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ANAEROBIC MICROBIAL ACTIVITIES INCLUDING HYDROGEN MEDIATED ACETOGENESIS WITHIN NATURAL GAS TRANSMISSION LINES

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Microbially influenced corrosion (MIC) is being increasingly recognised as a serious problem. To investigate the role of MIC, radiotracer activity and lipid biomass measurements were performed on samples from off-shore and on-shore natural gas transmission systems. These measurements evaluated the biomass and metabolism of microbial communities residing inside transmission pipelines. Aqueous and nonaqueous hydrocarbon samples from liquid separators, sludge catchers and nodules attached to pipe walls were aseptically recovered and inoculated into anaerobic tubes for radiotracer time course experiments or preserved with chloroform-methanol for total lipid analyses. MPN enrichments and phospholipid biomass determinations estimated microbial populations of 10^4–10^7 cells per gram in several samples. 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. [^{14}C]Acetate was slowly mineralised to $^{14}CO_2$ without significant methane production. [^{14}C]Acetate was produced by fermentation of [^{14}C]glucose, [^{14}C]palmitate and by hydrogen mediated acetogenesis in the presence of [^{14}C]CO_2. In one location acetogenesis from hydrogen and carbon dioxide accounted for 0·7 mmol·l^{-1} of acetate production per week. These results demonstrated that microorganisms could utilise natural gas impurities to produce organic acids. This activity could adversely affect the structural integrity (MIC) of high pressure natural gas pipelines.

KEY WORDS: corrosion, acetogenesis, anaerobic, microbial

INTRODUCTION

Microbially influenced corrosion (MIC) has been recognised as a mechanism through which the structural integrity of metals may be compromised (Tatnall, 1981; Pope *et al.*, 1984, 1989; Iverson & Olson, 1984; Hamilton, 1985). MIC in anaerobic environments has been attributed to sulphate reducing bacteria (SRB) using organics or hydrogen for energy (Tatnall, 1981; Pope *et al.*, 1984; Hamilton, 1985; Pankhania, 1988). Iron has been implicated as a source of electrons for sulphate reduction with concomitant corrosion (Cord-Ruwisch & Widdel, 1986). Daniels *et al.* (1987) reported that iron stimulated electron donation for methanogenesis, suggesting a mechanism by which methanogens could facilitate corrosion. The production of volatile fatty acids by fermentative microorganisms has also been implicated in corrosion (Pope *et al.*, 1984, 1989; Little *et al.*, 1986). It appears that several mechanisms may exist for anaerobic MIC of metals.

Analytical tools used to diagnose microbial contributions to corrosion have typically relied on microscopic observations of bacteria, pitting patterns, production of sulphide, or recovery and growth of specific microorganisms. The recovery of micro-

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organisms demonstrates their presence, but provides little insight into the significance or mechanism of MIC. For example, spores, cysts, or resting cells, which are metabolically inactive, are not likely to cause considerable corrosion. Both the presence and activity of microorganisms are probably required for MIC.

Growth experiments enriching trophic groups of microorganisms could lead to false security. SRB represent a diverse group of microorganisms capable of utilising many different energy sources and possessing various salt and nutritional requirements. Most media designed for the growth of *Desulfovibrio* species are incapable of growing *Desulfobacter* species and other sulphate reducing bacteria (Widdel, 1980). Microscopic observations may demonstrate the presence of microorganisms but cause and effect may not be clear. Many groups of microorganisms are capable of multiple functions within an environment. For example, methanogenic bacteria could consume acetic acid resulting in decreased corrosion, or derive energy from the oxidation of iron adversely affecting the structural integrity of the steel. Measuring sulphide or acid accumulation could document microbial processes contributing to metal loss but few studies examine microbial activities related to corrosion.

