IDENTIFICATION OF SULFATE REDUCING BACTERIA (SRB) AND THEIR ROLE IN MICROBIOLOGICALLY INDUCED CORROSION ON THE M.F.E. UNIT OPERATED BY FASKEN OIL AND RANCH, LTD. OF MIDLAND, TEXAS

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The M.F.E. Unit is located in the Permian Basin production area of the United States to the northwest of Midland, Texas approximately 15 miles and is just to the east of U.S. highway 1788. Production from this unit is sour, hydrogen sulfide containing, from the Greyburg formation. Hydrogen sulfide is soluble in the produced water from this unit and typically averages several hundred parts per million in solution in the water analyzed at the individual production wellhead in accordance with American Petroleum Institute RP-45. Wells are produced via rod/pump with generally an oil to water ratio of less than 1:4 being experienced. Under these conditions, it is generally accepted that a "water wet'' environment will exist on the exposed metal surfaces down hole and due to the presence of moderate to high levels of hydrogen sulfide "acid gas"; it would be highly probable that an active corrosion process would exist on the exposed metal surfaces down hole. Operator and service company experience confirms this premise.

Based upon the presence of an active hydrogen sulfide corrosion process inside the wells of this unit, the chemical service company involved with this unit is engaged in an active corrosion mitigation program involving the periodic batch truck treatment of these wells with a proven film persistent corrosion inhibitor and has experienced success in the mitigation of the down hole corrosion related failure problems on the unit. Corrosion monitoring is accomplished by the chemical service company via weight loss coupon analysis as well as Sulfate Reducing Bacterial (SRB) testing via the American Petroleum Institute (API), field test procedure for the analysis of SRB, RP-38 Section A-11. Corrosion monitoring via weight loss coupon has indicated that the corrosion mitigation program established by the service company is effective and the tests for SRB also indicated that proper control of these potential corrosion causing agents was well established.

After several years of successful corrosion mitigation on the M.F.E. Unit there was noted by the operator and service company to be occurring a slight increase in down hole corrosion related failures especially in the lower portions of the rod string and down hole pump areas. These failures appeared to be associated with a particular section of the unit and was suspected to be associated with injection water breakthrough from a particular injection well. The failures involved were analyzed by the service company and found to be caused by predominantly hydrogen sulfide under deposit type corrosion that had the appearance of SRB related pitting.

This pitting is typically characterized by a "stair-stepped'' and brightly colored appearance with iron sulfide corrosion by product coating each successive step in the process. Experience with field and laboratory growth of SRBs on a metal surface by the authors has yielded a typical SRB corrosion process by which the SRBs initially attach to the metal surface becoming "sessile", or attached to the surface, in nature. The attachment mechanism for each specific strain of SKB has been found by experimental study of the authors to be specific for that specific strain. Once the SRB strain has become "sessile", it begins to multiply and grow producing hydrogen sulfide as part of its metabolic process which in turn corrodes the metal surface underneath the SRB deposit. Iron sulfide corrosion by product is produced which is a solid that precipitates rapidly onto the metal surface and once this layer of corrosion by product builds up and as the SRB colony continues to grow, the focus of the hydrogen sulfide attack shifts to a lesser iron sulfide coated area within the affected area, thereby deepening the pit and partially passivating the initial corroded area.

By this method, the SRB pit "stair-steps'' through a series of shallower peripheral areas which are partially passivated by the iron sulfide while the central and ever deeper area of the pit continues to corrode at a higher under deposit corrosion rate until a failure occurs. The iron sulfide coated area generally remains "cathodic" or passive to the non coated or less

iron sulfide coated metal surfaces inside the pit thereby increasing the corrosion rate and helping to remove iron sulfide corrosion by product from the "anodic" or more active corrosion area speeding up the corrosion reaction and deepening the pit.

In every case studied to date by the authors, there appears to be an entire microbial ecosystem that supports the growth of one or more strains of SRB involved. Numerous types of microbes appear to act symbiotically to produce the necessary conditions for "sessile" or "attached" SRB growth. It has also been discovered that certain select strains of SRB lend themselves to the establishment of sessile colonies and ecosystems while others tend to prefer to grow in ''planktonic'' or "free swimming" environments. The research conducted to date appears to indicate distinct strains of very corrosively harmful SRB that tend to thrive and grow in an optimum fashion under sessile conditions while other SRB strains thrive and grow best in a planktonic or free swimming environment. Although all strains of SRB by definition produce hydrogen sulfide by the reduction of sulfate, these sessile forms of SRB create their own specific type of severe under deposit type corrosion on iron containing metal surfaces as described in this paper.

