culture Media		ATP Test		Microscopy
	APB/mL	SRB/mL	pg/mL	Bacteria/mL	Bacteria/mL
Water Source					
Source Water #1	10 ⁵	10 ⁴	489	4.9x10 ⁵	3.9x10 ⁶
Source Water #2	10 ³	10	498	5.0x10 ⁵	1.5x10 ⁵
Source Water #3	10 ²	10 ³	26	2.6x10 ⁴	3.6x10 ⁴
Source Water #4	10 ⁵	10 ³	807	8.1x10 ⁵	1.2x10 ⁶
Source Water #5	10 ²	10	117	1.2x10 ⁵	3.5x10 ⁵
Water Tanks					
Tank Water #1	10 ³	10 ³	318	3.2x10 ⁵	1.3x10 ⁶
Tank Water #2	< 10	< 10	4.6	4.6x10 ³	4.4x10 ³
Tank Water #3	10 ³	10 ²	555	5.6x10 ⁵	1.9x10 ⁶
Tank Water #5	10 ⁴	10 ⁴	20,259	2.0x10 ⁷	4.7x10 ⁶
Oil Based Muds (15% water)					
Mud In #1	< 10	< 10	7.3	7.3x10 ³	Too many solids to count
Mud Out #1	< 10	< 10	29	2.9x10 ⁴	
Mud In #3	< 10	< 10	823	8.2x10 ⁵	
Mud Out #3	< 10	< 10	28	2.8x10 ⁴	
Mud In #4	< 10	10	21	2.1x10 ⁴	
Water Based Muds					
Mud In water #5	10 ⁴	< 10	105	1.1x10 ⁵	Too many solids to count
Mud Out water #5	10 ⁴	< 10	128	1.3x10 ⁵	
Pit Water Post Drilling Completion					
Pit Water #1	= 10 ⁶	= 10 ⁶	5321	5.3x10 ⁶	2.3x10 ⁷

A six-bottle dilution series was inoculated for APB and SRB culture media. The results are expressed as number of positive bottles in the dilution series.

The ATP assay confirmed high concentrations of bacteria in the water sources and water based drilling mud while still at the well location. The drilling mud samples were difficult to process with microscopy or culture media bottles because of the solids interference from the samples.

During the survey, the oil-based drilling muds were also analyzed although bacteria presence was not anticipated as it was presumed that these fluids would have minimal water content. The ATP results did reveal the presence of bacteria in all oil-based mud drilling process. This was not surprising considering that the oil-based muds were comprised of approximately 15% water, providing more than enough water for bacterial activity.

The quick results from the ATP assay allowed the team to set a plan in motion for a biocide selection study and implementation of a microbiocide program to treat the drilling fluids. The complete time from start to finish by using the ATP test to identify the initial problem was reduced by 50%.

Case History#3: A Winning Combination... Biocide Treatment and Fast Results From the ATP Assay Save the Day for a Frac Job in the Haynesville Shale

A complete set of frac tanks filled with cross-linked fracturing gel turned to liquid in a 24-hour period for an operator in the Haynesville Shale. The operator suspected that the UV light pretreatment of the source water was not effective and bacteria were responsible for the degradation of the frac gel. The operator's engineer was concerned about the possibility of another frac gel degradation failure at their next frac job and requested that the frac tanks be pretreated with a glutaraldehyde-based biocide. The operator wanted confirmation of the efficacy of the treatment and it was decided to use the ATP assay for onsite validation of the efficacy of this biocide application. The tanks were treated with 250-ppm glutaraldehyde and exposed to the biocide for approximately 18 hours. Samples were collected and the ATP assay was run to assess onsite kill efficacy of the biocide treatment (Figure 1, Table 3). Culture media was also inoculated for validation of the ATP results. The ATP results indicated 98% and 99% kill rate for the total bacterial population for the two frac tanks monitored. At the end of the culture media bottle incubation period, the results verified that a 99.90% reduction or greater was achieved for the SRB and APB populations in the 2 frac tanks.

