Techniques for assessing microbial activities associated with microbially influenced corrosion

by

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ABSTRACT

Radiolabeled tracer techniques have been developed which measure the activities of microorganisms involved in microbially influenced corrosion (MIC). General microbial metabolism was demonstrated by [1-^{14}C]acetate incorporation into lipids and by [^{14}C]CO_2 production from [U-^{14}C]glucose. Specific activities related to corrosion included [^{35}S]SO_4 reduction to [^{35}S]HS-, [^{14}C]acetate from [^{14}C]glucose and hydrogen mediated [^{14}C]acetate from [^{14}C]CO_2. Acetogenesis from hydrogen and carbon dioxide impurities, accounted for 0.2-0.7 mmol/L of acetate production per week from some natural gas pipeline samples. These techniques were capable of assessing general microbial activities as well as specific activities which could adversely affect the structural integrity of metals.

INTRODUCTION

Sulfate reducing bacteria are considered to be major contributors to microbially influenced corrosion (MIC) in anaerobic environments[1,2,3,4]. Iron may serve as a source of electrons for sulfate reduction[5] and iron was also shown to stimulate methanogenesis[6], suggesting that methanogens could facilitate corrosion processes. Volatile fatty acid accumulation from fermentative microorganisms has also been implicated in corrosion[2,7,8]. Several mechanisms may exist for anaerobic MIC of metals.

Microbial contributions to corrosion have typically been examined by microscopic observations of bacteria, pitting patterns, production of sulfide, or recovery and growth of specific microorganisms. Presence of microorganisms provides little insight into their significance or mechanisms by which they contribute to MIC. Microorganisms which are metabolically inactive do not present major challenges to the structural integrity of metals. Functioning microorganisms may be capable of stimulating or attenuating corrosion processes. For example, methanogenic consumption of acetic acid may decrease corrosion, whereas energy derived from the oxidation of iron may adversely affect the structural integrity of metals. Measuring the rates of sulfide or acid accumulation could model microbial processes contributing to metal loss.

We have developed procedures for measuring carbon and electron flow through anaerobic microbial communities suspected of facilitating corrosion. These studies ascertained corrosive products produced in aqueous samples, sludge, or scrapings of natural gas collection and distribution pipelines. Many sample locations contained active microbial communities which produced corrosive end products such as sulfide or acetic acid.

EXPERIMENTAL METHODS AND MATERIALS

Experimental sites.

Samples from natural gas production platforms and transmission systems were examined. At each production platform the natural gas was separated from the liquids by a high pressure separator (HPS), pressurized to 60-70 atmospheres and metered. The aqueous and hydrocarbon liquids were often reinjected into the transmission system. At one on-shore processing facility approximately 2 x 105 L of water; predominantly process waters and brines, arrived each day. Upon arrival at an on-shore processing facility liquids and sludge were separated from the gas, the gas was dehydrated in a HPS and a low pressure separator (LPS), and prepared for distribution. After on-shore processing the natural gas remained dehydrated. One on-shore distribution pipeline was also examined. Fluids, wall scrapings of nodules, and sludge were collected. Subsurface samples were obtained as a part of the U. S. Department of Energy Subsurface Science Program as described previously[9,10].

Gases, chemicals and isotopes.

Nitrogen and N_2-CO_2(90:10%) were greater than 99.9% pure. In the laboratory all gases were passed through copper-filled Vycor furnaces (Sargent-Welch Scientific Co., Skokie, IL) to remove traces of oxygen. All chemicals used were of reagent grade and were obtained from Mallinckrodt (Paris, KY) or Sigma Chemical Co. (St. Louis, MO). Resi-analyzed glass-distilled solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). [1-^{14}C]Acetate (56 mCi/mmol) and [^{35}S]SO_4 (481 mCi/mmol) were purchased from New England Nuclear Corp. (Boston, MA). [2-^{14}C]Acetate 56 mCi/mmol), [^{14}C]bicarbonate (54 mCi/mmol), and [U-^{14}C]glucose (2.8

mCi/mmol) were obtained from Amersham Corp. (Arlington Heights, IL). Experiments utilized crimp-top tubes or serum vials (Bellco, Vineland, NJ or Wheaton, Millville, NJ) sealed with butyl rubber septa.