Considerable concern was expressed by the operator of the M.F.E. Unit as the analyses results provided by the chemical service company indicated that SRB were under control and in very low numbers down hole in the producing wells yet the chemical service company's analyses of failures on the effected producing wells indicated that SRB corrosion was the primary cause of the failures. Treatment for SRB type Microbiologically Induced Corrosion or " MIC" utilizing chemical service company proven techniques were recommended and indicated by the failure analyses but could not be properly monitored as the tests conducted for the enumeration of SRB via the standard API procedure did not originally indicate that a change in SRB numbers occurred after treatment.

At this point, a third party consulting firm operated by one of the authors was contacted to provide guidance and additional expertise involving SRB related MIC. The first step followed by the consultant was to observe the actual failures from the effected producing wells to establish that the corrosion pitting involved was indicative of SRB corrosion attack. Once this was established and agreed upon by all parties, the next step was to investigate field production and chemical vendor corrosion mitigation procedures to ensure that established industry guidelines were being properly observed on the M.F.E. Unit. After establishing that these programs were adequate, the next step followed was to investigate the monitoring methods utilized by the chemical service company as well as the technique followed by their field and laboratory personnel to ensure that standard practices were being followed. This was confirmed and agreed upon by all parties.

In the process of this review of procedures with the chemical service company and the operator, several problems were uncovered in the standard API procedure for field estimation of SRB utilized by the chemical service company on the M.F.E. Unit. Since the technique utilized by the chemical service company for the enumeration of SRB on the M.F.E. unit was the standard oil field practice as recommended in the **API** procedure RP-38 Section A-II. a brief review of this procedure is provided. The procedure involved utilizes a standard **API** growth medium or broth containing among other essential ingredients, soluble iron. In addition to this soluble iron, an iron nail is added to the growth medium. The reasoning behind the addition of the soluble iron to the growth medium is that SRB, during their metabolic growth process, produce hydrogen sulfide which will react with the soluble iron in the broth to produce a black precipitate, iron sulfide, which would act as an indicator for a positive presence of SRB on the test. The iron nail is added as a back up indicator for SRB presence as iron sulfide build up on the nail and subsequent corrosion of the nail's surface would also be an indication of a positive SRB presence in the inoculated broth.

The procedure for performing the API **RP-38** Section A-II test method is a serial dilution process and involves obtaining a water sample from the source whose SRB content is unknown and utilizing anaerobic sterile techniques, transferring a one (1) milliliter sample of the source water with a sterile hypodermic syringe into a sterile serial dilution bottle containing nine (9) milliliters of the specific API SRB growth broth accomplishing a 1:9 dilution of the sample. This bottle is thoroughly mixed and a one (1) milliliter sample from this bottle is transferred to a new serial dilution bottle containing nine (9) milliliters of the specific growth broth. This produces a dilution factor of the original sample of 1:100. The procedure is repeated transferring the diluted sample to fresh serial dilution bottles until the desired dilution factor is obtained. In the case of the chemical service company involved, the desired dilution factor at the end of the procedure was 1:100,000. By experience, the chemical service company had established that'a positive test by this procedure in a serial dilution bottle greater than 1:100,000 was unneeded.

The first problem encountered by the chemical service company with this procedure involves the sour, hydrogen sulfide containing, nature of the produced water from the M.F.E. Unit. With a soluble hydrogen sulfide content of several hundred parts per million. the first two and in some cases three bottles of the serial dilution test turned black, indicating a

positive test for SRB, due to a reaction between the hydrogen sulfide, contained in the produced water and still present in ample quantity in the subsequent dilutions, and the soluble iron in the API SRB broth either instantaneously or within a short time period after the inoculation. Since the produced water has hydrogen sulfide already present and at such a high soluble concentration, the iron nail in the first two and in some cases three serial dilution bottles also corroded fairly quickly also indicating a positive test for SRB by procedure. These two indications of SRB growth were considered a "false positive" test result and dismissed as they occurred in too short a period of time to be considered the result of SRB growth. The chemical service company reported these tests as positive only if they could establish an indicator reaction due to growth in the third or fourth serial dilution bottle and only if this positive result occurred over a period of at least two to three days after the initial inoculation of the test bottles. It was impossible for the chemical service company to detect SRB content in the producing wells if their SRB content was sufficient only to actively cause growth in the first two or sometimes the first three bottles due to this "false positive" reaction encountered.

In the case of SRB and MIC most industry personnel experienced with this type of corrosion and the API RP-38 test procedure agree that any presence, even in the first bottle of the serial dilution test, of these type of bacteria is an indication of possible MIC at some point in the effected system.