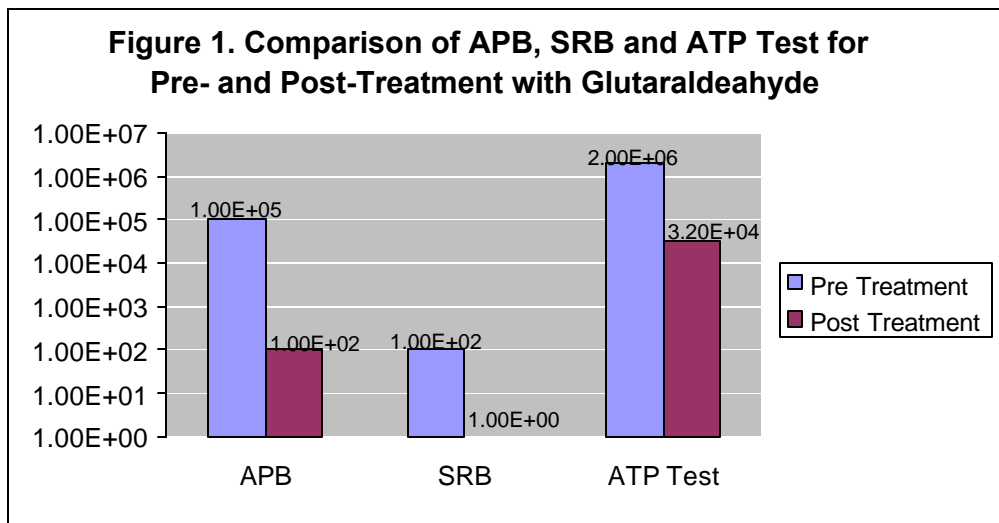


Table 3. Results From Glutaraldehyde Biocide Treatment

	APB	SRB	ATP Test
Tank #1			
Before Treatment	10 ⁵	10 ²	2.0 X 10 ⁶
After Treatment	10	BDL	1.3 X 10 ⁴
% Reduction	99.99	100	99.35
Tank #2			
Before Treatment	10 ⁵	10 ²	2.0 X 10 ⁶
After Treatment	10 ²	BDL	3.2 X 10 ⁴
% Reduction	99.90	100	98.40

The operator's engineer was pleased with the ATP results and requested that the ATP test be used to monitor the pre- and post-treatment waters from a UV light bacterial control process. The fresh water was processed through the UV light unit and samples were collected and evaluated by ATP assay and bacterial culture media (Figure 2, Table 4).

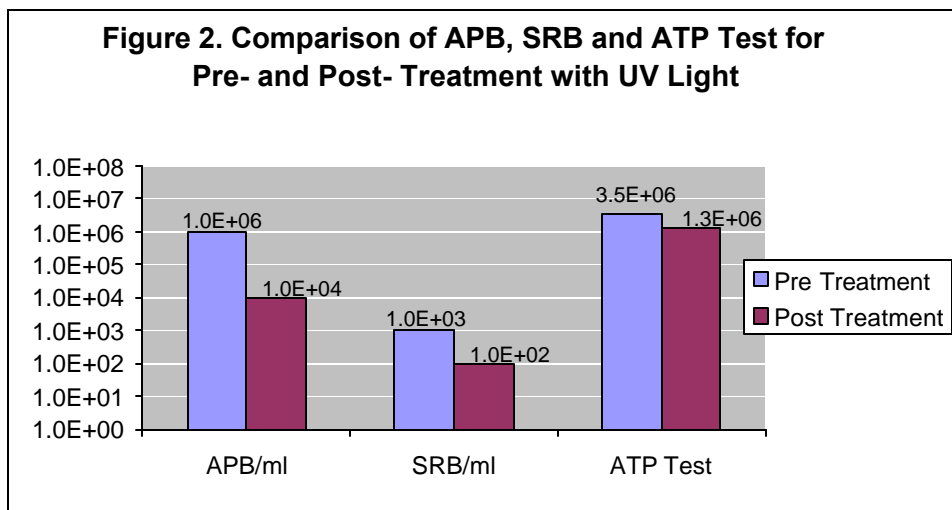


Table 4. Results from the Comparison of Pre- and Post- Treatment with UV Light

	Culture Media		ATP Test	Microscopy
	GHB/mL	SRB/mL	Bacteria/mL	Bacteria/mL
Before UV Light Treatment	10 ⁶	10 ³	3.5x10 ⁶	2.2x10 ⁶
After UV Light Treatment	10 ⁴	10 ²	1.3x10 ⁶	5.1x10 ⁵
% Reduction	99%	90%	63%	77%

The ATP results show a 63% reduction in the total bacterial population in the fresh water from the UV light treatment. The culture media results indicated a 90% reduction (1 log unit) in the SRB and a 99% reduction (2 log units) in the GHB populations. It is important to note that the ATP assay will be measuring all surviving bacteria, both those that are injured and healthy, culturable and non-culturable, while the culture media will be evaluating a portion of two sub-populations (SRB and GHB) of the total bacterial consortium present in the water sample. In addition, even with the 99% reduction observed in the APB populations and the 63% reduction in total bacterial populations measured by the ATP assay, a significant concentration of GHB and total bacteria still remain in the water following UV treatment, i.e. 10⁴ and 10⁶ bacteria/mL respectively. When this water is used to prepare a cross-linked fracturing gel, which is a readily biodegradable bacterial food source, and is then stored in a frac tank for hours to days before being pumped downhole during the frac job, the surviving bacterial populations will proliferate, resulting in degradation of the frac gel and subsequent issues downhole such as biogenic hydrogen sulfide production and microbially induced corrosion. As such, ATP can be used alongside other microbiological tools to quantify the efficacy of antimicrobial initiatives.