Internal corrosion of off-shore natural gas transmission systems is a severe problem. An extensive network of pipelines transporting products from off-shore platforms in the Gulf of Mexico to on-shore purification systems were installed in the 1970's. The off-shore pipeline systems are required to transport natural gas, liquid hydrocarbons and considerable volumes of process waters. The off-shore natural gas collection system contains an aqueous component with portions of the system stagnating for weeks at a time. Lateral pipes connecting platforms to trunk lines are often too small in diameter for the use of cleaning devices resulting in sludge and fluid accumulations.

The goal of this study was to examine mechanisms by which resident microorganisms could contribute to the internal corrosion of the gas pipeline. Microbial phospholipids were examined for biomarkers characteristic of sulphide producing microorganisms without requiring growth (Dowling *et al.*, 1986; White *et al.*, 1979), and carbon and electron flow through the microbial community were also studied. These studies ascertained corrosive products produced in aqueous samples, sludge, or scrapings of pipeline walls. Hydrogen and carbon dioxide are known components of the product gas and specific experiments were designed to investigate the production of acetate from hydrogen and carbon dioxide. Active microbial communities were detected in many samples which were capable of producing acetate from palmitate, glucose and hydrogen plus carbon dioxide.

MATERIALS AND METHODS

Site Description

Several pipeline companies operate off-shore natural gas transmission systems servicing greater than 200 natural gas production platforms located in the Gulf of Mexico. At each platform the natural gas was separated from the liquids by a high pressure separator (HPS), pressurised to 60–70 atmospheres and metered. The aqueous and hydrocarbon liquids were often reinjected into the transmission system for transportation to intermediate pumping stations and subsequently to the on-shore separation facility. In this study HPS aqueous samples were obtained from 3 separators on one production platform (HPSC-4, 5, 6, Table 1) and from separators on five additional platforms operated by different gas producing companies. The high pressure

Table 1 Measures of general microbial activities obtained from natural gas transmission systems

Sample	*^{14}C-1-Acetate incorp. into micro. lipids dpm·day^{-1}*	*^{14}C-U-Glucose mineralisation to $^{14}CO_2$ dpm × 10^3·day^{-1}*	*^{14}C-1-Palmitate mineralisation to$^{14}CO_2$ dpm × 10^3·day^{-1}*	*^{14}C-2-Acetate mineralisation to$^{14}CO_2$ dpm × 10^3·day^{-1}*
Production Platforms				
HPSC-4	50	7	1·5	1·8
HPSC-5	43,700	93	bdl	19
HPSC-6	3,925	3	bdl	2·8
HPS V	4,550	340	nd	40
HPS W	141	27	nd	7·5
HPS X	5,700	19	nd	79
HPS Y	bdl	bdl	bdl	bdl
HPS Z	160	13	nd	40
Pump Stations				
SSA-2	344	10·5	13	1·4
SSA-6	14	bdl	bdl	bdl
SSA-6 scr	434	bdl	bdl	bdl
SSA-7	488	12	4	2·1
SSA-7 scr	24	bdl	bdl	bdl
HPS	125	4	1·8	bdl
Casing scr	2,280	174	nd	5·6
On shore				
HPS	199	nd	nd	7·5
LPS	58	nd	nd	7·5
LPS scr	440	nd	nd	39
Sludge	2,160	73.5	nd	3.7
Catcher	52	nd	nd	nd
Catcher scr	168	nd	nd	nd

nd = not determined; bdl = below detectable limits; HPS = high pressure separator; SS = side stream sample loop; scr = scrapings of pipe walls inclusive of nodules; LPS = low pressure separator.
Limits of detection for each assay was approximately 150 dpm.

natural gas and the reinjected liquids entered 15–30 cm diameter lateral pipelines which connected to larger pipes downstream. At intermediate locations lateral pipelines from several platforms entered pumping stations where liquids were again separated from the gas, the gas repressurised, and liquids reinjected. Two pumping stations operated by different natural gas transmission companies were examined in these studies. One pumping station contained three lines which could be sampled (SSA-2, 6, 7, Table 1). Both aqueous and hydrocarbon fractions were sampled from separators, side stream loops from separators, and pipe casing. Major off-shore transmission pipes exiting the pumping stations were approximately 1 m in diameter, the gas was at 60 atmospheres pressure, and gas velocities approached 1 m·s^{-1}.

