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

FINAL 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:	Various chemicals that inhibit the growth and/or the metabolism of corrosion-associated microbes such as sulfate reducing bacteria, denitrifying bacteria, and methanogenic bacteria were evaluated to determine their ability to inhibit corrosion in experiments utilizing pure and mixed bacterial cultures, and planktonic cultures as well as mature biofilms.
Results:	Planktonic cultures are easier to inhibit than mature biofilms but several compounds were shown to be effective in decresing the amount of metal corrosion. Of the compounds tested hexane extracts of *Capsicum* pepper plants and molybdate were the most effective inhibitors of sulfate reducing bacteria, bismuth nitrate was the most effective inhibitor of nitrate reducing bacteria, and 4-((pyridine-2-yl)methylamino)benzoic acid (PMBA) was the most effective inhibitor of methanogenic bacteria. All of these compounds were demonstrated to minimize corrosion due to MIC, at least in some circumstances.
Conclusions:	The results obtained in this project are consistent with the hypothesis that 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. This approach of controlling MIC by controlling the metabolism of biofilms is more environmentally benign than the current approach involving the use of potent biocides, and warrants further investigation.

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EXECUTIVE SUMMARY

The objective of this project was to develop/identify environmentally benign products that could prevent and/or control microbiologically influenced corrosion (MIC) in the interior of metal pipelines in the gas and oil industry.

A key hypothesis of the project was that eradicating all microorganisms using potent biocides was not necessary to prevent/minimize MIC. Instead, the inhibition of key metabolic functions of one or more type of corrosion-associated microorganism may suffice to prevent/minimize MIC.

A variety of compounds that may selectively inhibit corrosion associated microorganisms were investigated in this project including extracts of pepper plants, nitrate, nitrate analogues, compounds that selectively inhibit sulfate reducing bacteria, and compounds that selectively inhibit methanogenic bacteria. Sulfate reducing bacteria (SRB) have traditionally been thought to be the key causative agents of MIC; however, recent research regarding the microbial ecology of corrosion-associated microorganisms indicates that denitrifying bacteria, methanogenic bacteria, and acid producing bacteria may frequently be present in greater abundance in natural gas pipeline environments than SRB. The quantitative contribution of various types of microorganisms to MIC is unknown and an objective of this project was to gain an increased understanding of the contributions of various types of microorganisms to MIC so that more effective and more environmentally benign strategies to control MIC could be formulated.

Preliminary research prior to this project indicated that extracts of pepper plants of the genus *Capsicum* possessed biocidal activity and could be the basis of a natural, and therefore more environmentally benign, biocide than those currently in use for the control of MIC. Research in this project confirmed that extracts of *Capsicum* plants do indeed have biocidal properties, but they appear to selectively kill and inhibit sulfate reducing bacteria and have little effect on other corrosion-associated microorganisms such as denitrifying bacteria. Extracts of pepper plants contain a multitude of compounds which vary in type and concentration among various species of pepper plants. The exhaustive chemical characterization of pepper plants and a determination of the biological activities of each of the compounds was beyond the scope of this project. However it was determined that hexane extracts of Chile de Arbol and Serrano peppers yielded the best results as regards killing SRB and preventing metal corrosion using pure and mixed cultures.

The ability of various selective metabolic inhibitors to control MIC was tested using weight loss determinations and electrochemical methods to quantify corrosion. Commercially available equipment to quantify corrosion using electrochemical techniques was obtained from InterCorr Corporation, as these devices are routinely used in the gas and oil industry to monitor corrosion. Our laboratory experiments allowed us to determine both electrochemical parameters and metal weight loss for each condition tested. Some experiments showed good correlations between electrochemical and weight loss data and indicated that compounds that selectively inhibit various types of corrosion-associated microorganisms can minimize corrosion. However, metal corrosion resulting from contact with a mixed microbial community and an aqueous environment is a complex process and consistent results were not obtained in all experiments.

While further testing to confirm these findings is needed, the results obtained in this project support the hypothesis that significantly altering the biochemical activity of complex microbial communities in biofilms by disrupting sulfate reduction, nitrate reduction, or methanogenesis can diminish microbiologically influenced corrosion in the anaerobic environments typically found in gas and oil pipelines. Of the compounds tested, hexane extracts of *Capsicum* plants and molybdate were the most effective inhibitors of sulfate reducing bacteria, bismuth nitrate was the most effective inhibitor of nitrate reducing bacteria, and 4-((pyridine-2-yl)methylamino)benzoic acid (PMBA) was the most effective inhibitor of methanogenic bacteria. All of these compounds were demonstrated to minimize corrosion due to MIC, at least in some circumstances.

Inhibitory compounds of all types were most effective in preventing/minimizing corrosion in tests of clean metal surfaces subsequently exposed to microorganisms. Metal surfaces that were already covered with established biofilms were more difficult to treat, but certain metabolic inhibitor chemicals were demonstrated to diminish the amount of corrosion caused by mature biofilms.

The results obtained in this project support an approach to minimize MIC by altering the composition/metabolism of microbial communities rather than using biocides to kill all microorganisms. Controlling MIC by controlling the metabolism of biofilms will be a more environmentally benign approach than the current use of potent biocides, and this approach to controlling MIC warrants further investigation.

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 corrosion-associated microorganisms for their abilities to control MIC. Specifically, the natural products that were examined in this project were those compounds that can be extracted from the seeds and pods of pepper plants of the genus *Capsicum.* Additionally, other chemicals that may have the ability to selectively inhibit the metabolic processes of particular groups of microorganisms were also 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 (*1-5*). 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 (*6*). 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 (*2-4, 7-12*).

It is known that several types of microorganisms can cause corrosion when these microorganisms are tested as pure cultures: sulfate reducing bacteria (*13-15*), acid producing bacteria (*16*), iron respiring bacteria (*8, 17-19*), denitrifying bacteria (*4, 20-22*), and methanogenic bacteria (*23, 24*). 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 (*25, 26*). Therefore a goal of research in this project 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 that 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 (*27-29*). 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 (*14*). Another compound described in the literature as being capable of selectively inhibiting sulfate reducing bacteria is anthraquinone (*30, 31*) which works by uncoupling ATP synthesis from electron transfer reactions associated with hydrogen-dependent sulfate respiration. Additionally, preliminary 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 (*29*). Another selective inhibitor of methanogenesis is 4-((pyridine-2-yl) methylamino)benzoic acid (PMBA) which inhibits ribofuranosyl-aminobenzene-5'-phosphate synthase, disrupting the first step in the synthesis of methanopterin and thereby inhibiting

methanogenesis (*32*). Thus, BES and PMBA can selectively inhibit methanogenic bacteria, but can inhibit these bacteria without killing them (*29*).

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.

This project then examined a variety of chemicals that may selectively inhibit various types of corrosion-associated microorganisms and subsequently reduce corrosion. These investigations were designed to gain an increased understanding of the contributions of various types of microorganisms to corrosion and to identify environmentally benign compounds that are useful in controlling microbiologically influenced corrosion.

MATERIALS AND METHODS

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 (*4*).

