DEVELOPMENT OF AN ENVIRONMENTALLY BENIGN MICROBIAL INHIBITOR TO CONTROL INTERNAL PIPELINE CORROSION

TWELFTH QUARTER REPORT

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ABSTRACT

Title:	Development of an Environmentally Benign Microbial Inhibitor to Control Internal Pipeline Corrosion
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Objective:	The overall program objective is to develop and evaluate environmentally benign agents or products that are effective in the prevention, inhibition, and mitigation of microbially influenced corrosion (MIC) in the internal surfaces of metallic natural gas pipelines. The goal is to develop one or more environmentally benign (a.k.a. "green") products that can be applied to maintain the structure and dependability of the natural gas infrastructure.
Approach:	Previous testing indicated that the growth, and the metal corrosion caused by pure cultures of sulfate reducing bacteria were inhibited by hexane extracts of some pepper plants. This quarter tests were performed to determine if chemical compounds other than pepper extracts could inhibit the growth of corrosion-associated microbes and to determine if pepper extracts and other compounds can inhibit corrosion when mature biofilms are present.
Results:	Several chemical compounds were shown to be capable of inhibiting the growth of corrosion-associated microorganisms, and all of these compounds limited the amount of corrosion caused by mature biofilms to a similar extent.
Conclusions:	It is difficult to control corrosion caused by mature biofilms, but any compound that disrupts the metabolism of any of the major microbial groups present in corrosion-associated biofilms shows promise in limiting the amount/rate of corrosion.

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EXECUTIVE SUMMARY

The main goal of this project is to develop an environmentally benign product that could prevent and/or control microbiologically influenced corrosion (MIC) in the interior of metal gas pipelines. The main activities for this quarter were the evaluation of various chemicals other than hexane extracts of pepper plants to inhibit bacterial survival/growth of corrosion-associated microorganisms. Additionally, experiments were performed using mature biofilms to determine if various chemicals and/or hexane extracts of pepper plants could inhibit MIC, using mixed cultures of bacteria obtained from a natural gas pipeline.

Previous results obtained in this project indicated that hexane extracts of pepper plants could selectively inhibit the growth of sulfate reducing bacteria and that the use of pepper plant extracts could result in decreased metal corrosion. These previous experiments used conditions in which the pepper extract and the bacteria were added at the same time. Experiments performed during the past quarter included an examination of an expanded range of chemicals that may be useful as selective inhibitors of corrosion-associated microorganisms. The chemical compounds examined included a variety of molybdenum-containing compounds that may selectively inhibit sulfate-reducing bacteria, anthraquinone that may selectively inhibit sulfate-reducing bacteria, some structural analogues of nitrate that may serve as selective inhibitors of denitrifying bacteria, 2-bromoethanesulfonic (BES) acid that may selectively inhibit methanogenic bacteria, and glutaraldehyde a general biocide. Growth tests were performed with pure cultures of sulfate reducing bacteria and denitrifying bacteria which demonstrated that nearly all molybdenum-containing compounds inhibit the growth of sulfate reducing bacteria, and that ammonium cerium nitrate and bismuth nitrate are capable of inhibiting the growth of denitrifying bacteria.

Previous corrosion testing performed in this project, and indeed most of the corrosion testing done to investigate MIC, utilized an experimental approach in which the inhibitory chemical and the bacterial suspension are added to the metal coupon at the same time. However, experiments performed this quarter included the use of mature biofilms that more accurately mimic the environment present in natural gas pipelines. It is well known that established biofilms are much more difficult to treat with biocides/inhibitors than bacterial cells in solution. The results obtained with our tests of mature biofilms indicate that the majority of corrosion occurred

while the biofilm was becoming established and prior to the addition of inhibitory chemicals. However, the addition of various inhibitors decreased the amount of subsequent corrosion caused by these mature biofilms. Of the seven chemical compounds tested in mature biofilm experiments all were successful in decreasing corrosion to a similar degree. These results may be interpreted to indicate that disruption of the metabolism of any of the major microbial groups present in the biofilm caused a similar decrease in corrosion. These results are consistent with the hypothesis that it may be possible to minimize MIC by altering the composition and/or the metabolism of biofilms rather than by attempting to kill all microorganisms. Further testing is needed to confirm the ability of selective inhibitors to minimize corrosion, but decreasing corrosion using selective inhibitors may allow the use of compounds that are far less toxic than biocides for the control of MIC.