Upon arrival at an on-shore processing facility liquids and sludge were separated from the gas in the sludge catcher, 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. At one on-shore processing facility examined in this study, greater than 2×10^{12} BTU of gas (2×10^9 cu ft) entered each day. Included with that gas was 6×10^5 l of petroleum, considerable sludge, and approximately 2×10^5 l of water; predominantly process waters and brines. Fluids, wall scrapings of nodules, and sludge were examined from the sludge catcher, HPS and LPS.

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 Company, Skokie, IL) to remove traces of oxygen. All chemicals used were of reagent grade and were obtained from Mallinckrodt (Paris, KY) or Sigma chemical company (St Louis, MO). Resi-analysed glass-distilled solvents and reagents were purchased from J T Baker Chemical Company (Phillipsburg, NJ). [1-^{14}C]Acetate (56 mCi·mmol^{-1}) and [^{35}S]SO_4 (481 mCi·mmol^{-1}) were purchased from New England Nuclear Corporation (Boston, MA). [2-^{14}C]Acetate 56 mCi·mmol^{-1}), [^{14}C]bicarbonate (54 mCi·mmol^{-1}), and [U-^{14}C]glucose (2·8 mCi·mmol^{-1}) were obtained from Amersham Corporation (Arlington Heights, IL). Experiments utilised crimp-top tubes or serum vials (Bellco, Vineland, NJ or Wheaton, Millville, NJ) sealed with butyl rubber septa. Incubations were at ambient temperature for 20 min to 1 week. 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%, appoximating the partial pressure available in the gas transmission system. Reductants included 0·05% cysteine-HCl or sodium sulphide. Resazurin was the redox indicator.

Field Studies

Sediment aliquots were inoculated for anaerobic microbial activity experiments within 30 min of arrival on-shore. All isotope solutions (1·0–50 uCi) were sterilised prior to use and transferred with gastight syringes (Hamilton Company, 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 *in situ* temperature of 23–30°C. Acetate incorporation experiments contained approximately 2 g sample, 4·0 μCi of [1-^{14}C]acetate and 1·0 ml of anaerobic sterile distilled water. Hydrogen mediated acetogenesis experiments contained 5·0 μCi [^{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 mineralisation experiments contained 1·0 μCi of the radiotracer, 1 g of sample and 1·0 ml of anaerobic sterile distilled water. Sulphate reduction experiments contained 1·0 μCi 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 NaOH. Time course experiments were performed in duplicate and consisted of at least 6 time points between 20 min and 7 days.

Analytical Procedures

Mineralisation experiments were acidified with 2·0 ml of 3·0 M HCl and the headspace of experimental tubes was analysed by gas chromatography-gas proportional counting as described by Nelson and Zeikus (1974). Aliquots of the aqueous phase of experimental tubes were analysed by HPLC with subsequent scintillation counting to quantify radiolabelled acetate accumulation. A Rezex organic acid column, (Phenomenex, Rancho Palos Verde, CA) 300 × 7·8 mm, with 0·01 N H_2SO_4 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 by a modification (White *et al.*, 1979) of the single phase

chloroform-methanol method of Bligh and Dyer (1959). The lipid fraction was evaporated to dryness and portions were counted by liquid scintillation counting to determine the amount of radioactivity incorporated into microbial lipids. Sulphate reduction assays were inhibited in the field with NaOH. Upon return to the laboratory the tubes were acidified with HCl. Sulphide was sparged from the tubes with nitrogen gas and the sulphide trapped in acidified zinc acetate. Aliquots of the zinc acetate solution were counted on the scintillation counter.