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

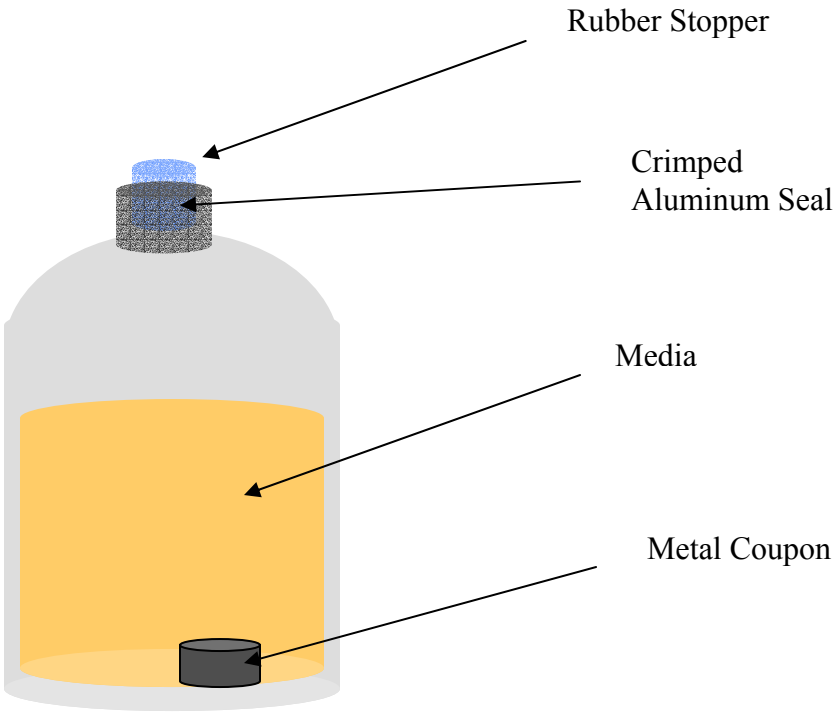


FIGURE 1. DESIGN OF THE BATCH TESTS

Origin of MIC consortium. Field samples were received from a gas company in Colorado who was experiencing microbiologically influenced corrosion. The samples, (CO-WW and CO-RD), were 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. This mixed culture was maintained in the lab by subculturing into fresh general corrosion media every 3-to-4 weeks.

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) (*33*), 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 (*34*).

Analytical Methods. Total protein was measured by the Lowry method (*35*); sulfide was measured using the methylene blue method (*36*); iron (II) was measured by the ferrozine method (*37*); and nitrite was measured using the method of Montgomery and Dymock (*38*).

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 individually 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)

Mature Biofilm Experiments. Metal coupons containing mature biofilm were prepared by exposure to a mixed community of corrosion-associated microorganisms for a period of one week 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-to-12 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.

Pepper Plant Extracts. Three varieties of *Capsicum sp*. commonly known as Chile de Arbol, Serrano, and Habanero were used to obtain plant extracts. The Soxhlet extraction method was used with hexane, methylene chloride, aqueous acid (pH 2.0), and aqueous alkali (pH 12.0). Fresh Serrano and Habanero peppers were ground in a spice grinder, dried at 70°C overnight, and ground again to form a powder. Chile de Arbol were purchased dried were powdered using a spice grinder. Each type of pepper was extracted with each solvent producing a total of 12 separate pepper extracts. Forty grams of pepper powder were extracted with 350 mL of solvent

conducted in four replicates. The extraction process was completed in 18 hours and the replicates were combined together. The extracts were concentrated using a Buchii Rotavapor (Model R-205), and excess solvent was evaporated using TurboVap LV Evaporator.

HPLC Analysis. The extracts were then analyzed by HPLC equipped with a photodiode detector. A Supelcosil LC-18 (25cm x 4.6mm; five-micron particle size; Supelco) analytical column was used at 50^{o}C temperature. Peaks were obtained between 28 minutes and 51 minutes. The eluent was composed of: Solvent A .01 M H3PO4 (80%), Solvent B Methanol (20%), at a flow rate of 1.0ml/min, holding for two minutes and ramping gradually by 51 minutes to obtain 0% solvent A and 100% solvent B and holding for four minutes. A wavelength of 285 nm was used to detect the capsaicin and dihydrocapsaicin peaks.

Growth Inhibition (Antimicrobial) Screening. The microdilution assay method (*39*) was used to determine the growth inhibition of the pepper components. They were subjected to antimicrobial screening against two sulfate reducing bacteria, *D. vulgaris* and *D. desulfuricans*. The test compounds were serially diluted 50% with sterile distilled water in 96-well microtiter plates. For the inoculum, an overnight bacterial culture was centrifuged at 4,000 rpm for five minutes, washed with a 50mM phosphate buffered saline, pH 6.8, and the optical density was adjusted at 600 nm to obtain a final cell concentration of 10^{6}cfu/mL per well. ATCC # 1249 Modified Baar's Medium was used as a source of cell nutrients. The multi-well plates were then placed inside a modular anaerobic chamber, purged with nitrogen gas, and incubated at 30^{o}C for *D. vulgaris* and 37^{o}C for *D. desulfuricans*. Optical density at 600 nm was measured using the MRX II (Dynex Technologies) plate reader from 0 up to 168 hours. Growth inhibition was defined as the test compound limiting turbidity to <0.05 absorbance at 600nm (*40*). The initial12 crude extracts were also tested against the denitrifier *Comamonas denitrificans* using this procedure.

High-Precision Liquid Chromatography for Fractionation of Pepper Extracts. Pepper extracts were analyzed with High Performance Liquid Chromatography (HPLC) with a Photo Diode Array lamp (PDA) at 285nm. Extracts were analyzed following a 10% dilution; this was done because the initial extract was too thick to inject. Using the Supleco Supelcosil LC-18 column (25cm x 4.5mm x 5um) the analysis of the extracts was done with a flow rate of 1.0

ml/min and an initial solvent ratio of 80% A (0.01M Phosphoric Acid) and 20% B (HPLC grade Methanol). The flow rate remained the same but the solvent ratio changed with time. The ratio changed gradually to equal 100% B by 57min of run time and then slowly went back to the original ratio by 66min. For each extract myristic, stearic, oleic, palmetic, caffeic, cinnamic, and coumaric acids were qualitatively analyzed along with capsaicin and dihydrocapsaicin. These final two compounds act as internal standards and concentration marker for comparative analyses. Gas chromatography - mass spectrometry (GC/MS) was used to determine what compounds were present in pepper plant extracts and fractions.

Biofilm Formation. The crude extracts were tested against the two sulfate-reducing bacteria for their ability to inhibit biofilm formation. The methods used to look at the effects on biofilm formation and its measurements are discussed in detail by (*41*), (*42*) and (*43*). The test compounds were incubated with bacterial cells at 10^8 cfu/mL (final concentration) and allowed to form biofilm up to 120 hrs. ATCC # 1249 Modified Baar's Medium was used as a source of cell nutrients. The biofilm assay was done by staining formed/residual biofilm with 1% crystal violet for 25 minutes, washing the wells with sterile distilled water and dissolving the cell-associated dye by adding 200 μL of dimethyl sulfoxide (DMSO) per well (*44*). Quantification of the formed biofilm was done by optical density measurement at 600 nm using the MRX II (Dynex Technologies) plate reader and comparing the results with the untreated biofilm.

Continuous Flow Cell System. Biofilm formation of *D. vulgaris* was studied using the continuous flow cell system (Stovall Life Science, Inc., Greensboro, NC). The sterile system consisted mainly of a 3-channel flow cell with individual channel dimensions of 1 mm D x 4 mm W x 40 mm L and the attached microscope coverslip #1 (0.13-0.16 mm thick) that served as the substratum for biofilm growth. The TEST and CONTROL biofilms were grown in separate channels at 37°C supplied with Modified Baar's Medium that was prepared under 97% N_2 and 3% H_2. Inocula used were from overnight cultures that were diluted to obtain a final cell concentration of 10^8 cfu/mL as measured at OD_{600}. The medium was placed in a water bath and pumped into the system using the IsmaTec low flow peristaltic pump at a flow rate of 3mL/hr. The bubble trap near the influent side prevented the air bubbles from reaching the flow cell. After equilibrating the system with the culture media, the flow was stopped and the inoculum

(0.5mL) was injected at the inlet of each channel. The flow cell was turned upside down to allow the cells to establish on the glass surface. After 1 hr, it was inverted to the right-side up position and medium flow was re-started.

Biofilm development for the TEST channel was monitored periodically for 7 days using light microscopy (Carl Zeiss Axioskop 2 *plus*, Germany) equipped with time lapse program from Axiovision (Carl Zeiss, Inc.) as the software for image analysis. The Time Lapse software enables the camera to take photos at desired time intervals automatically for the whole duration of the experiment. For the CONTROL channel, photographs were taken manually at the beginning and the end of the run.