BACKGROUND

The overall objective of this project is to develop, test, and apply environmentally benign agent(s) to control microbial-caused corrosion on the internal surfaces of metal (iron or steel) pipes used for natural gas production and transmission. The overall hypothesis being tested in this project is that agents exist in nature that inhibit some or all of the steps executed by microorganisms in the formation of biofilm and/or microbial processes resulting in metal corrosion. As biofilm formation is an absolute prerequisite for the initiation and production of microbiologically influenced corrosion (MIC), blocking biofilm formation or propagation will block or mitigate MIC. Similarly, if key metabolic processes of microorganisms that contribute to MIC can be inhibited then corrosion can be prevented/diminished.

The general approach is to evaluate natural products isolated from plants and chemical compounds that may act as selective inhibitors of various types of microorganisms for their abilities to block the attachment, physiology, or reproduction of microbial agents that are responsible for MIC. Specifically, the natural products that are examined in this project are those compounds that can be extracted from the seeds and pods of pepper plants. These plants are members of the Genus *Capsicum.* These pepper extracts will be tested for their environmental impact and effects, effective concentrations, modes of application, and stability against pure and mixed cultures of corrosion-associated microorganisms under simulated field conditions. This project will determine the feasibility of producing an environmentally benign MIC control agent derived from pepper plants. Additionally, other chemicals that may have the ability to selectively inhibit the metabolic processes of particular groups of microorganisms will also be evaluated to obtain information regarding the contribution of various microbial groups to corrosion, and the use of selective inhibitors to control corrosion with compounds that may be less toxic than general biocides.

Corrosion is a leading cause for pipe failure, and is a main component of the operating and maintenance costs of gas industry pipelines [5, 11, 13, 16, 27]. Quantifying the cost of corrosion generally in the gas industry, and more specifically the cost associated with microbial corrosion, is not easily done and is controversial. Corrosion was estimated in 2001 to cost the gas industry about $13.4 billion/yr and of this as much as $2 billion/yr may be due to MIC [18]. Basic research to increase our understanding of the microbial species involved in microbial corrosion, and their interaction with metal surfaces and with other microorganisms will be the

basis for the development of new approaches for the detection, monitoring, and control of microbial corrosion. A thorough knowledge of the causes of microbiologically influenced corrosion (MIC) and an efficient and effective means of detecting and preventing corrosion is lacking. It is well recognized that microorganisms are a major cause of corrosion of metal pipes, but despite decades of study it is still not known with certainty how many species of microorganisms contribute to corrosion, how to reliably detect their presence prior to corrosion events, or how to rapidly assess the efficacy of biocides/mitigation procedures [1, 2, 10, 11, 13, 16, 25, 26, 29].

It is known that several types of microorganisms can cause corrosion when these microorganisms are tested as pure cultures: sulfate reducing bacteria [3, 7, 14], acid producing bacteria [22], iron respiring bacteria [1, 20, 21, 31], denitrifying bacteria [16, 17, 28, 30], and methanogenic bacteria [4, 9]. However, the relative contribution of these various types of microorganisms to corrosion in complex microbial communities typical of biofilms present in gas pipelines is unknown [32, 33]. Therefore a goal of recent research was to obtain data concerning the ability various chemical compounds to selectively inhibit certain types of microorganisms and to then determine the effects on metal corrosion. It is hoped that improved data can be obtained that quantifies the effect of different types of microorganisms on corrosion. Such data will identify those types of microorganisms are of greatest priority when monitoring MIC and which types of microorganisms should be preferentially targeted by biocides intended to control MIC.