Total phospholipids were extracted as above and separated on silicic acid columns. Fractions were collected, subjected to methanolysis (White *et al.*, 1979), evaporated to dryness under a stream of N_2 gas, and the methyl esters of the fatty acids were stored at $-20°C$. The esters were analysed directly by capillary-gas liquid chromatography (GC) as previously described (Bobbie & White, 1980). Fatty acid methyl esters (FAME) were solubilised in hexane with methylnonadecanoate (19:0) as the internal injection standard. The FAME were analysed on a Hewlett Packard 5880 GC at 50°C in the splitless mode using a nonpolar cross-linked methyl silicone fused silica glass capillary column (50 m × 0·2 mm internal diameter). The GC oven was temperature programmed from 80°C to 150°C at a rate of $10°C\,min^{-1}$, and then $3°C\,min^{-1}$ to 240°C, and then $5°C\,min^{-1}$ to 280°C and held for 5 min. Hydrogen gas was used as the carrier gas at a flow of $1·0\,ml\,min^{-1}$. Peak areas were quantified using a Nelson Analytical (Perkin Elmer) laboratory data system operated with an internal standard program. Tentative peak identification was based upon relative retention times on a nonpolar column and compared to standards. Standards were procured from Supelco Incorporated (Bellefonte, PA) and applied Science Laboratories Incorporated (State College, PA). Gas chromatography-mass spectrometry verification of selected samples was performed on a Hewlett-Packard 5996A system fitted with a direct capillary inlet as previously described (Guckert *et al.*, 1986; Nichols *et al.*, 1987). Fatty acids are designated by the total number of carbon atoms: number of double bonds followed by the position of the unsaturation. Suffixes *c* and *t* indicate cis and trans geometry while *i* represents iso-branching.

RESULTS

Anaerobic samples from eight high pressure separators on production platforms, HPS separators, side streams, casing from off-shore pumping stations, and on-shore separators were examined for microbial activities during time course experiments. As shown in Table 1, radiolabelled acetate incorporated into lipids demonstrated that the resident microbial community within the off-shore transmission lines was metabolically active. The highest rates of acetate incorporation were observed in samples from production platforms. The platforms reinjected process water containing microorganisms, nutrients and electron acceptors such as sulphate, with the gas for transportation to shore. Lower activities observed from pumping station samples were likely related to depletion of electron acceptors and nutrients as gas was transported toward the on-shore facility. Samples from HPS Y showed no acetate incorporation or any other activity (Table 1). Beside HPS Y was a tank of glutaraldehyde with a line attached to the HPS indicating the use of a biocide. Although samples from HPS Y did not possess microbial activities, organic matter was not removed raising the expectation that there could have been microbial activity further downstream of the HPS Y. However, results from HPS Y suggested that, within a limited area, microbial activities could be inhibited with biocides.

Radiolabelled carbon dioxide was detected from glucose, palmitate and acetate (Table 1) demonstrating that oxidation of organic matter occurred in the off-shore transmission system. After 7 day incubations no radiolabelled 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.

Oxidation of the methyl position of acetate to carbon dioxide was indicative that these environments possessed an electron acceptor of higher redox than carbon dioxide. Sulphate could enter the transmission system at production platforms with process sea water or from formation waters. However, it would be expected that sulphate would have been rapidly utilised and not available at the pump stations or at the on-shore facility. Sample locations far removed from production platforms produced less carbon dioxide from the methyl-position of acetate even though carbon dioxide was produced from glucose. The exception to the trend was a highly active sediment sludge at the processing facility (Table 1). These results implied that a microbial community consisting of fermentative and acetate oxidising anaerobes was present in gas transmission pipelines. The low rate of acetate oxidation compared to glucose oxidation also suggested that acetate may accumulate in these environments.