Case History #4: You CAN Teach an Old Dog New Tricks! Survey of Saltwater Disposal Facilities – Produced Fluids, Interface and Oil Samples

An East Texas operator was experiencing H₂S production and pad formation in their saltwater disposal system (SWD) tanks. The operator requested an evaluation of the SWDs to determine if bacteria were one of the components of the pad material and if they were contributing to the H₂S production. The ATP assay and bacterial culture media were used for the system survey. The tanks at SWD #1 had been cleaned 2 weeks before the survey. SWD #2 was operating under normal conditions.

Table 5. Survey Results for Two Saltwater Disposal Systems

Sample Location	Culture Media		ATP Assay	H ₂ S in Gas Phase
	GHB/mL	SRB/mL	Bacteria/ml	
SWD #1				
Tank Bottom	10 ⁵	10 ⁵	2.4x10 ⁶	5
Water from Pad	10 ⁵	10 ⁵	6.8x10 ⁶	5
Oil from Pad	---	---	1.5x10 ⁸	5
SWD #2				
Water from Saltwater Bath	10 ⁵	10 ⁵	5.5x10 ⁵	115
Oil from Saltwater Bath	---	---	1.3x10 ⁶	115
Good Oil Tank	---	---	9.9x10 ⁴	175
Sales Tank Oil	---	---	3.3x10 ⁶	28

Despite the cleaning in the first SWD facility, ATP analysis identified that high concentrations of bacteria had already accumulated in the oil and water phases of the pad. Hydrogen sulfide was starting to accumulate in the headspace of the tank. High concentrations of bacteria were also observed in the oil, water, and pad samples for SWD facility #2. This facility also contained high concentrations of H₂S in the tank headspace. The high concentrations of bacteria observed by the ATP assay were confirmed several weeks later when the results of the bacterial culture media were determined. The ATP analysis allowed for evaluation of the oil layer in the system. The culture media technology does not work well in oily samples.

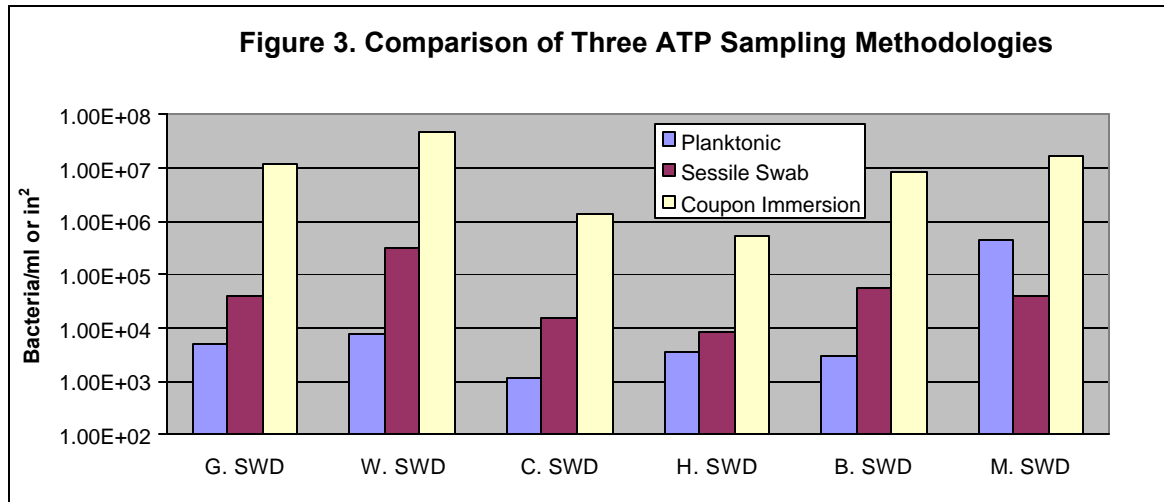
Case History #5: To Dip or Not to Dip... That is the Question! Sessile Sampling ATP Results

An East Texas operator was experiencing microbially influenced corrosion failures in their saltwater disposal facility flowlines. Sessile sampling from corrosion coupons installed throughout the system routinely provided little to no recovery of viable bacteria in salt-adjusted bacterial culture media (Table 6, culture media results). This low recovery of sessile bacteria did not correlate with the severity of the issues observed in the system. Baker Hughes personnel decided to try the ATP assay for enumeration of sessile bacteria from the coupons. Two different methodologies were evaluated to assess which provided the best recovery of sessile bacteria. The first method involved

swabbing the corrosion coupon, and placing the deposition and cotton swab tip into a filter-sterilized dilution blank prepared from system water. ATP was analyzed and bacterial culture media was inoculated from the sessile swab sample. For the second method, the coupon was directly immersed into the lysing solution for the ATP assay (UltraLyse 7) and allowed to sit for 5 minutes with agitation. A portion of this solution was analyzed. The results for all analyses are summarized in Table 6 and Figure 3.