A survey of the literature revealed that molybdenum compounds, such as sodium molybdate, can act as selective inhibitors of sulfate reducing bacteria, and that 2-bromoethane sulfonic acid (BES) can selectively inhibit methanogens [15, 23, 24]. Molybdenum compounds can interact with an enzyme involved in the metabolism of sulfate, ATP sulfurylase, and cause non-productive consumption of ATP, which causes a metabolic/energy drain on sulfate reducing bacteria. Thus molybdenum will selectively inhibit sulfate reducing bacteria, but it can have significant inhibitory effects without killing sulfate reducing bacteria. However, not all sulfate reducing bacteria are inhibited by molybdenum [7]. Another compound described in the literature as being capable of selectively inhibiting sulfate reducing bacteria is anthraquinone [6, 8] which works by uncoupling ATP synthesis from electron transfer reactions associated with hydrogen-dependent sulfate respiration. Additionally, proprietary studies performed at GTI

indicate that certain extracts of pepper plants can selectively inhibit the growth of sulfate reducing bacteria (data not shown).

BES is an analogue of an enzyme cofactor that is essential to methanogenesis, HS-coenzyme M. Thus BES can selectively inhibit methanogenic bacteria, but it can inhibit these bacteria without killing them [24].

Other bacterial groups of interest as regards MIC are denitrifying bacteria and acid producing bacteria. Acetylene can be used to inhibit denitrification, but the use of acetylene may not be specific for the inhibition of denitrifiers and acetylene does not lend itself to use in a pipeline environment. Accordingly, we examined several chemical compounds that may act as structural analogues of nitrate and selectively inhibit denitrification. The compounds that may serve as selective inhibitors of denitrifying bacteria include ammonium cerium nitrate (ACS), bismuth nitrate, and bismuth oxide. Acid producing bacteria are a very diverse group of bacteria employing several different pathways to produce several different acidic compounds. Thus, while it would be desirable to selectively inhibit acid producing bacteria there are no selective inhibitors described in the literature and no clear way to develop such an inhibitor because of the diversity of this bacterial group.

Materials and Methods

Pepper Extract Stock Solutions. Three extracts were selected for batch tests. The extracts and the extraction procedure were described in previous reports. Extract 1 (obtained from Chile de arbol peppers) contained caffeic and coumaric acids as its primary identifiable constituents; extract 2 (Serrano peppers) contained several unknown organic acids; extract 3 (Habañero peppers) contained stearic, palmitic, and oleic acids. Stock solutions of 1%, 5%, and 10% of each extract were made in hexane, and inoculated into anaerobic growth media.

Origin of MIC consortium. A field sample was received from a gas company who was experiencing microbiologically influenced corrosion. The sample was inoculated into a general corrosion media (see below) with or without mild steel metal coupons and incubated at room temperature. This mixed microbial community was then used as the source of bacterial cells and biofilms for subsequent experiments to test the ability of various inhibitory compounds to prevent growth and/or metal corrosion.

Conditions for bacterial growth. General Corrosion Media consisted of:

Sodium nitrate	0.25 g
Sodium thiosulfate	0.20 g
Sodium acetate	0.30 g
Sodium citrate	0.20 g
Yeast extract	0.10 g
Lactate (1.5 M stock)	6 ml
$CaCl_2$	0.7 g
Casamino acids solutions	1 ml (10% stock)
Vitamin B1 solution	2 ml (0.5mg/ml stock)
Arginine solution	2 ml (10 mg/ml stock)
Glutamate solution	2 ml (10 mg/ml stock)
Glutamine solution	1 ml (20 mg/ml stock)
Serine solution	4 ml (10 mg/ml stock)
$MgSO_4$	0.25 g
Trace metals solution	10 ml (Na_2EDTA-$2H_2O$, 0.026 g/L; H_3BO_3, 0.0037 g/L; NaCl, 0.0006 g/L; $FeSO_4$-$7H_2O$, 0.0017 g/L; $CoCl_2$-$6H_2O$, 0.0012 g/L; $Ni(NH_4)(SO_4)$-$6H_2O$, 0.002 g/L; Na_2SeO_4-$2H_2O$, 0.001 g/L; Na_2SeO_4(anhydrous), 0.00028 g/L; $MnSO_4$-H_2O, 0.00022 g/L; $ZnSO_4$-$7H_2O$, 0.00029 g/L; $CuSO_4$-$5H_2O$, 0.00005 g/L)
Salts solution	20 ml (add slowly, otherwise the Ca will precipitate) [$(NH_4)_2SO_4$ 1.19 g; $K_2HPO_4.3H_2O$, 1.3 g; KH_2PO_4, 0.449 g; $NaHCO_3$, 0.168 g]