Phospholipid fatty acid methyl-esters have often been used as indicators of microbial biomass (Smith *et al.*, 1986; White *et al.*, 1979) and they were detected in a variety of samples from the gas transmission lines. It has been estimated that 1 g dry weight of cells contains 50 μmol of phospholipids, each with two fatty acids for approximately 100 μmol PLFAME (White *et al.*, 1979). Results in Table 2 indicated microbial populations of 10^4–$10^7 \cdot g^{-1}$ of separator and sludge catcher contents. If microorganisms were contributing to corrosion then PLFAME would be expected to be observed in aqueous samples and nodules. As shown in Table 2, nodule contents scraped from the sides of metal pipes contained substantial PLFAME accounting for more than 10^8 bacteria $\cdot g^{-1}$ of nodule. Unique PLFAMES and minor peaks were difficult to detect in these samples due to a large background of hydrocarbons. The high ratio of 18- to 16-carbon PLFA shown in Table 2 was likely due to subtraction of hydrocarbon components rather than being indicative of the microbial community structure. The preponderance of iso- and anteiso- 15:0, 16- and 18- PLFA are typical of microbial communities. The 10 me16:0 and cy17:0 PLFA observed in side stream samples (SSA-2, 3, 6) are indicators of SRB.

Mass spectral verification of microbial phospholipids is shown in Figure 1. The lower diagram shows the total ion chromatogram from the GC-MS in a full scan mode of 40–500 m/z. The internal standard was 19:0 and the chromatogram was then compared to a mixture of external fatty acid standards. The total ion chromatogram was similar to gas chromatography in that hydrocarbons were detected. The upper diagram (Fig. 1b) shows results from an ion extraction program focusing on the single mass of 74 m/z which identified fatty acids linked to the methyl-ester derivitising agent. Considerable noise, particularly in the 17-18 carbon-length region, which was likely generated by hydrocarbon contaminants was eliminated by the ion extraction programme. Phospholipid fatty acids which were extracted from the oily materials and then derivitised by methyl-esters verified the presence of fatty acids typical of microorganisms.

Quantifying loss of metal from the inside of pipes is a difficult but definitive measure of corrosion. Biological activities characteristic of processes which could result in the loss of metal were sought. Reduction of $[^{35}S]SO_4$ to $[^{35}S]HS^-$ was measured in samples from production platforms but sulphate reduction rates were much lower or below detectable limits at the pumping stations (Table 3). The lower sulphate reduction rates

Table 2 Microbial phospholipid determinations from natural gas pipeline samples

Data expressed as mole percent *Samples* *PLFAME*[1]	*HPS*	*Nodule A*[2]	*Nodule B*[2]	*S.C. water*	*LPS*[3]	*HPS*[3]	*SSA-2*	*SSA-3*	*SSA-6*
i 14:0	nd	nd	nd	nd	nd	nd	2·07	nd	1·0
i 15:0	nd	1·2	1·4	2·3	nd	nd	0·7	nd	1·2
a 15:0	nd	18·0	23·8	1·8	11·2	0·9	1·0	nd	4·6
15:0	nd	nd	nd	nd	nd	nd	4·6	nd	2·4
16:1ω9c	nd	nd	nd	nd	nd	nd	1·2	nd	1·0
16:1ω7c	nd	nd	nd	nd	nd	nd	0·9	0·1	nd
16:0	15·0	28·5	23·6	42·6	nd	nd	49·0	67·0	40·0
10 me 16:0	nd	nd	nd	nd	nd	nd	nd	nd	0·6
i 17:0	4·0	1·5	1·25	6·7	nd	2·3	nd	3·4	2·4
a 17:0	6·5	16·0	19·4	5·3	nd	5·2	nd	2·0	4·2
cy 17:0	nd	nd	nd	nd	nd	nd	0·3	0·2	0·6
17:0	nd	nd	nd	nd	nd	nd	3·2	0·5	2·3
18:1ω9c	39·0	6·4	3·6	10·8	44·6	47·7	1·1	0·5	3·6
18:1ω7c	15·0	13·9	12·7	5·5	22·5	24·3	nd	0·5	1·5
18:0	20·0	14·7	14·3	25·1	21·7	21·7	29·4	23·1	27·5
pmol·gdw^{-1}	191	11,152	5,957	232					
pmol·ml^{-1}					11	34	15	4,795	305

nd = not detected; [1]Phospholipid fatty acid methyl-esters determined as described in the text; [2]A and B nodules were from the on-shore sludge catcher; [3]LPS and HPS samples contained considerable hydrocarbons making dry weight determinations difficult. Dry weight would have been much less than 5%.