To solve this problem, it was decided that the removal of the soluble hydrogen sulfide from the source producing well samples would be desirable before the inoculation of the API serial dilution test broth.
This would insure that a "false positive" test result on the first two or three serial dilution bottles would not occur due to the reaction of soluble hydrogen sulfide from the sample with the soluble iron or the iron nail in the API serial dilution broth or bottle. This procedure had to be conducted anaerobically as the introduction of air and oxygen into the sample was known to inhibit the growth of the anaerobic SRB strains that were sought by the test. The hydrogen sulfide removing chemical agent or "scavenger" should also not be corrosive to the metal nail and should be selected so as to not directly effect the growth of any of the known strains of SRB detected by this test method. To determine this, an alternate test procedure other than the API RP-38 serial dilution test method would need to be utilized to ensure that accurate results were obtained. In the case of this study, two alternate test methods were utilized. The first test method utilized was an immunoassay technique marketed originally by Conoco, Inc. under the name of "RAPIDCHEK". The second test method utilized was a selective media growth system developed by the author and labeled as RCCI plate media and RCCI serial dilution broth. Pure cultures of SRB strains isolated by the author were utilized and laboratory simulated M.F.E. produced water was prepared without the addition of hydrogen sulfide and actual field samples of M.F.E. produced water containing hydrogen sulfide were also utilized in this study.

As a result of this study a proprietary technique for the removal of hydrogen sulfide from field produced water samples under anaerobic conditions that did not effect the SRB population or the growth of that population in the API RP-38 SRB standard broth was developed and employed by the chemical service company. **Also,** as a result of this study several other major problems with the API RP-38 SRB standard broth and test method were uncovered.

The first problem uncovered was the time it took to run the API RP-38 SRB test, 28 days, in comparison with the "RAPIDCHEK" system, approximately 30 minutes, and the RCCI plate media and serial dilution broth methods, 24-48 hours. The unusually long period required to incubate the API RP- 38 SRB test to obtain results meant an unusually and economically costly lag period between the initiation of the test and the application of remedial chemical treating procedures. Failures due to SRB MIC were occurring on the M.F.E. Unit producing wells effected at a rate faster than the test itself. Even if the "false positives" of the API RP-38 SRB test were eliminated, the test itself was too slow to drive preventative chemical treating for any uncovered SRB problems. One could only be reactive to the problem and not proactive in preventing the problem. The "RAPIDCHEK' system would seem to be the most proactive solution as its results could be obtained within 30 minutes of sample acquisition. Through the use of comparative testing it was shown that the "RAPIDCHEK' system was grossly inaccurate at the detection of the strains of SRB found in the M.F.E. Unit compared to the API RP-38 SRB test method as well as the RCCI plate media and serial dilution methods. The "RAPIDCHEK' system and the RCCI plate media and serial dilution methods do not require hydrogen sulfide scavenging since nether test method involves soluble iron as an indicator of growth.

A third problem with the API RP-38 SRB standard broth test found during the study of the produced water in the M.F.E. Unit was indicated in the number of SRB strains that it could detect as well as the proper enumeration of known SRB content as compared to the RCCI plate media method. The RCCI plate media and serial dilution methods caused growth of 15 different strains of SRB in the M.F.E. Unit produced waters while the API RP-38 SRB growth broth caused growth of none of the different SRB strains. Results from the RCCI plate media and serial dilution methods produced significantly higher and more consistent results than the API RP-38 SRB standard broth test.

A fourth problem found with the API RP-38 SRB standard broth test was indicated in the difficulty and time expenditure required to properly match the broth total dissolved solids with that of the field produced water. Failure to properly match the field water total dissolved solids content with standard previously prepared sterile API RP 38 SRB broth test bottles such as those purchased by most chemical service companies due to the readily commercially available status of this product, will result in the instantaneous destruction of many viable SRB cells due to the "osmosis effect". The cell wall of the SRB's either implode or explode due to extreme differences in the total dissolved solids content of the water inside their cells compared to the total dissolved solids of the broth or media they are thrust into. The RCCI plate media and serial dilution methods utilize sterile produced water from the actual produced water sample that is previously bulk prepared and thereby avoids this problem completely. SRB should always be cultivated in broth and/or media that closely match the produced water environment or matrix that they naturally are growing within.