Add 935 ml of dH_2O and autoclave. Transfer to a sterile 1 L bottle and let cool.

Add:	
Glucose/succinate/glycerol	10 ml (Glucose, 18 g; Sodium succinate, 5 g; Glycerol, 25 ml)
Ferric citrate (500 mM stock)	5 ml
Reducing agent solution	1 ml (Thioglycolate, 0.1 g; Ascorbate, 0.1 g; Add 10 ml of dH_2O and filter sterilize)

ATCC Culture Medium 292 (Baar's medium) was used to cultivate sulfate reducing bacteria (SRB) and contained: 3.5 g sodium lactate, 0.5 g NH_4Cl, 1.0 g K_2HPO_4, 2.0 g $MgSO_4 \cdot 7H_2O$, 1.0 g $CaSO_4$, and 1.0 L tap water. A 5% (wt/vol) solution of ferrous ammonium sulfate and a 10% (wt/vol) solution of yeast extract were sterilized separately and added aseptically to the above base (1.0 ml of each per 100 ml of the medium). Heterotrophic Anaerobic Bacteria (HAB) medium per liter contained 2.0 g glycerol, 10.0 g peptone, 10.0 g tryptone, 0.2 g sodium thiosulfate, 0.2 g magnesium sulfate, and 0.5 g K_2HPO_4. Denitrifying bacteria medium (DNB) per liter contained 1.0 g of asparagine, 5.0 g of sodium citrate, 2.0 g of potassium nitrate, 2.0 g of K_2HPO_4, 2.0 g of magnesium sulfate, 0.01 g of calcium chloride, and 0.01 g of $FeCl_3$. Acid producing bacteria (APB) medium per liter contained 5.0 g of D-glucose, 0.2 g of sodium thiosulfate, 10.0 g of tryptone, 10.0 g of proteose peptone, 2.0 g of glycerol, 0.2 g of magnesium sulfate, 0.5 g of K_2HPO_4, and 0.01 g of phenol red. Methanogenic bacteria (MET) medium per liter contained 4.0 g of sodium hydroxide, 2.0 g of yeast extract, 2.0 g of trypticase peptones, 0.5 g of mercaptoenthanesufonic acid, 1.0 g of ammonium chloride, 0.4 g of potassium phosphate trihydrate (dibasic), 1.0 g of magnesium chloride hexahydrate, 0.4 g of calcium chloride dihydrate, 1.0 g of resazurin, 10 ml of mineral solution, 10 ml of vitamin solution [16].

Growth on metal coupons was conducted on a 1/16-inch thick C1018 mild steel coupon with a density of 7.87 g/cm^3 (Catalog number G10180 – CO100, Metal Samples Co., Munford, AL).