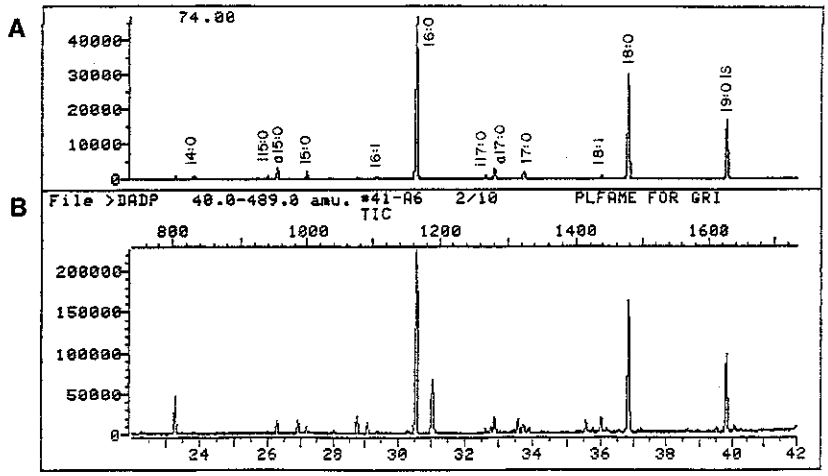

Fig. 1 Gas chromatographic and mass spectral tracing of phospholipid fatty acid profiles from aqueous hydrocarbon samples of natural gas transmission systems. (A) Single ion extraction profile for m/z = 74; the diagnostic peak for phospholipid fatty-acid methyl-esters (PLFAME). (B) Total ion chromatogram 40–500 m/z showing hydrocarbon contamination in addition to fatty acids.

at the pumping stations may have reflected depletion of the sulphate pool within the closed pipeline. The slow acetate mineralisation rate as compared to the glucose mineralisation rate led to the hypothesis that biologically produced acetate could accumulate within the aqueous portions of the pipe. Acetate formation from carbohydrates and alcohols as well as acetate production from hydrogen mediated acetogenesis were examined. As shown in Table 3, acetate was produced from both glucose and hydrogen plus carbon dioxide. Glucose was not expected to be a major substrate in gas transmission systems but was used merely as an indicator of fermentative potential. Acetate production from carbon dioxide however, represented an acid producing mechanism available to resident microorganisms which could impact the structural integrity of high pressure pipelines.

The recovery of radiolabelled acetate produced from carbon dioxide is shown in Figure 2. Control experiments exhibited background counts of 10–20 dpm per elution fraction. One day time points from HPS V revealed considerable radiolabelled volatile fatty acids. The peak at elution fraction 58 was tentatively identified as formate. The peak at elution fraction 64 was demonstrated to be acetate by both total and radiolabelled acetate standards. At one day approximately 1500 dpm of acetate was produced from the 5 μCi of $^{14}CO_2$ ($1{\cdot}1 \times 10^7$ dpm·tube^{-1}). After 7 d the peak at fraction 58 was below detectable limits whereas the acetate peak had increased ten fold to 15,000 dpm.

The carbon dioxide pool size in the HPS and LPS samples was 6–8 mmol·l^{-1} but 150 mmol·l^{-1} in the sludge catcher water. Assuming two moles of carbon dioxide were consumed per mole of acetate produced, samples from HPS V could have fixed 10% of the carbon dioxide pool into acetate within one week accounting for the production of 0·7 mmol·l^{-1} of acetate per week. Other aqueous, scraping, and sludge samples accounted for less than 0·2 mmol·l^{-1} of acetate produced per week from carbon dioxide (Table 3). 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 Specific activities and trophic groups of microorganisms related to internal corrosion