To check for cell viability, Live/Dead *Bac*Light (Molecular Probes, Eugene, OR) was used. The biofilms were stained by injecting the dyes near the inlet of the flow cell. Prior to injection, the cells were rinsed with distilled water and both ends of the flow cells were clamped. With epifluorescent microscopy, dead cells appear fluorescent red while live ones are fluorescent green.

RESULTS AND DISCUSSION

Characterization of Extracts of Pepper Plants

Preliminary experiments performed prior to this project suggested that pepper plants may contain biocidal compounds and may be useful in controlling MIC. Accordingly, the ability of extracts of pepper plants to inhibit MIC was further investigated in this project. A total of 12 extracts from three varieties of *Capsicum sp*. (such as Chile de Arbol, Serrano, and Habanero) were obtained by Soxhlet extraction using hexane, methylene chloride, aqueous acid, and aqueous alkali.

Figure 2 shows the photos of the peppers used while Figure 3 shows the extracts from them.

Chile de Arbol Habanero Serrano

FIGURE 2. CAPSICUM SP. VARIETIES USED IN SOXHLET EXTRACTIONS

Extracts of pepper plants contain a multitude of compounds which vary in type and concentration among various species of pepper plants. The exhaustive chemical characterization of pepper plants and a determination of the biological activities of each of the compounds was beyond the scope of this project. However an example of the fractionated extract by HPLC is presented in Figure 4. Table 1 lists some of the compounds identified by GC-MS in pepper extracts, while Table 2 lists the amounts of capsaicin and dihydrocapsaicin in various pepper extracts.

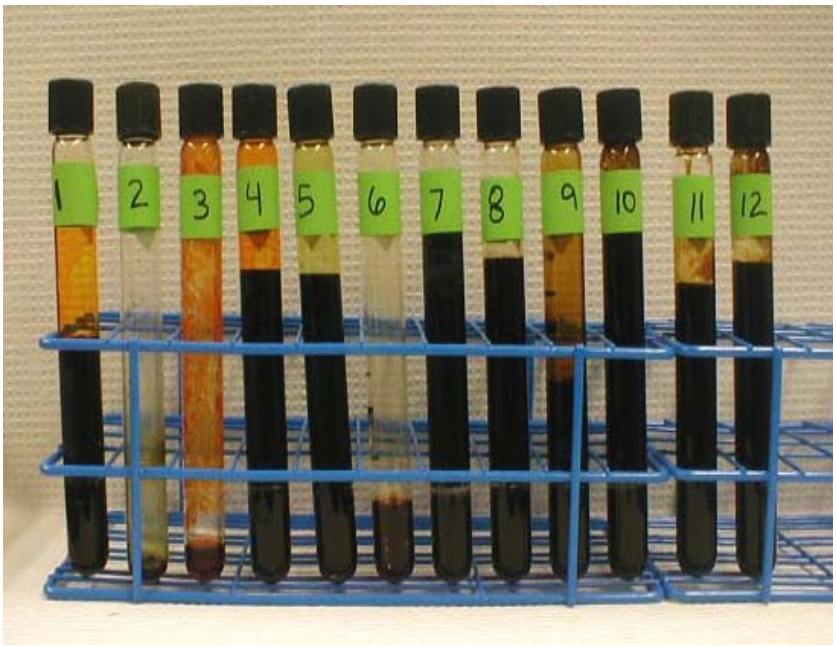


FIGURE 3. EXTRACTS OBTAINED USING VARIOUS SOLVENT SYSTEMS

Soxhlet Extraction of Fresh/Dry *Capsicum sp*.

1. Chile de Arbol with Hexane
2. Serrano with Hexane
3. Habanero with Hexane
4. Chile de Arbol with Methylene Chloride
5. Serrano with Methylene Chloride
6. Habanero with Methylene Chloride
7. Chile de Arbol with Aqueous Acid
8. Serrano with Aqueous Acid
9. Habanero with Aqueous Acid
10. Chile de Arbol with Aqueous Alkali
11. Serrano with Aqueous Alkali
12. Habanero with Aqueous Alkali

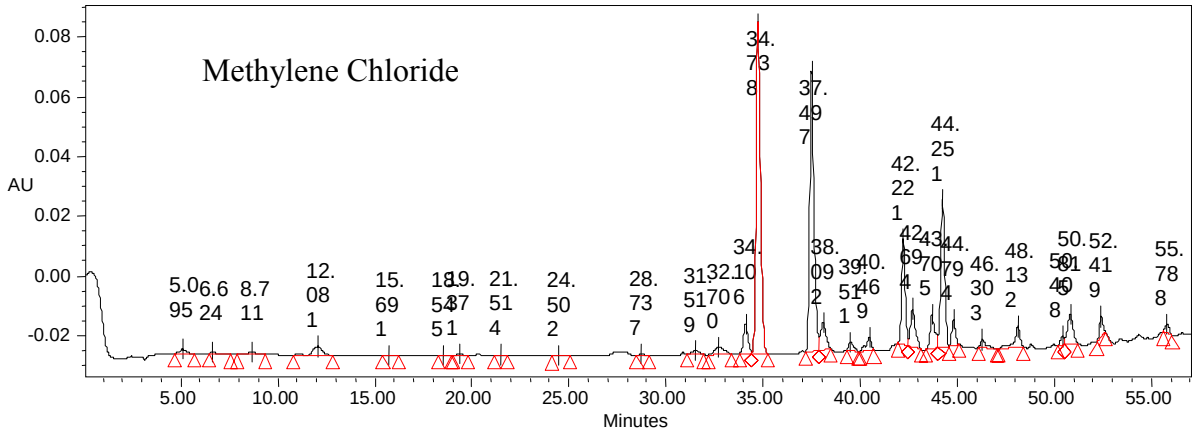


FIGURE 4. EXTRACTION AND HPLC OF HABANERO PEPPERS EXTRACTED WITH METHYLENE CHLORIDE.

TABLE 1. COMPOUNDS DETECTED IN THE HABANERO FRACTIONS USING GC-MS

Name	Formula	Molecular weight	Cas #
1,(2-aminophenyl)-3-methoxy-1-Propanone	$C_{10}H_{13}NO_2$	179	NA
N-butyl-Benzenesulfonamide	$C_{10}H_{15}NO_2S$	213	3622-84-2
N-oxide N-[(4-methylphenyl)methylene]-Benzenamine	$C_{14}H_{13}NO$	211	19865-55-5
Nonivamide	$C_{17}H_{27}NO_3$	293	2444-46-4
Natural Capsaicin	$C_{18}H_{27}NO_3$	305	404-86-4
Hexyl Vanillate	$C_{14}H_{20}O_4$	252	84375-71-3
bis(dihexylamide) Pentanediolic Acid	$C_{29}H_{58}N_2O_2$	466	NA

TABLE 2. AMOUNTS OF CAPSAICIN AND DIHYDROCAPSAICIN IN PEPPER PLANT EXTRACTS

EXTRACT TYPE	CAPSAICIN MG/L	DIHYDROCAPSAICIN MG/L
Arbol in Hexane	**1071**	**525**
Serrano in Hexane	**642**	**288**
Habenero in Hexane	**1215**	**691**
Arbol in Methylene Chloride	**1536**	**432**
Serrano in Methylene Chloride	**1613**	**678**
Habenero in Methylene Chloride	**5360**	**3418**
Arbol in DMSO/Methanol	**322**	**103**
Habenero in DMSO/Methanol	**328**	**103**

Ability of Pepper extracts to Inhibit MIC

The ability of pepper extracts to inhibit corrosion was investigated in experiments employing the sulfate reducing bacteria *Desulfovibrio vulgaris* grown in general corrosion medium (GCM) in the presence of of metal coupons and in the presence or absence of pepper extracts. The results of initial tests are shown in Figure 5. Corrosion caused by *D. vulgaris* was inhibited by the presence of the pepper extracts. The corrosion rates for the pepper extract-treated batch tests was similar to the sterile media control (rate = 1.5 mils yr-1); therefore, the corrosion observed in the batch tests was due to the media itself and not the action of the bacteria. The corrosions rates of *D. vulgaris* batch tests were almost 3-fold lower in tests treated with the respective extracts than tests in which no extract was added (Figure 5). There was no significant difference between the 3 extracts with regards to inhibiting corrosion; the average

corrosion rate for each of the three extracts ranged between 1.2 - 1.7 mils yr^{-1}. The average corrosion rate for the non-treated controls (*D. vulgaris* without pepper extract) was 3.6 mils yr^{-1}.