Plate Counts and Most Probable Numbers Analyses. Microbial population size was estimated by standard plate counts (all organisms) and by Most Probable Numbers (MPN) analyses (SRB, acid-producing bacteria). For plate counts, 100 μl samples from each batch test were serially diluted and spread onto solid R2A agar plates (Difco Laboratories; Detroit, MI). Plates were incubated for 7 days in an anaerobic chamber after which plates having between 30 -300 colony forming units (cfu) were counted. The population size of the culture was estimated by

multiplying the number of cfu by the dilution factor. For MPN estimates, a 1-ml sample of each batch test was added to 10 ml of modified Postgate's B medium or Acid-producing medium (Dixie Testing & Products; Houston, TX); 1 ml of the resulting bacterial suspension was sequentially diluted by 10X increments to a final 10^{-5} or 10^{-12}-fold dilution. Each series was performed in triplicate. Anaerobic dilution tubes were scored for SRB by noting the presence of a black FeS precipitate after 14 days at 26°C. Anaerobic dilution tubes were scored for acid-producing bacteria by observing a color change in the medium from red to yellow (indicating a decrease in pH) after 14 days at 26ºC. MPN were determined using the program MOST PROBABLE NUMBER CALCULATOR© Version 4.04 (Klee, 1996).

Analytical Methods. Total protein was measured by the Lowry method (Lowry, 1951); sulfide was measured using the methylene blue method (Clesceri et al., 1998); iron (II) was measured by the ferrozine method (Stooky, 1970); and nitrite was measured using the method of Montgomery and Dymock (1961).

Metal Coupons and Weight Loss Measurements. Circular metal coupons were purchased from a commercial source (Metal Samples Co.; Munford, AL). Coupons were made of 1018 steel, had a diameter of 0.400 inches, and were ⅛ inch thick. The density of the coupons was 7.87 g/cm^3 and the surface area was 2.64 cm^2. Prior to experiments, the coupons were weighed. Upon the conclusion of experiments, coupons were removed from the batch tests and cleaned according to ASTM standard G1-90 (for metals composed of iron and steel). The coupons were subjected to a series of six solutions, either acidic or basic and at varying temperatures. One modification was made to the ASTM method: 5M sodium hydroxide was used at a temperature of 100°C for the last cleaning step. The coupons were then rinsed in deionized water, allowed to dry at room temperature overnight in a dessicator, and then weighed the next day. The corrosion rate of the metal coupons were calculated according to the following formula:

$$\text{Corrosion Rate} = (K \times W)/(A \times T \times D) \quad (1)$$

where:

K = 3,450,000 (constant used to determine corrosion rate in mils per year)
T = time of exposure (h)
W = weight loss (g)
D = density (g/cm^3)
A = surface area (cm^2)

Experiments. Metal coupons containing mature biofilm were prepared by exposure to a mixed community of corrosion-associated microorganisms for a period of two weeks to allow a mature biofilm to form on the metal coupons. Sterile 125-ml serum bottles were filled with 99 ml of growth media and a metal coupon containing a mature biofilm. The media was amended to achieve a final concentration of 0.1% for each compound tested. The experiments were concluded after 4 weeks and the coupons' weight loss was measured. Control cultures – inoculated but lacking inhibitors – and sterile (non-inoculated) media controls were also performed. Each test was performed in triplicate.

RESULTS AND DISCUSSION

Evaluation of various compounds to inhibit the growth of sulfate reducing bacteria and denitrifying bacteria. While selective inhibitors of sulfate reducers, methanogens, and denitrifiers are known from the literature it was of interest to test alternative compounds to see if improved selective inhibitors could be identified that may allow better selective inhibition of the targeted group of microorganisms and/or may be more compatible with use in a gas pipeline environment. Several chemical compounds were tested to identify alternative selective inhibitors for sulfate reducing bacteria and potential inhibitors of denitrifying bacteria. Those compounds are listed in Table 1, where the results of microbial growth tests with a mixed inoculum derived from a gas pipeline sample are shown. The compounds listed in Table 1 were tested at a concentration of 5 mM to determine their ability to inhibit the growth of a gas pipeline microbial culture in either sulfate reducing medium or denitrifier medium. Glutaraldehyde, a general biocide widely used in the gas industry was included as a control. Cultures were incubated anaerobically at room temperature for three weeks.