Field studies.

Sediment aliquots were inoculated for anaerobic microbial activity experiments within 30 min of arrival on-shore or at land surface. All anaerobic studies were performed using strictly anaerobic techniques with a N_2-CO_2 (90:10%) atmosphere. Hydrogen was added to experimental tubes at 12%, approximating the partial pressure available in the gas transmission system. Reductants included 0.05% cysteine-HCL or sodium sulfide. Isotope solutions (1.0-50 uCi) were sterilized prior to use and transferred with gastight syringes (Hamilton Co., Reno, Nev.). Time course experiments were performed in duplicate using sterile crimp-top anaerobic tubes. All incubations were at ambient temperature which was similar to the <u>in situ</u> temperature of 23-30°C. Acetate incorporation experiments contained approximately 2 g sample, 4.0 uCi of [1-^{14}C]acetate and 1.0 ml of anaerobic sterile distilled water. Hydrogen mediated acetogenesis experiments contained 5.0 uCi [^{14}C]CO_2, 2 g sample and 1.0 mL of sterile anaerobic distilled water. At t=0 and appropriate time points, duplicate tubes were inhibited with 3.0 ml methanol and were frozen. Acetate and glucose mineralization experiments contained 1.0 uCi of the radiotracer, 1 g of sample and 1.0 mL of anaerobic sterile distilled water. Sulfate reduction experiments contained 1.0 uCi of [^{35}S]SO_4, 1 g of sample and 1.0 ml of anaerobic sterile water. At appropriate time points tubes were inhibited by the addition of 1.0 mL of 2.0 M sodium hydroxide. Time course experiments were performed in duplicate and consisted of at least six time points between 20 min and 7 days.

Analytical procedures.

Mineralization experiments were acidified with 2.0 mL of 3.0 M hydrochloric acid and the headspace of experimental tubes was analyzed by gas chromatography-gas proportional counting as described by Nelson and Zeikus[11]. Aliquots of the aqueous phase of experimental tubes were analyzed by HPLC with subsequent scintillation counting to quantify radiolabeled acetate accumulation. A Rezex organic acid column,(Phenomenex, Rancho Palos Verde, CA) 300 x 7.8 mm, with 0.01 N sulfuric acid as the mobile phase was used to separate acetate, formate, and lactate from glucose, bicarbonate and metabolic products.

Acetate incorporation into lipids was determined from time course experiments which were extracted and analyzed as described previously[10,12]. Sulfate reduction assays were inhibited in the field with sodium hydroxide. Upon return to the laboratory the tubes were acidified with hydrochloric acid. Sulfide was sparged from the tubes with nitrogen gas and the sulfide trapped in acidified zinc acetate. Aliquots of the zinc acetate solution were counted on the scintillation counter.

RESULTS

Anaerobic samples from high pressure separators (HPS) located on natural gas production platforms, off-shore pumping stations, and on-shore transmission systems were examined for microbial activities during time course experiments. As shown in Table 1, radiolabeled acetate incorporated into lipids demonstrated that the resident microbial communities were metabolically active. Several platforms reinjected process waters containing microorganisms, nutrients and electron acceptors such as sulfate,

with the gas for transportation to shore. Addition of process waters to the gas-line system likely provided microbial inocula and nutrients capable of sustaining microbial communities. Glucose was not substrate expected to be important in gas transmission systems but was used in these experiments as a substrate used by many types of microorganisms. These anaerobic communities proved capable of mineralizing glucose. Samples from HPSC-1 were capable of oxidizing the glucose pool within days while samples from HPSC-6 would have required many months to oxidize the glucose pool.