The final problem isolated during the study of SRB Microbiologically Induced Corrosion (MIC) on the M.F.E. Unit also involves the test method utilized with the API RP-38 SRB standard broth test. The standard API RP-38 SRB serial dilution broth test calls for sampling the produced water by drawing a one (1) milliliter sample from a wellhead produced water sample via a sterile hypodermic syringe. This type of sampling is excellent for the enumeration of planktonic or "free swimming" bacterial types. Field studies involving cultivation of SRB from the interior pipe surfaces of the effected production wellheads sampled by swabbing the interior surfaces with sterile cotton tips as well as cultivation of solids obtained by swabbing weight loss coupon surfaces as they were extracted from the MIC effected production wellheads indicate that most of the SRB strains isolated by these procedures are sessile or "attached" to the interior pipe surfaces. Five different strains of sessile SRB were isolated from these interior pipe and weight loss coupon swabbed SRB analyses utilizing the RCCI plate media and serial dilution methods with only one of these same sessile strains being indicated as present in a sessile adapted version created by our study group utilizing the API RP-38 SRB serial dilution test. In this adapted method, swab samples obtained from the interior wellhead surfaces and weight loss coupons from the MIC effected producing wells are placed in a total dissolved solids solution matching the matrix of the individual produced water involved. The sample is agitated violently for one (1) minute and a one (1) milliliter sample of the fluid is hypodermically pulled and inoculated into the first API RP- 38 SRB serial dilution test bottle. The test is then followed as in a normal serial dilution procedure. The resultant growth of this test after the 28 day recommended incubation were then taken and isolated by SRB strain utilizing RCCI proprietary procedures with the results reported only in the number of strains isolated.

Utilizing the chemical vendor modified version of the API RP-38 SRB serial dilution test as well as the RCCI plate media and serial dilution tests along with the modified sampling procedures adapted for both planktonic and sessile SRB strain types on the M.F.E. Unit has enabled the establishment of a viable and cost effective chemical treatment program for the control of SRB initiated MIC on the effected wells. Failures due to SRB MIC have been dramatically reduced and the speed of analyses of these procedures have been dramatically improved making them proactive instead of reactive.

In conclusion, there were several problems found with the American Petroleum Institute field test procedure for SRB, RP-38 A-11. The procedure as stated is not applicable in sour, hydrogen sulfide containing systems where the soluble hydrogen sulfide causes "false positive" results in the test. The procedure as stated is slow requiring 28 days of incubation which places the chemical treating vendor and the operator in a reactive situation regarding the control of SRB populations and their resultant MIC. The procedure as stated does a poor job at the cultivation of many strains of planktonic SRB and a very poor job at the cultivation of sessile strains of SRB. In our opinion, this procedure should be placed under scientific review and should not be a Recommended Practice of the American Petroleum Institute.

REFERENCES

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RP-38 SRB Serial Dilution Test Results on the M.F.E. Unit

SAMPLE POINT	INCUBATION PERIOD	TEST RESULTS as Colonies per milliliter
1) Capitan Reef	28 days	100*
2) MFEU #105	28 days	1000"
3) MFEU #106	28 days	1000*
4) MFEU #112	28 days	1000"
5) MFEU #203	28 days	1000*
6) MFEU #206	28 days	1000*
7) MFEU #209	28 days	1000*

* False positive test results indicators reacted in short period of time

RCCI Serial Dilution Bottle Test

SAMPLE POINT	INCUBATION PERIOD	TEST RESULTS in Colonies per milliliter
1) Capitan Reef	48 hours	1,000,000
2) MFEU #105	48 hours	10,000
3) MFEU #106	48 hours	1,000,000
4) MFEU #112	48 hours	10,000,000
5) MFEU #203	48 hours	1,000,000
6) MFEU #206	48 hours	1,000,000
7) MFEU #209	48 hours	1,000,000

RCCI Plate Media Test for SRB

SAMPLE POINT	INCUBATION PERIOD	TEST RESULTS in Colonies per milliliter
1) Capitan Reef	48 hours	1,000,000
2) MFEU #105	48 hours	100,000
3) MFEU #106	48 hours	1,000,000
4) MFEU #112	48 hours	10,000,000
5) MFEU #203	48 hours	1,000,000
6) MFEU #206	48 hours	1,000,000
7) MFEU #209	48 hours	1,000,000

Proprietary RP-38 Adjusted Test removing hydrogen sulfide

SAMPLE POINT	INCUBATION PERIOD	TEST RESULTS in Colonies per milliliter
1) Capitan Reef	28 days	1,000
2) MFEU #105	28 days	100
3) MFEU # 106	28 days	1,000
4) MFEU #112	28 days	10,000
5) MFEU #203	28 days	1,000
6) MFEU #206	28 days	1,000
7) MFEU #209	28 days	1,000

Sessile SRB COMPARISON Tests all Procedures Listed

Sample point Isolated	SRB Method	Incubation Period	Test Results in number of Strains
1) MFEU	RP-38 SRB	28 days	0
2) MFEU	RP-38 SRB Adjusted(-)H2S	28 days	1
3) MFEU	" RAPIDCHEK'	30 minutes	0
4) MFEU	RCCI serial dilution	48 hours	15
5)) MFEU	RCCI plate media	48 hours	15