Table 1. Inhibition of Growth of Gas Pipeline Microorganisms with Various Compounds.

TEST COMPOUND	**INHIBITION OF GROWTH**
SRB INHIBITORS	
SODIUM MOLYBDATE	+
AMMONIUM MOLYBDATE	+
MOLYBDENUM TRIOXIDE	+
MOLYBDENUM OXIDE	+
MOLYBDENUM DICHLORIDE DIOXIDE	-
MOLYBDENUM DISILICATE	+
MOLYBDENUM SULFIDE	+
MOLYBDENUM ACETATE DIMER	+
MOLYBDENUM ACETYLACETONATE	+
DENITRIFICATION INHIBITORS	
AMMONIUM CERIUM NITRATE	+
BISMUTH OXIDE	+/-
BISMUTH NITRATE	+
GENERAL BIOCIDE	
GLUTARALDEHYDE	+

The results shown in Table 1 indicate that nearly every molybdenum-containing compound tested resulted in inhibition of growth of the gas pipeline culture in sulfate reducing medium. The inhibition of growth suggests that these compounds are potentially useful as selective inhibitors of SRB, however; additional testing is needed to confirm the selective nature of this growth inhibition. Likewise, the results in Table 1 indicate that ammonium cerium nitrate and bismuth nitrate are potentially useful as selective inhibitors of denitrifying bacteria.

Evaluation of the ability of various compounds to reduce metal corrosion rates in microbial growth experiments.

Before proceeding to develop chemical formulations of compounds that may be useful to control MIC in the gas industry it is necessary to determine the effect of these inhibitors on the corrosion of metal. Experiments were set up in which a gas pipeline sample was used to inoculate microbial growth media that contained mild steel metal coupons. These samples were incubated at room temperature for 44 days to allow growth of microorganisms and the onset of corrosion. The metal coupons were carefully weighed prior to the experiment and then cleaned according to standard procedures after the 44 day incubation and reweighed. Corrosion was recorded as weight loss of the metal coupons, and the results are shown in Table 2. The concentration of chemicals tested as selective inhibitors or general inhibitors of MIC were: 100 mM glutaraldehyde, 15 mM sodium molybdate, 5 mM BES, and 1 mM ammonium cerium nitrate. The results shown in Table 2 indicate that glutaraldehyde yields the best corrosion inhibition, while inhibition of methanotrophs and denitrifiers provides better corrosion reduction than inhibition of sulfate reducing bacteria. However, not all of the data in Table 2 are consistent with this interpretation and further testing is required before firm conclusions can be drawn. However, these results are promising as they indicate that inhibiting certain types of microbial activity/metabolism has a greater effect on corrosion than others. Obtaining conclusive data that correlates the contributions of various types of microorganisms to metal corrosion will be of great value in developing improved products for corrosion control and for improved methods of monitoring MIC-associated microorganisms.

Table 2. The effect of biocides and inhibitors on corrosion rates of metal coupons in 44 days. Glu = glutaraldehyde, SM = sodium molybdate, BES = 2-bromoethane sulfinic acid, ACN = ammonium cerium nitrate.

COMPOUND	CORROSION RATE (MILS/YR)
NO INHIBITOR	3.24
GLU	0.68
SM	2.16
BES	1.36
ACN	1.62
SM + BES	1.78
SM + ACN	2.27
BES + ACN	3.16
SM + BES + ACN	1.67