Production of acetic acid could directly impact the structural integrity of pipeline steels and as shown in Table 1, samples from production platforms were capable of fermenting carbohydrates to acetic acid. Natural gas contains traces of hydrogen and when pressurized to 60 atm. the hydrogen partial pressure could support microbial metabolism. Production of acetic acid from hydrogen plus carbon dioxide represented a mechanism by which readily available natural gas contaminants could be biologically converted to a corrosive agent. Samples from HPSC-1 could convert the carbon dioxide pool to acetic acid within days, while samples from other platforms exhibited little ability for hydrogen mediated acetogenesis. Sulfate reduction has commonly been associated with MIC. As shown in Table 1, radioactive sulfide was detected from radiolabeled sulfate.

Activities observed from pumping station and on-shore samples were lower than activities observed from production platforms (Table 2.). Decreased activities were likely related to depletion of electron acceptors and nutrients as gas was transported toward the on-shore facility. Radiolabeled acetate was incorporated into microbial lipids at a slow rate and carbon dioxide was detected from glucose, demonstrating that oxidation of organic matter occurred in the transmission system. After seven day incubations no

radiolabeled methane was detected from either glucose or acetate from any site, suggesting that methane was not a significant microbial end product formed in off-shore natural gas collection systems. Sulfate entering the transmission system at production platforms with process sea water or from formation waters was expected to be rapidly utilized and not available at the pump stations or at the on-shore facility. As shown in Table 2, sulfate reduction was similar to the other microbial activities in that they were lower than at the production platforms.

The recovery of radiolabeled acetate produced from carbon dioxide is shown in Figure 1. Control experiments exhibited background counts of 10-20 dpm per elution fraction. One day time points revealed radiolabeled volatile fatty acids including a peak at elution fraction 40 which was tentatively identified as formate (data not shown). Results shown in Figure 1 are from a seven day time point. The peak at elution fraction 50 was demonstrated to be acetate by both total and radiolabeled acetate standards. After seven days the peak at fraction 40 was below detectable limits whereas the acetate peak had increased five fold. Additionally, a peak appeared at elution fraction 69 which was tentatively identified as butyrate.

The carbon dioxide pool size within gas transmission lines averaged 6-8 mmol/L. Assuming two moles of carbon dioxide were consumed per mole of acetate produced, samples could have fixed 10% of the carbon dioxide pool into acetate within one week accounting for the production of 0.5 mmol/L of acetate per week. Other aqueous, scraping, and sludge samples accounted for 0.2 - 0.7 mmol/L of acetate produced per week from carbon dioxide (data not shown). In addition, 5% of the glucose pool was converted to acetate each day suggesting that fermentative oxidation of organic acids and alcohols to acetate could substantially contribute to acetate production and

accumulation.

Table 3 shows microbial activities measured from external samples of on-shore natural gas transportation systems. Samples 1-4 were scrapings of nodules which were incubated with the various isotopes over time course experiments. A soil sample is included as a control showing microbial activities which were in close proximity to the gas pipeline. Samples 3 and 4 showed significant incorporation of acetate into microbial lipids and elevated levels of glucose mineralization. Sulfate reducing activities were measurable from three of the four scrapings and from the local soil suggesting that sulfate reducing bacteria were present and capable of contributing to MIC.

Table 4 compares microbial activities from natural gas transmission samples with those from lake sediments and subsurface environments. Sediments from eutrophic lakes[10,13] exhibited high levels of anaerobic microbial activities including sulfate reduction rates of mmoles/l sediments each year. Additionally, microbial activities within sediments appear reproducible in that aliquots from different locations or obtained at different times generally exhibited microbial activities which varied less than a factor of three[13]. Subsurface sediments have generally fit into two major categories, either subsurface clays and unsaturated zones[10], or subsurface aquifers. Subsurface aquifer sediments typically exhibited activities within two orders of magnitude of those from lake sediments. In contrast, the low water availability confining clay zones exhibit activities barely above detection limits. Within 3 m of spatial distance microbial activities in subsurface sediments may vary five orders of magnitude. The variability of microbial activities observed from natural gas transmission systems was similar to subsurface sediments. Many samples exhibited