The mode of inhibition is most likely toxicity of the pepper extracts towards *D. vulgaris*. As the "cells/ml, initial" data in Table 2 show, approximately 95% of the inoculated bacteria are killed by extract 1 at 0.01% (20,378 cells/ml vs. 436,000 in the no-extract control); the efficiency of killing is even higher (>99.99%) in all other extracts, and in higher concentrations of extract #1. **This result is significant because it indicates that with a very brief contact time of less than one minute the pepper extracts tested here are capable of killing planktonic *D. vulgaris***. At the conclusion of the experiment, all of the extract-treated cultures are below the detection limit for *D. vulgaris*; assuming a detection limit of 1 cell/ml, the total population in each of these cultures must be less than 100 viable cells (vs. over 100,000 in the controls). There was little sulfide produced indicating that metabolic activity of *D. vulgaris* was inhibited in these conditions as well. The protein data and the sulfide data are consistent with the MPN data indicating that the *D. vulgaris* cells were killed by exposure to the pepper extracts so that no corrosion or production of metabolites was observed that differed significantly from sterile controls. Dissolved iron (Fe^{2+}) increased from initial values in most tests, which could suggest that that the organisms might be able to survive by utilizing iron as an electron acceptor; however, the fact that this value also increases substantially in the non-inoculated control (presumably due to the "baseline" corrosion/deterioration of the coupon) makes this difficult to substantiate.

TABLE 3. CHEMICAL CONCENTRATIONS AND MPN COUNTS OF *DESULFOVIBRIO VULGARIS* CULTURES TREATED WITH SOLUTIONS OF PEPPER EXTRACTS FROM 3 SEPARATE PEPPER PLANT SPECIES.

The final concentrations of the extracts were 0.01, 0.05, and 0.1%. Control cultures with bacteria but lacking pepper extracts (designated as CON Inoc) and sterile media controls that contain no bacteria or pepper extract (designated as CON N-I) were also conducted.

Pepper Extracts	Extract Conc. (Final)	pH Initial	pH, Final	Protein (mg/ml), Initial	Protein (mg/ml), Final	Nitrite (mM), Initial	Nitrite (mM), Final	Sulfide (mM), Initial	Sulfide (mM), Final	Iron (mM), Initial	Iron (mM), Final	Cells/ml, Initial	Cells/ml, Final
Extract 1	0.01%	7.0	7.0	0.0090	0.0989	0.0000	0.0000	0.0000	0.1	0.0055	0.0214	20,378	0
Extract 1	0.05%	7.0	7.0	0.0134	0.1046	0.0000	0.0000	0.0000	0.1	0.0064	0.0231	334	0
Extract 1	0.10%	7.0	7.0	0.0057	0.1146	0.0000	0.0000	0.0000	0.1	0.0067	0.0274	43	0
Extract 2	0.01%	7.0	7.0	0.4778	0.5712	0.0000	0.0000	0.0000	0.0000	0.0079	0.0207	43	0
Extract 2	0.05%	7.0	7.0	0.4977	0.4464	0.0000	0.0000	0.0000	0.0000	0.0096	0.0134	0	0
Extract 2	0.10%	7.0	7.0	0.5068	0.4352	0.0000	0.0000	0.0000	0.0000	0.0011	0.0146	0	0
Extract 3	0.01%	7.0	7.0	0.4405	0.4864	0.0000	0.0000	0.0000	0.0000	0.0086	0.0085	9	0
Extract 3	0.05%	7.0	7.0	0.6714	0.5491	0.0000	0.0000	0.1	0.0000	0.0151	0.0080	0	0
Extract 3	0.10%	7.0	7.0	0.6995	0.5370	0.0000	0.0000	0.2	0.0000	0.0189	0.0082	1	0
CON (Inoc)	xx	7.0	7.5	0.3873	2.3908	0.0000	0.0000	0.0000	1.4	0.0006	0.0331	436,275	>100,000
CON (N-I)	xx	7.0	7.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0144	0	0

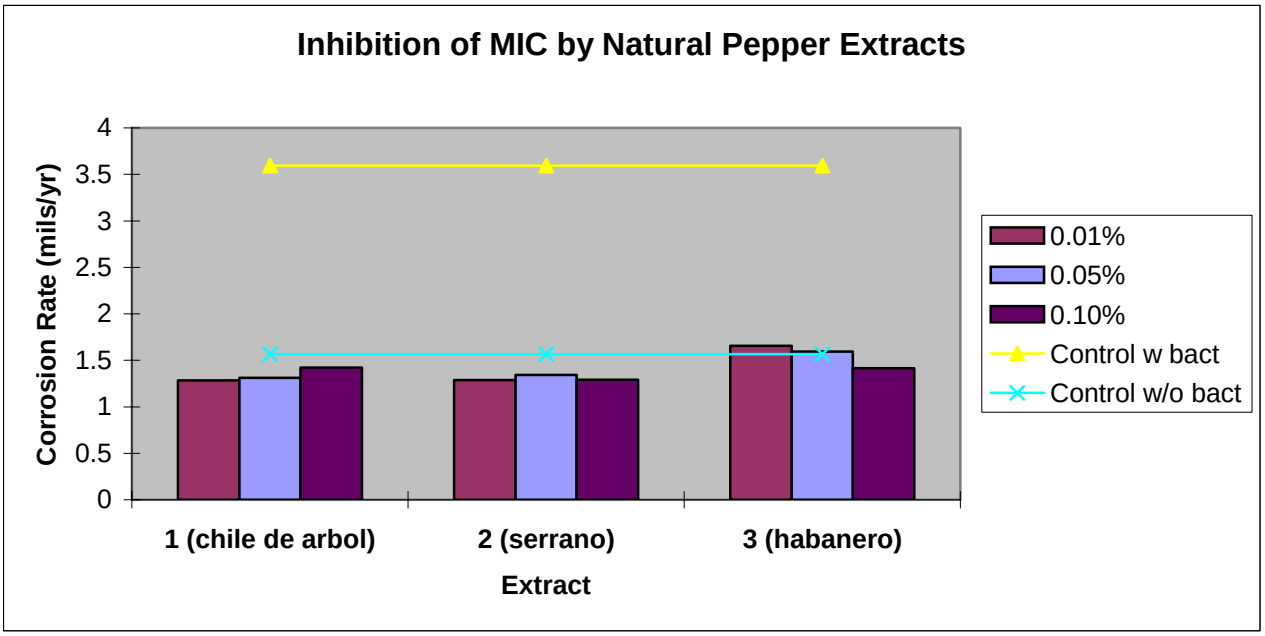


FIGURE 5. CORROSION RATES OF *DESULFOVIBRIO VULGARIS* CULTURES TREATED WITH SOLUTIONS OF PEPPER.

Extracts from 3 separate pepper plant species. The final concentrations of the extracts were 0.01, 0.05, and 0.1%. Control cultures (with bacteria but lacking pepper extracts) and sterile media controls (no bacteria, no extract) were also conducted.