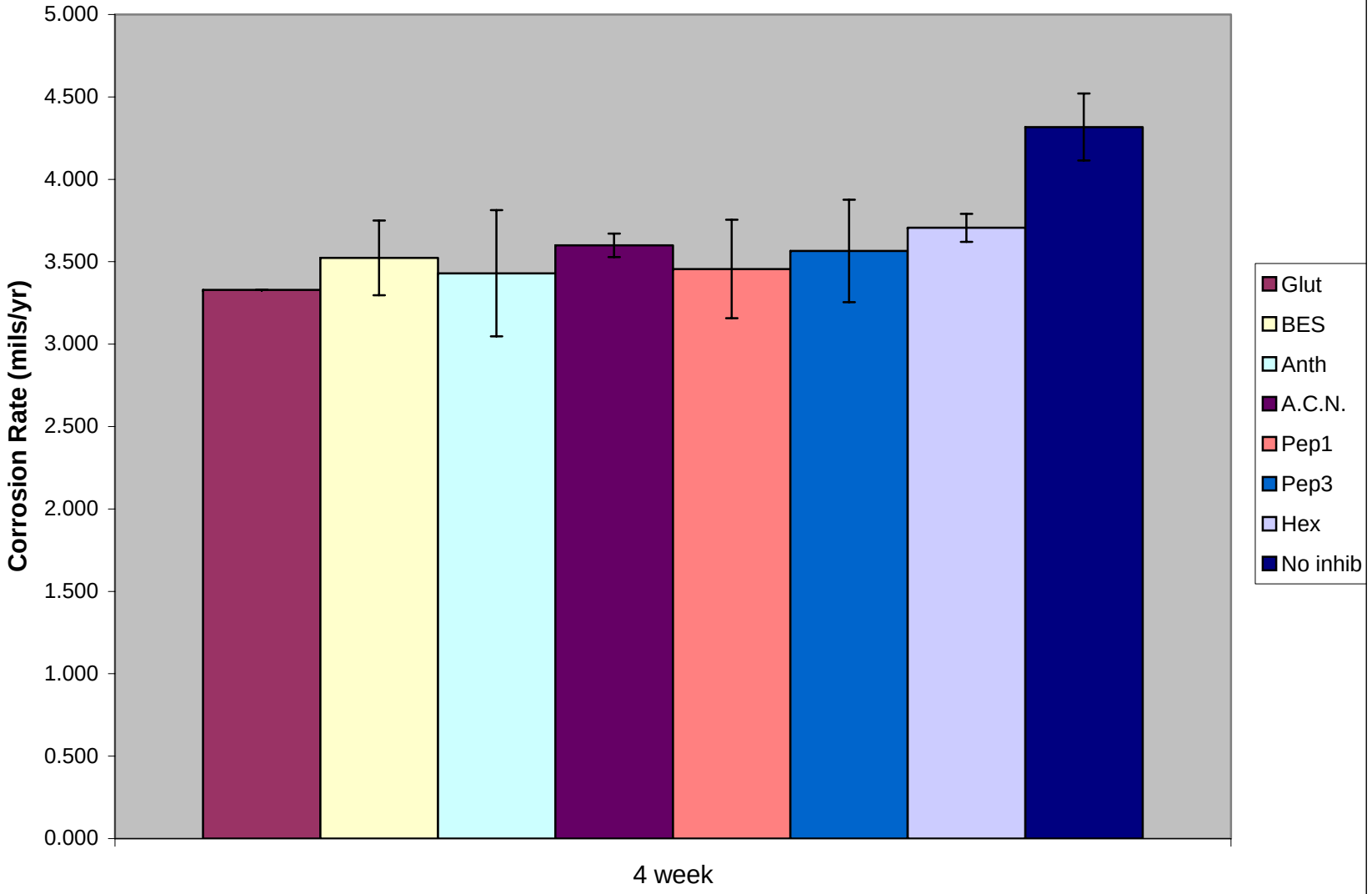
Evaluation of the ability of various compounds to reduce metal corrosion rates in experiments with mature biofilms.