DISCUSSION

Pope et. al,[2,8] detected the presence of microorganisms in aqueous and nodule samples from off-shore natural gas transmission pipelines by observing turbidity in media after serial dilutions. Two trophic groups of microorganisms were identified; sulfate reducing and fermentative acid producing bacteria. Detection of bacteria does not demonstrate their role in the corrosion of steel. Inactive cells, cysts, or spores, probably contribute little towards corrosion. Microbial activities likely control rates and extent of MIC. This study demonstrated utility of field techniques which were capable of assessing general activities of the resident communities and specific microbial activities which could impact pipeline integrity.

Lake sediments depleted of sulfate typically exhibit methane as the predominant reduced end product of anaerobic decomposition. In contrast to lake sediments, methanogenesis did not appear as a major end product of anaerobic metabolism in these gas pipelines. The insignificance of methanogenesis likely resulted from less favorable energetics of producing methane against a 60 atmosphere gradient. In sulfate limited portions of the system hydrogen mediated acetogenesis may have been a favorable alternative to methanogenesis and sulfate reduction.

Glucose and acetate turnover in freshwater sediments has been determined to be in the order of seconds to minutes[13]. In these pipeline samples, degradation of acetate and glucose took days. Glucose is not a likely substrate in off-shore pipeline environments but fermentation of complex alcohols, acids, and unsaturates are possible. Mass spectral analysis of pipeline contents (Gas Research Institute, unpublished data) revealed that a large range of compounds including fatty acids, alcohols and

unsaturated hydrocarbons were available for oxidation by sulfate reducers or fermentative microorganisms.

Hydrogen metabolism in anaerobic sediments occurs within seconds and the pool size is generally maintained below 10 umol/L [13] with the terminal product typically being methane or sulfide. Hydrogen has been detected in natural gas at concentrations above 0.1% and at 60 atmospheres of pressure hydrogen could be readily abundant. Acetate formation from hydrogen and carbon dioxide is typical in environments with high hydrogen partial pressures or low hydrogen consumption by methanogens and sulfate reducers[13]. Presence of hydrogen utilizing acetogens and degradable organic matter in these anaerobic natural gas pipelines result in the production and accumulation of acetate as a major end product of microbial metabolism.

Acetate accumulation, as suggested by these studies, can contribute to acidification which could increase the corrosion of steel. Reduction of sulfate to sulfide and production of acetate from hydrogen and carbon dioxide are specific microbial activities which have been demonstrated to occur in pipelines and can influence corrosion. The abundance of hydrogen, water and carbon dioxide coupled with low sulfate reduction rates in downstream portions of the pipeline suggests an available niche suitable for hydrogen mediated acetogenesis. The production of acetate was not restricted to the aqueous contents of the pipe but also occurred in wall scrapings of nodules. Acetate production by microorganisms within nodules supports the hypothesis that microbially produced endproducts can contribute to metal deterioration.

This study demonstrated that microorganisms were present and metabolically active in aqueous, sludge and wall scraping samples from natural gas transmission systems. The availability of water, hydrogen, carbon dioxide, and hydrocarbons appeared to be capable of supporting

microbial biomass and activities. The production of acetate by fermentation of organic substrates and hydrogen mediated acetogenesis provides mechanisms for products of microbial metabolism which could impact the integrity of the off-shore gas transmission lines. Most importantly this study demonstrated the utility and significance of measuring microbial activities which can impact the integrity of steel structures.

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Table 1. Microbial activities from off-shore natural gas platforms which could be associated with MIC.