Corrosion rates by *C. denitrificans* were not inhibited by the presence of the pepper extracts. In most cases, corrosion rates were actually higher than rates observed in non-treated control cultures and sterile media controls (Figure 6). The average corrosion rate for the *C. denitrificans* controls that did not receive pepper extract was 3.6 mils yr^{-1}, whereas the rates for the extract-treated tests ranged from 2.4 - 9.3 mil yr^{-1}. The organic acids in the extracts may have been utilized as carbon sources or electron donor by *C. denitrificans* cells; additional tests would need to be conducted to determine whether this was actually the case. The presence of the pepper extracts may, however, inhibit nitrate reduction by *C. denitrificans* (Table 4). Concentrations of nitrite, the first metabolite of the denitrification pathway, increased as much as 10-fold in the batch tests after 2 weeks (Table 4) when pepper extract was present, but no nitrite accumulation was observed in the absence of pepper extract. At 4- and 6-weeks, the nitrite levels had returned to initial concentration values indicating that the cells were using nitrite as an electron acceptor (data not shown). Protein levels also increased, as did culture density in most samples, but not to

comparable levels. Not surprisingly, sulfide levels were below detection; however, iron (II) concentration did increase, suggesting that iron may have served as an alternate electron acceptor for the organisms (Table 4). The pH of the cultures decreased from 7, at the onset of the incubation, to approximately 6. The slight acidity may be one reason for the increased corrosion rates in these cultures.

TABLE 4. CHEMICAL CONCENTRATIONS OF *COMOMONAS DENITRIFICANS* CULTURES TREATED WITH SOLUTIONS OF PEPPER EXTRACTS FROM 3 SEPARATE PEPPER PLANT SPECIES.

The final concentrations of the extracts were 0.01, 0.05, and 0.1%. Control cultures with bacteria but lacking pepper extracts (designated as CON Inoc) and sterile media controls that contain no bacteria or pepper extract (designated as CON N-I) were also conducted.

Pepper Extract	Extract Conc. (Final)	pH Initial	pH, Final	Protein (mg/ml), Initial	Protein (mg/ml), Final	Nitrite (mM), Initial	Nitrite (mM), 2 weeks	Sulfide (mM), Initial	Sulfide (mM), Final	Iron (mM), Initial	Iron (mM), Final	cfu/ml, Initial	cfu/ml, Final
Extract 1	0.01%	6.5	7.5	0.2217	0.3420	0	0	0	0.0000	0.0080	0.0126	0	0
Extract 1	0.05%	6.5	7.0	0.2817	0.3827	0	8	0	0.0000	0.0055	0.0328	0	211667
Extract 1	0.10%	6.5	6.0	0.2647	1.7640	0.1	2.7	0.0001	0.0000	0.0065	0.0787	0	1195000
Extract 2	0.01%	6.5	6.3	0.3765	0.3505	0.0004	4	0.0001	0	0.0125	0.0762	35100	475000
Extract 2	0.05%	6.5	6.2	0.3756	0.3978	0.0005	2.4	0.0001	0	0.0128	0.0771	43167	3431333
Extract 2	0.10%	6.5	6.1	0.3841	0.3774	0.0003	1.5	0.0001	0	0.0055	0.0796	115	406500
Extract 3	0.01%	6.5	6.0	0.3537	0.5687	0	0.3	0.0001	0.0002	0.0103	0.1347	26033	935000
Extract 3	0.05%	6.5	6.2	0.3662	0.6211	0.1	0.3	0.0001	0.0002	0.0122	0.1266	5763	628333
Extract 3	0.10%	6.5	6.1	0.5022	0.7868	0	0.5	0.0002	0.0003	0.0171	0.1199	63	913333
Con (Inoc)	xx	7.0	6.0	0.0467	0.4037	0	0.1	0.0003	0	0.0059	0.0723	4227	8833333
Con (N-I)	xx	7.0	7.0	0.1125	0	0	0	0	0	0.0000	0.0144	0	0

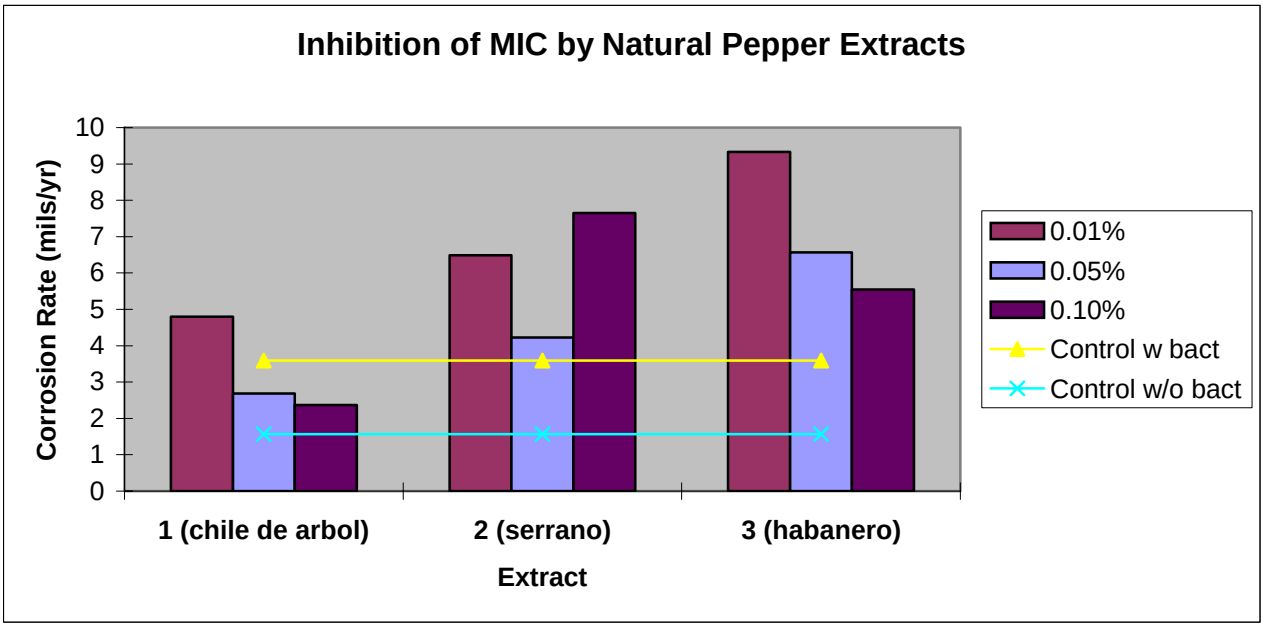


FIGURE 6. CORROSION RATES OF *COMOMONAS DENITRIFICANS* CULTURES TREATED WITH STOCK SOLUTIONS OF 1, 5, AND 10% PEPPER EXTRACTS FROM 3 SEPARATE PEPPER PLANT SPECIES.

The final concentrations of the extracts were 0.01, 0.05, and 0.1%. Control cultures (with bacteria but lacking pepper extracts) and sterile media controls (no bacteria) were also conducted.

To further investigate the ability of pepper extracts to inhibit MIC experiments were performed with mixed cultures of corrosion associated microorganisms derived from natural gas pipelines. Figure 7 shows the corrosion rates for the Mixed Culture CO-WW081503 with 0.01, 0.05, and 0.10% (final concentration) of the 3 pepper extracts. The corrosion rates in cultures treated with Chile de Arbol or Serrano extracts were not significantly different than the sterile media control whereas the corrosion rates in cultures treated with Habanero extracts were higher. Unexpectedly, the corrosion rates in untreated cultures (bacteria-only controls, with no pepper extract) were significantly lower than the treated samples or in the sterile media. The reasons for this observation are not clear, as previous tests in our lab using pure laboratory strains resulted in relatively high corrosion rates. One possibility is that the biofilm formed in these samples had a protective effect (lacking in the sterile control) but the pepper extracts affect biofilm composition/metabolism such that this protective effect can become lost.