The results reported in Table 2 were obtained by adding the selective inhibitor compounds, along with the microbial innoculum, at the start of the experiment. This experimental approach could allow the inhibitors to act by preventing biofilm formation, and it has been widely observed that biofilms are far more difficult to treat than planktonic cells. Indeed, it has been stated that once biofilms are established they cant be effectively eradicated with biocides [12, 19]. Accordingly, additional experiments were performed in which the mixed MIC culture was used to establish biofilms for about two weeks on mild steel coupons. These mature biofilms were then challenged with various microbial inhibitor compounds (each at 0.1%) and the amount of metal corrosion occurring during a further 4-week incubation at room temperature was determined. The results shown in Table 3 and Figure 1 demonstrate that all seven of the microbial inhibitor compounds reduced corrosion as compared with the untreated control; however, none of the seven treatments yielded results significantly different from each other. The majority of the corrosion that occurred took place in the 2-week incubation during which time the biofilm was established (see Table 3, To weight loss data). At the concentration of the chemicals tested here (0.1%) it was not possible to eradicate the established biofilms, but it was possible to minimize subsequent corrosion.

Table 3. The ability of various compounds to inhibit corrosion caused by established biofilms. BES = 2-bromoethane sulfinic acid, ACN = ammonium cerium nitrate, Pep1 = hexane extract of Chile de Arbol pepper, Pep3 = hexane extract of Habenero chile pepper.

Sample I.D	Final Conc. (%)	A, Weight Loss (g)	K, constant	T, Exp time (hrs)	A, Surface Area (cm^2)	D, Density (g/cm^3)	Corrosion Rate (mils/yr)	Standard Deviation
T_0		0.01887	3450000	312	2.63648	7.87	10.054	2.330
Glutaraldehyde (9 day)	0.1	0.01980	3450000	504	2.63648	7.87	6.532	0.198
BES (9 day)	0.1	0.01930	3450000	504	2.63648	7.87	6.367	0.493
Anthraquinone (9 day)	0.1	0.01963	3450000	504	2.63648	7.87	6.477	0.505
ACN (9 day)	0.1	0.02203	3450000	504	2.63648	7.87	7.269	1.060
Pep1 (9 day)	0.1	0.02070	3450000	504	2.63648	7.87	6.829	0.249
Pep3 (9 day)	0.1	0.01947	3450000	504	2.63648	7.87	6.422	0.076
Hexane (9 day)	0.1	0.01963	3450000	504	2.63648	7.87	6.477	0.481
No inhibitor (9 day)		0.02100	3450000	504	2.63648	7.87	6.928	0.405
Glutaraldehyde (4 week)	0.1	0.01970	3450000	984	2.63648	7.87	3.329	0.000
BES (4 week)	0.1	0.02085	3450000	984	2.63648	7.87	3.523	0.227
Anthraquinone (4 week)	0.1	0.02030	3450000	984	2.63648	7.87	3.430	0.382
ACN (4 week)	0.1	0.02130	3450000	984	2.63648	7.87	3.599	0.072
Pep1 (4 week)	0.1	0.02045	3450000	984	2.63648	7.87	3.456	0.299
Pep3 (4 week)	0.1	0.02110	3450000	984	2.63648	7.87	3.565	0.311
Hexane (4 week)	0.1	0.02193	3450000	984	2.63648	7.87	3.706	0.085
No inhibitor (4 week)		0.02555	3450000	984	2.63648	7.87	4.317	0.203

Figure 1. Ability of Various Compounds to Inhibit MIC in Established Biofilms: 4 Weeks



CONCLUSIONS AND FUTURE EXPERIMENTS

Quantifying various types of bacteria that may be present in gas pipeline samples is a difficult challenge. The results reported here indicate that it is easier to reduce metal corrosion by addition of inhibitors prior to the formation of biofilms, but even after biofilms have become established inhibitors can minimize metal corrosion. Additional tests will be required to more fully document the ability of various compounds to selectively inhibit various types of microbial groups/pathways and to determine the contribution of various microbial groups to metal corrosion. However, one interpretation of the data presented here is that biofilms are comprised of a complex mixture of microbial species and the disruption of any of the major microbial groups in the biofilm environment affects the overall health/activity of the biofilm and results in a decreased corrosion rate. Thus it may be possible to achieve control of MIC by modifying microbial metabolism with environmentally benign compounds rather than by seeking to eradicate microorganisms with toxic biocides. This will be further evaluated in future tests.

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