Table 6. Microbiological Survey Results for SWD Coupon Locations

Sample Location	Culture Media (from Sessile Swab)		ATP Analysis		
	GHB/ml	SRB/ml	Planktonic Water Sample	Sessile Swab	Coupon Immersion
G SWD	10 ⁴	10 ³	5.0x10 ³	3.0x10 ⁴	1.2x10 ⁷
W SWD	10 ⁴	10 ²	7.7x10 ³	2.3x10 ⁵	4.7x10 ⁷
C SWD	< 10	< 10	1.1x10 ³	1.1x10 ⁴	1.4x10 ⁶
H SWD	10 ²	< 10	3.4x10 ³	6.4x10 ³	5.5x10 ⁵
B SWD	< 10	<10	3.0x10 ³	4.2x10 ⁴	8.3x10 ⁶
M SWD	< 10	< 10	4.6x10 ⁵	3.0x10 ⁴	1.7x10 ⁷



As predicted based on prior sampling, the culture media provided consistently low recovery of the sessile bacteria populations with the highest bacterial concentration, 10⁴ GHB/ml, present on the G and W SWD coupons. The produced fluids from these SWDs have a high TDS and an average chloride level of 100,000 ppm or greater. Culturing of bacteria from high-TDS waters has been known to be more challenging in many fields. Coupon immersion consistently provided 2 to 3 logs higher bacterial recovery than the sessile swab sampling methodology. The ATP assay verified that sessile bacteria are present on the coupons at concentrations which can contribute to microbially induced corrosion, concentrations many times higher compared to the planktonic population contained in the surrounding bulk fluid.

DISCUSSION AND CONCLUSIONS

Oil and gas producers are under constant pressure to optimize operating costs, minimize environmental impacts and protect capital infrastructure. As such, stakeholders throughout the industry continually strive to investigate new technologies that offer potential to meet one or all of these objectives.

The threat of microbial attack is becoming more recognized as a primary consideration in oil and gas production operations. The tools available for biological detection in oil and gas applications have traditionally been limited to microscopic examination and culture-based visual counting techniques. As has been described, each of these methods has pros and cons. Microscopic examination has traditionally been the best means by which to quantify total microorganisms in a wide variety of sample types. It is however not easily portable and hence usually requires shipment of samples from the field to a laboratory, is restricted to trained users, is unable to distinguish living from dead biomass, and requires a substantial capital investment. Serial dilution culture tests are, by contrast, substantially easier to use and can be used in the field. However, they measure only select portions (GHB and SRB) of the total population, are known to recover only a very small percentage of the targeted populations within each media type, and require days or weeks to obtain feedback.

Second-generation ATP measurements offer an excellent complement to these existing tools. It is a portable methodology that can be operated by any user in the field, providing immediate feedback on total living microorganisms. From a standpoint of features, the second-generation ATP test bridges the gap between microscopic examination and serial dilution culture tests. The ATP test also provides bacterial enumeration capability for a diverse range of samples including fluids (including oil- or water-based drilling muds, oils, emulsions, interfacial pads, and even certain treatment chemicals), solids (deposits, soils or suspended particles), and surfaces (walls of equipment or coupons).

The greatest benefit to the use of second-generation ATP measurements is the real-time results. The ATP assay provides service companies the ability to pinpoint the problem area in a system, apply a treatment, and support the efficacy of a treatment in less than one week compared with what traditionally requires weeks or months. This rapid feedback will keep a chemical provider on the cutting edge of service. It also provides feedback that allows expedient adjustment to a system (for example, biocide dosage) to prevent a minor problem from becoming a major issue with the possibility of MIC and the spread of bacterial introduction downstream of the original problem.

The case studies demonstrate the accuracy of enumeration of total bacteria as compared to the traditional methods of culture media and microscopy. The ATP assay measures total living bacteria in a system while epifluorescence microscopy measures total living and dead cells. The case histories consistently showed the microscopic estimate to be within one log of the concentration measured by the ATP assay. The ATP assay results were consistently higher than the populations recovered with bacterial culture media. Culture media measures only the culturable portion of subpopulations of the total bacterial count, and always present the potential for false negatives due to unculturable organisms or the absence of a key nutrient.

Based on the case histories presented above, second-generation ATP measurement achieves the objectives of cost minimization, environmental impact mitigation, and product quality protection in bacteria management programs. The data presented demonstrated that second-generation ATP measurement compares very well with the traditional tests. This does not mean, however, that ATP measurements should outright replace the traditional tests. Rather, it should be incorporated into the microbial 'tool kit' to augment the ability of a field operator or engineer to diagnose or survey oil and gas production processes.

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