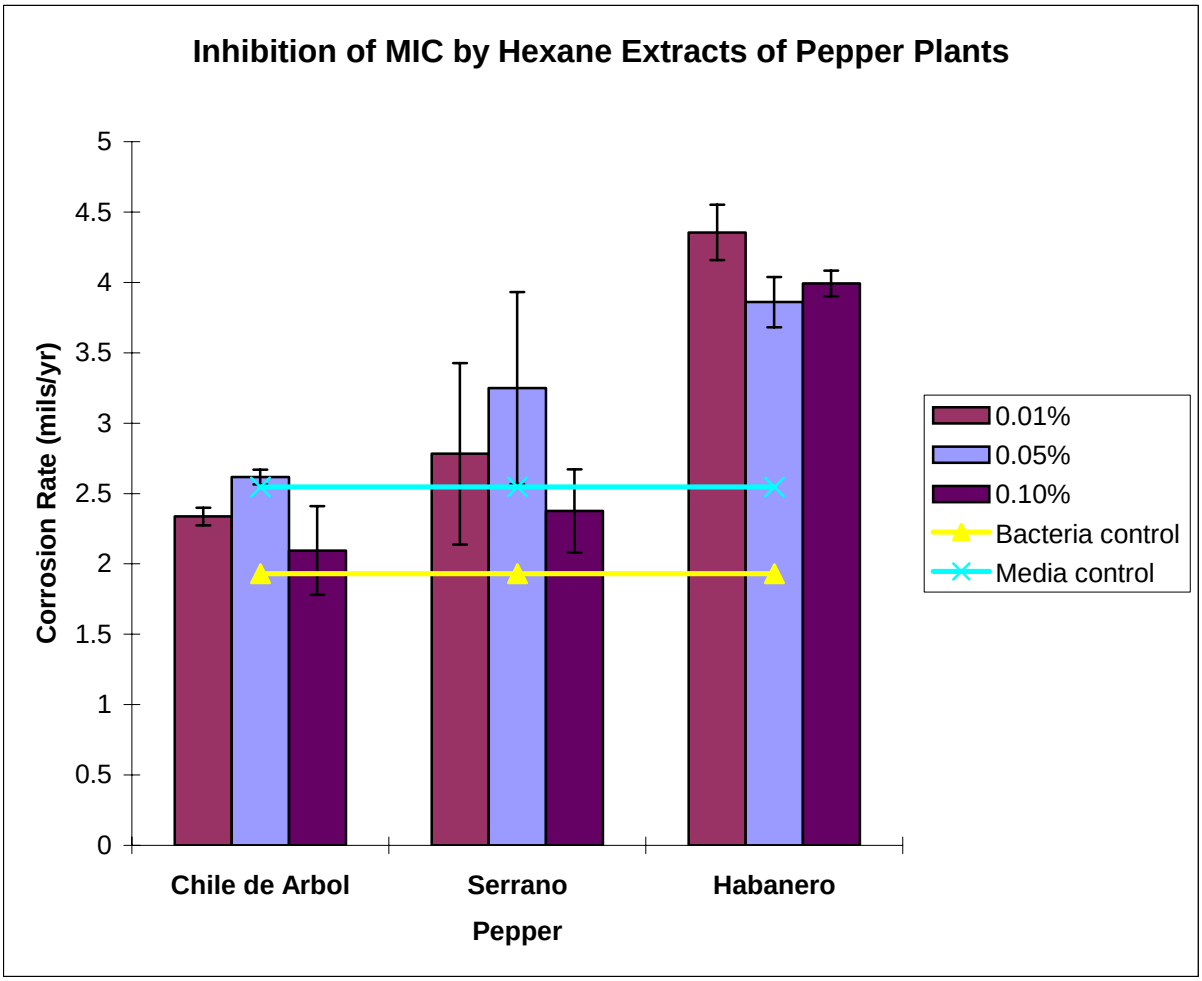


FIGURE 7. CORROSION RATES OF CO-WW CULTURES TREATED WITH SOLUTIONS OF PEPPER EXTRACTS FROM 3 SEPARATE PEPPER PLANT SPECIES.

The final concentrations of the extracts were 0.01, 0.05, and 0.1%. Control cultures (with bacteria but lacking pepper extracts) and sterile media controls (no bacteria, no extract) were also conducted.

Treatment with the pepper extracts affected the growth and metabolic activity of the CO-WW081503 microbial consortia. Standard plates counts from samples taken from the untreated cultures (bacteria controls) estimated the culture density to be approximately 1 x 10^4 cfu ml^{-1} at the onset of the incubation period. The culture density increased to 1.2 x 10^8 cfu ml^{-1} at 2 weeks in the absence of pepper extract. In contrast, cell densities were two to three orders of magnitude lower- approximately 1 x 10^5 cfu ml^{-1}, 1 x 10^6 cfu ml^{-1}, and 1 x 10^6 cfu ml^{-1}- in cultures treated

with Chile de Arbol, Serrano, and Habañero extracts, respectively, after 2 weeks. There was no growth detected in sterile media controls.

The pH of all samples declined somewhat during the six-week incubation. This finding suggested the growth of acid-producing bacteria, and indeed, MPN analyses of the batch test revealed their presence. Densities of acid producing bacteria reached a maximum of 1 x 10^8 cells ml^{-1} in untreated control cultures, but were approximately 10 -100X lower in treated samples (data not shown). MPN analyses also detected the presence of SRB, but only in significant densities in untreated cultures (max. 6.0 x 10^5 cells ml^{-1}) and samples treated with Habañero extracts (max. 1.1 x 10^5 cell ml^{-1}). These results are consistent with the hypothesis that compounds present in extracts of Chile de Arbol and Serrano peppers are capable of inhibiting SRB, but extracts of Habanero are less effective than the other two pepper extracts (Figure 7). The absence of SRB in cultures containing Chile de Arbol or Serrano is consistent with these results.

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 5, where the results of microbial growth tests with a mixed inoculum derived from a gas pipeline sample are shown. The compounds listed in Table 5 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 5. 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 5 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. Likewise, the results in Table 5 indicate that ammonium cerium nitrate and bismuth nitrate are potentially useful as selective inhibitors of denitrifying bacteria.

Additional tests were performed to determine the ability of ammonium cerium nitrate and bismuth nitrate to inhibit denitrifying bacteria. Denitrifiers have been identified, using molecular biological methods developed at GTI, as major components of the bacterial biofilms in a number of corroded pipeline samples (*26*), and other researchers have linked active denitrifying bacteria to corrosion (*4, 20, 21*). A pure culture of the denitrifying bacteria *Comomonas denitrificans* was

used in resting cell and growth experiments. The denitrifying medium described in the materials and methods section was used as is or with equivalent concentrations of ammonium cerium nitrate or bismuth nitrate substituted for potassium nitrate. All experiments were performed using anaerobic serum bottles and a helium headspace. Denitrification was monitored by analyzing the headspace gas to quantify the concentration of nitrogen formed as a result of the metabolism of nitrate/nitrate analogues by *Comomonas denitrificans.* Resting cell experiments were performed by first growing *Comomonas denitrificans* in regular denitrifying media and harvesting cells in the mid-log phase growth. Concentrated cell suspensions were obtained by centrifugation and used at about 10^9 cells/ml in overnight incubations at 30^{o}C in regular or modified denitrifying media. Growth tests were performed by inoculating regular or modified denitrifying medium with about 10^5 cells/ml of *Comomonas denitrificans* and incubating at 30^{o}C for 96 hours. The composition of the headspace gas for these tests are summarized in Table 6 which clearly illustrates that ACN does not effectively inhibit denitrification in the presence of growing cells, while bismuth nitrate does. Both salts considerably decreased denitrification by resting cells. The observation that resting cells of *Comomonas denitrificans* can produce 18.4% nitrogen in the presence of bismuth nitrate while no growth on bismuth nitrate was observed may indicate that this compound is not effectively metabolized by *Comomonas denitrificans* but that inactivation of denitrifying enzymes is not instantaneous. Apparently ACN does inhibit denitrification but does not eliminate denitrification. Growth of *Comomonas denitrificans* with ACN was somewhat slower than with regular denitrifying medium but eventually robust growth occurred and was nearly equivalent to the cell density achieved by this culture in regular denitrifying medium.