Sample	*$^{35}SO_4$ reduction* *dpm·day^{-1}*	*^{14}C-Acetate from ^{14}C-U-glu* *dpm × 10^3·wk^{-1}*	*^{14}C-Ace from $^{14}CO_2$* *dpm × 10^3·wk^{-1}*	*H_2 using Acetogens* *log·ml^{-1}*	*Sulfate Reducers V, B, D (1)*
Production platforms					
HPSC-4	1,020	++(2)	3·7	1	V, D
HPSC-5	450	+	bdl	bdl	V, B
HPSC-6	360	+	bdl	3	V
HPS V	nd	812	1,900	nd	nd
HPS W	nd	609	79	nd	nd
HPS X	nd	203	3·5	nd	nd
HPS Y	nd	bdl	bdl	nd	nd
HPS Z	nd	175	6·3	nd	nd
Pump stations					
SSA-2	150	+	bdl	bdl	V, B
SSA-3	nd	nd	62·5	nd	bdl
SSA-6	bdl	+	bdl	1	B
SSA-6 scr	bdl	nd	bdl	bdl	B
SSA-7	56	+	2	3	V, B, D
SSA-7 scr	bdl	nd	bdl	bdl	V, B
Separator	bdl	++	10·1	3	V, B, D
On shore					
HPS	nd	44	4·2	nd	nd
LPS	nd				
LPS scr	nd	nd	2	nd	nd
Sludge	nd	602	4·2	nd	nd
Catcher	nd	nd	70	nd	nd
Catcher scr	nd	nd	4·2	nd	nd

nd = not determined; bdl = below detectable limits.
(1) Presence of sulphate reducing species per ml; *Desulfovibrio* = V, *Desulfobacter* = B, *Desulfobulbus* = D.
(2) 0 to ++. Acetate production from [^{14}C]glucose, where 0 = $< 10^4$ dpm·wk^{-1}, + = $> 10^4$ dpm·wk^{-1} and ++ = $> 10^5$ dpm·wk^{-1}.

Experiments were performed to enrich hydrogen utilising acetogens and sulphate reducing bacteria from pipeline samples. As shown in Table 3, hydrogen utilising acetogens were detected in 5 of the 9 samples examined at densities of 10^1–10^3·ml^{-1}. SRB representing three genera were enriched from several samples, *Desulfovibrio, Desulfobacter*, and *Desulfobulbus*. Quantities of heterotrophic and sulphate reducing bacteria were estimated to be 10^2–10^5·ml^{-1} by other investigators using serial dilution experiments (Pope *et al.*, unpublished data). These results demonstrated the presence of microorganisms capable of reducing sulphate to sulphide, fermentation of organics to acetate and acetogenesis from hydrogen plus carbon dioxide.

DISCUSSION

Previous investigators (Pope *et al.*, 1984, 1989) 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; sulphate reducing and fermentative acid producing bacteria. PLFAME analyses demonstrated that microorganisms were present in gas transmission line samples without requiring growth of the bacteria.