Sample	[1-^{14}C]Acetate incorp. into micro. lipids dpm/day	[U-^{14}C]Glucose mineralization to $^{14}CO_2$ dpm x 10^3/day	[^{14}C]Acetate from [^{14}C]Glucose dpm/day	[^{14}C]Acetate from [^{14}C]CO_2 dpm x 10^3/day	[^{35}S]SO4 reduction dpm/day
HPSC-1	4600	340	800	270	n.d.
HPSC-2	400	27	630	11	n.d.
HPSC-3	5700	19	200	0.5	n.d.
HPSC-4	500	7	20000	0.6	1000
HPSC-5	44000	93	1600	bdl.	450
HPSC-6	3900	3	1300	bdl.	360

n.d. = Not determined, bdl. = Below detectable limits. HPS = high pressure separator. Limits of detection for each assay was approximately 150 dpm.

Table 2. Microbial activities from Pumping stations and on-shore processing facilities which could be associated with MIC.

Sample	[1-^{14}C]Acetate incorp. into micro. lipids dpm/day	[U-^{14}C]Glucose mineralization to $^{14}CO_2$ dpm x 10^3/day	[^{14}C]Acetate from [^{14}C]Glucose dpm/day	[^{14}C]Acetate from [^{14}C]CO_2 dpm x 10^3/day	[^{35}S]SO4 reduction dpm/day
Pumping stations					
A-2	344	11	400	bdl.	150
A-3	bdl.	bdl.	n.d.	9	n.d.
A-7	488	12	7000	0.3	56
A-8	bdl.	18	2000	bdl.	110
On Shore					
HPS	200	n.d.	6.3	0.6	n.d.
LPS	60	n.d.	n.d.	0.3	
Sludge	2200	n.d.	90	0.6	n.d.
Catcher	170	n.d.	n.d.	10	n.d.

n.d. = Not determined, bdl. = Below detectable limits. HPS = high pressure separator. LPS = low pressure separator. Limits of detection were approximately 150 dpm.

Table 3. Microbial activities from external samples of on-shore transportation facilities which could be associated with MIC.

Sample	[1-^{14}C]Acetate incorp. into micro. lipids dpm/day	[U-^{14}C]Glucose mineralization to $^{14}CO_2$ dpm x 10^3/day	[^{14}C]Acetate from [^{14}C]CO_2 dpm x 10^3/day	[^{35}S]SO4 reduction dpm/day
1	26	19	bdl.	140
2	bdl.	4	bdl.	64
3	1245	56	bdl.	bdl.
41	5258	154	bdl.	50
soil	20	27	180	240

bdl. = Below detectable limits.

1. Sample of external corrosion pit of a valve which also exhibited 78,000 dpm/day [^{14}C]CO_2 from [^{14}C]Acetate.

Table 4. Comparisons of microbial activities between lake sediments, deep subsurface environments and samples from natural gas transmission systems.

Sample	[1-^{14}C]Acetate incorp. into micro. lipids dpm x 10^3/day	[U-^{14}C]Glucose mineralization to $^{14}CO_2$ dpm x 10^3/day	[^{14}C]Acetate from [^{14}C]Glucose dpm x 10^3/day	[^{14}C]Acetate from [^{14}C]CO_2 dpm x 10^3/day	[^{35}S]SO4 reduction dpm x 10^3/day
Lakel sediments	>2000	>1000	>300	>50	>100
Subsurface clays[2]	<10	<5	<2	<5	<2
Subsurface aquifers[2]	>50	>20	>10	<5	<5
Off-shore MIC	.1-40	.1-300	.05-20	0-200	<1
On-shore external MIC	0-5	0-1	not det.	0-25	0-.3

not det. = not determined.

1. Results summarized from Phelps et al., 1985.

2. Results summarized from Phelps et al., 1989.

Figure 1.

Acetogenesis from hydrogen and carbon dioxide by samples from an off shore gas separator. Experimental tubes contained 5.0 uCi of $^{14}CO_2$, 12% hydrogen, 1.0 atmosphere nitrogen gas, 1.0mL of sterile anaerobic water and approximately 2 mL of sample. Elution fractions were collected at three drops per vial, cocktail added, and counted by scintillation counting.

ACETOGENESIS FROM H_2:CO_2 BY SAMPLES FROM TEST LOOP 41A-3