TABLE 6. HEADSPACE GAS COMPOSITION OF *COMOMONAS DENITRIFICANS* IN RESTING CELL AND GROWTH EXPERIMENTS EVALUATING POTENTIAL INHIBITORS OF DENITRIFICATION.

Gas Component Mole %	**Sterile Reg. Denit**	**Resting cells Reg. Denit**	**Resting cells ACN**	**Resting cells $BiNO_3$**	**Growth Reg. Denit**	**Growth ACN**	**Growth $BiNO_3$**
Nitrogen	2.03	85.8	27.8	18.4	82.1	71.3	0.8
CO_2	0.05	7.1	5.8	5.9	11.3	14.2	0.03
Helium	97.92	7.1	66.4	75.7	6.6	14.5	99.17

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 7. 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. These concentrations were based on literature values suggesting effective concentrations for these various compounds. The results shown in Table 7 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 7 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 7. THE EFFECT OF BIOCIDES AND INHIBITORS ON CORROSION RATES OF METAL COUPONS IN 44 DAYS.

Glu = glutaraldehyde, SM = sodium molybdate, BES = 2-bromoethane sulfonic 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

Monitoring Corrosion Using Two Methods: Electrochemicallly and by Weight Loss.

The InterCorr International Corporation makes SmartCET™ electrochemical devices to monitor corrosion. Their approach measures three distinct basic parameters (all related to corrosion current density), and derives additional ones from analysis of this data (InterCorr International 2004):

- **Linear Polarization Resistance (LPR)** monitoring involves measurement of the Polarization Resistance of a corroding electrode using small amplitude (~25mV) sinusoidal polarization of the electrodes. The slope of the response at the corrosion potential, the Polarization Resistance, is then inversely proportional to the corrosion current. LPR performs well in most aqueous environments, but cannot provide any useful information on localized/pitting corrosion, and sometimes underestimates low corrosion rates.
- **Electrochemical noise (EN)** refers to the fluctuations in current on the surface of a metal. Electrochemical noise arises due to relatively short-term variations in corrosion current and potential, which occur due to the changes in anodic and cathodic areas. This technique was originally developed to detect non-uniform or localized corrosion, such as pitting, crevice corrosion, and cavitation, but may also be used for general corrosion. General corrosion and passivation both give fairly flat profiles for EN over time; in contrast, pit formation leads to sharp, transient downward spikes in both current and potential, and pit propagation leads

to broader, more chaotic drops in both of these. EN techniques work well in aqueous environments, or when mixed-phase systems (such as hydrocarbons) are present; however, it is poorly suited to very low-conductivity systems, and tends to overestimate low corrosion rates.

- **Harmonic distortion (HM)** is a measure of non-linear current distortion arising during the LPR measurement. The data is used to provide a measure of the corrosion current, and to provide an on-line estimate of the corrosion rate calculation (Stern-Geary) constant, which is used in other corrosion rate calculations. HM is also not suitable for quantitation of pitting corrosion, although it can provide a qualitative "warning" that pitting is occurring.
- **Pitting factor (PF)** is derived from the EN and HD data, and quantitates the risk or likelihood (although not necessarily the extent) of localized attack (pitting) on the metal surface.
- **Skewness** and **Kurtosis** are two statistical parameters, derived from the EN data, which quantify the asymmetry of the fluctuations in the EN measurements. These parameters can be used to identify localized corrosion, and to provide an actual estimate of the rate in which it occurs.

Advantages of the SmartCET™ approach include an elimination of the requirement for temperature measurement or control, and the ability to derive actual quantitative data for localized pitting. The use of the InterCorr device to monitor corrosion in MIC experiments allows a quantitative determination of metal corrosion using both electrochemical and weight loss data for each condition tested. This is possible because each SmartCet probe contains three removable metal electrodes. Probes used were 1018 carbon steel, so as to be comparable to coupon experiments conducted in the various GTI corrosion-related projects. The electrodes enable the collection of electrochemical data of several types throughout the duration of the experiment and at the conclusion of the experiment the amount of weight loss by each electrode can be quantified yielding triplicate values for each data point. The amount of biomass/protein associated with each electrode can also be quantified.

The first InterCorr experiment performed in this project started 8/27/04 and lasted for one month. The microbial inoculum consisted of a bacterial consortium isolated from a corroded pipeline sample obtained from a Colorado utility company, designated CO-RD. The CO-RD-

derived consortium had been grown for approximately one week (in GCM) prior to its inclusion in this experiment. A total of six test reactors were used, with single reactors for non-inoculated controls and glutaraldehyde (0.1%), and duplicate reactors for hexane extract of Chile de Arbol pepper (0.1% pepper extract #1) and inoculated no-inhibitor positive controls. All electrodes were cleaned and weighed prior to set-up. Electrochemical data (LPR, HM, and EN corrosion rates, plus pitting factor) were collected throughout the run, which was terminated after approximately four weeks. At the conclusion, all electrodes were collected and cleaned, and re-weighed for weight-loss determinations. The results of the weight loss data for each of these conditions is shown in Figure 8 and in Table 8, which illustrate that this experiment yielded unexpected and variable results.

TABLE 8. WEIGHT LOSS AND ELECTROCHEMICAL RESULTS FROM THE 8/27/04 INTERCORR EXPERIMENT.

	Non-Inoculated	**No Inhibitor (#1)**	**No Inhibitor (#2)**	**Pepper (#1)**	**Pepper (#2)**	**Glutaraldehyde**
Wt. Loss*	0.0293	0.0213	0.0182	0.0219	0.0152	0.0220
LPRCR**	1.86	64.53	1.63	2.66	1.75	1.84
HNCR**	1.50	40.01	1.22	2.44	1.60	1.27
ENCR**	6.85	3.77	24.40	15.50	15.57	6.15
PF**	0.0197	0.0030	0.0783	0.0323	0.0857	0.0427

* = Average for three electrodes
** = Time-averaged values over the full course of the experiment

The standard deviations for almost all conditions are quite high and the amount of weight loss observed in the negative control (Not inoculated) generally exceeded the weight loss in the positive controls (No inhibitor). Although these observations called into doubt the usefulness of this experiment from the standpoint of testing putative inhibitors, an attempt was still made to see if the electrochemical data could be correlated to weight loss data, as this could still have been useful for validating the ability of one or more electrochemical parameter to predict weight loss. However, as the following data and graphs show (Figures 9 through 12), it was impossible to discern any sensible relationship between the weight losses of the electrodes and any of the four electrochemical parameters.