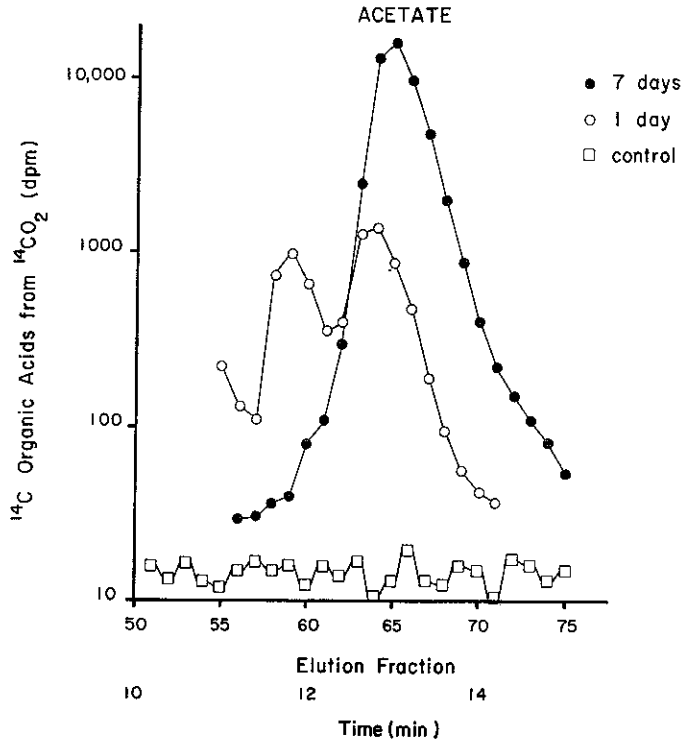

Fig. 2 Acetogenesis from hydrogen and carbon dioxide by samples from an off shore gas separator. Experimental tubes contained 5·0 μCi $^{14}CO_2$, 12% hydrogen, 1·0 atmosphere nitrogen gas, 1·0 ml sterile anaerobic water and approximately 2 ml sample. Elution fractions were collected at 3 drops per vial, cocktail added, and counted by scintillation counting.

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. Radiolabelled acetate incorporation into lipids is a general activity common to most organisms and varies dramatically between environments (Smith *et al.*, 1986; Phelps *et al.*, 1989). Glucose, palmitate and acetate mineralisation are activities common to many organisms. 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.

In sulphate rich sediments methanogenesis is a minor process (Winfrey & Zeikus, 1979; Lovley & Klug, 1983; Phelps & Zeikus, 1985) whereas in sulphate depleted sediments methane formation is the predominant reduced end product of anaerobic decomposition (Winfrey & Zeikus, 1979; Phelps & Zeikus, 1985). 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 may have been due to less favourable energetics of producing methane against a 60 atmosphere gradient. In sulphate limited portions of the system hydrogen mediated acetogenesis may have been the most favourable route for hydrogen utilisation.

Glucose and acetate uptake and turnover in fresh sediments has been determined to be in the order of seconds to minutes with pool sizes less than 100 μmol·l^{-1} (Lovley &

Klug, 1983; Phelps & Zeikus, 1985). 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 more likely (Schink, 1986). Mass spectral analysis of pipeline contents (Gas Research Insitute, unpublished data) revealed that a large range of compounds including fatty acids, alcohols and unsaturated hydrocarbons were available for oxidation by sulphate reducers or fermentative microorganisms.

Hydrogen metabolism in anaerobic sediments occurs within seconds and the pool size is generally maintained below 10 $\mu mol \cdot l^{-1}$ (Conrad *et al.*, 1985; Phelps & Zeikus, 1984) with the terminal product typically being methane or sulphide. Hydrogen has been detected in natural gas at concentrations above 0·1% and at 60 atmospheres of pressure hydrogen could be readily abundant. In these pipeline samples methanogenesis was insignificant. Acetate formation from hydrogen and carbon dioxide is typical in environments with high hydrogen partial pressures or low hydrogen consumption by methanogens and sulphate reducers (Phelps & Zeikus, 1984). Degradable organic matter in these anaerobic natural gas pipelines leads to the production and accumulation of acetate as a major end product of microbial metabolism.

Accumulation of acetate as a product of fermentation, as suggested by these studies, can contribute to acidification which could increase the corrosion of a steel pipeline. Reduction of sulphate to sulphide and production of acetate from hydrogen and carbon dioxide are specific activities which have been demonstrated to occur in pipelines and can influence corrosion. The abundance of hydrogen, water and carbon dioxide coupled with low sulphate 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 which contained nodules. Pope *et al.* (1989) have observed characteristic pitting in nodules containing microorganisms. Acetate production by microorganisms within nodules supports the hypothesis that microbially produced end products can contribute to metal deterioration.

The role of microbial acetate production in the internal corrosion or deterioration of pipelines has not been well documented. This study demonstrated that microorganisms were present and metabolically active in aqueous, sludge and wall scraping samples from off-shore 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 by which products of microbial metabolism could impact the integrity of the off-shore gas transmission lines.

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