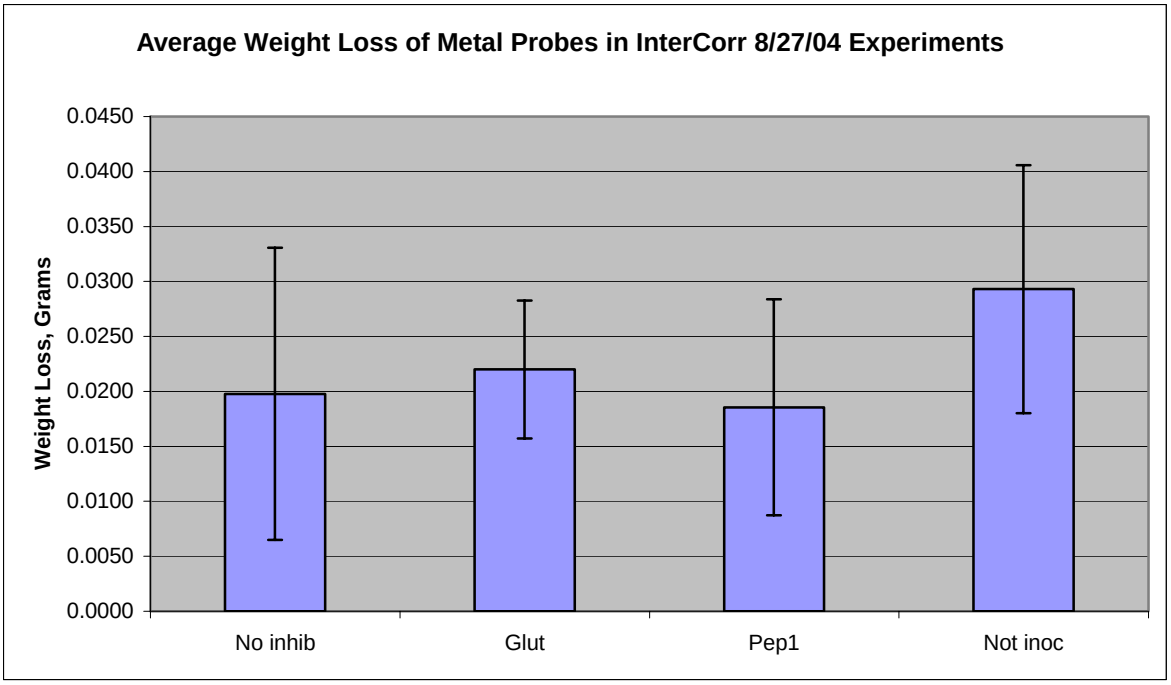
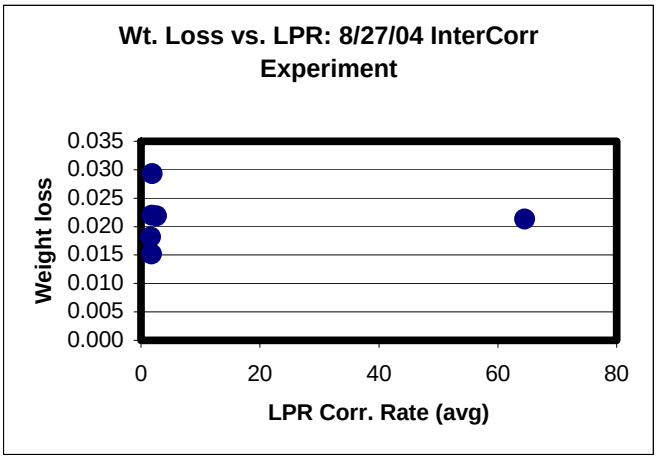


FIGURE 8. AVERAGE WEIGHT LOSS OF METAL PROBES IN INTERCORR 8/27/04 EXPERIMENTS.



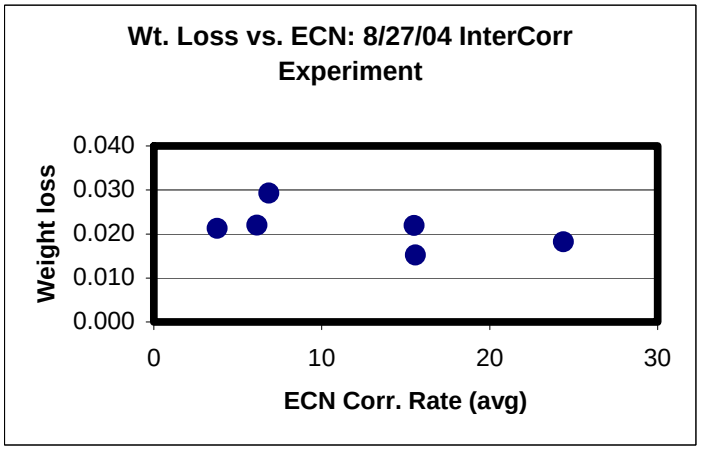
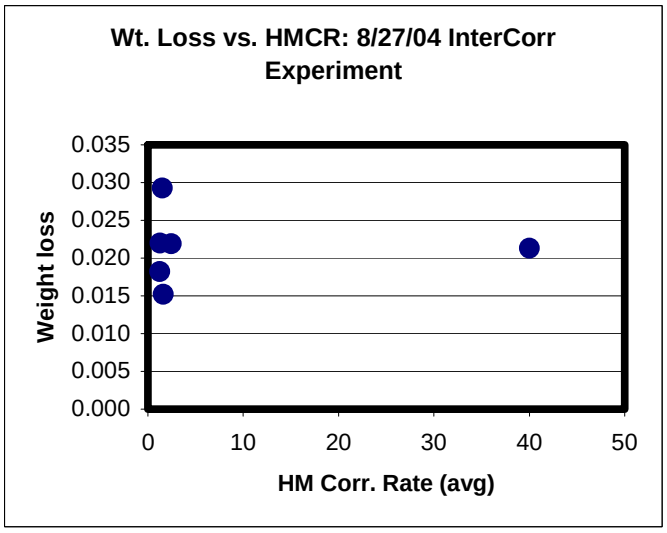


FIGURE 9. WEIGHT LOSS VERSUS LPR: 8/27/04 INTERCORR EXPERIMENT.



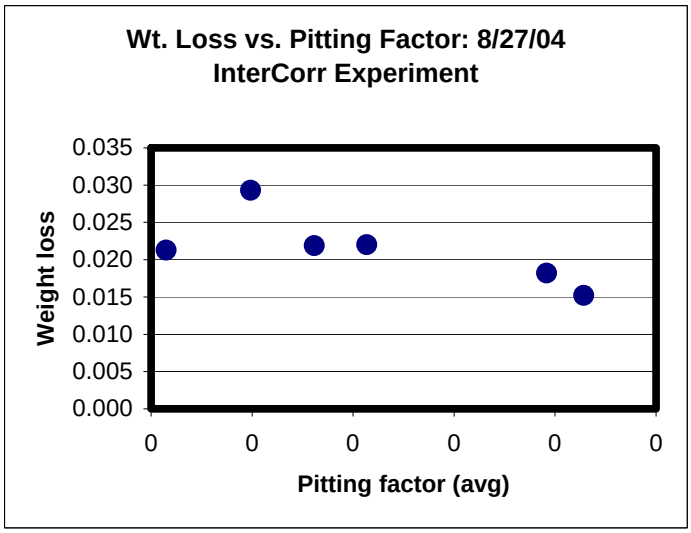


FIGURE 10. WEIGHT LOSS VERSUS ECN: 8/27/04 INTERCORR EXPERIMENT.

FIGURE 11. WEIGHT LOSS VERSUS HM: 8/27/04 INTERCORR EXPERIMENT.

FIGURE 12. WEIGHT LOSS VERSUS PITTING FACTOR: 8/27/04 INTERCORR EXPERIMENT.

The graphs showing the amount of general corrosion (LPR) and pitting corrosion detected electrochemically in each sample throughout the course of the experiment are shown in Figures that are attached as an appendix to this report. The graph of LPR rate during the course of the 8/27/04 InterCorr experiment shows that the amount of general corrosion detected in this experiment differed 25-fold among the six conditions with the No inhibitor #1 sample showing vastly higher general corrosion than any other sample. Clearly these electrochemical data do not agree with the weight loss data in Figure 8. The data in the graph of LPR data for the 8/27/04 InterCorr experiment also illustrate that the general corrosion rate detected electronically for each sample varied considerably throughout the course of the experiment. Often the levels of general corrosion detected electronically were higher in the first few days of the experiment, but reached lower and steady values at the end of the experiment. However, in the course of the experiment the corrosion rate rose and fell at different times in each sample. The result of these fluctuating electrochemical data is that it would not be possible to get an accurate determination of the amount of corrosion in a given sample by performing the experiment for a short period of time. Instead it seems necessary to utilize extended incubation times to obtain stable electrochemical readings. The data in the appendix for the pitting rate for the 8/27/04 InterCorr experiment show that no significant pitting corrosion was detected in any of these six samples. Figures 9 through 12 show graphs of electrochemical parameters LPR, ECN, HM, and pitting factor versus weight loss respectively and it can be seen that this experiment did not produce electrochemical data that appears to correlate with weight loss data.

One further InterCorr/SmartCET^{TM} run was conducted starting 10/28/04, in which several putative biocorrosion inhibitors were tested. The reactor vessels used for this experimentwere constructed of glass and Teflon allowing them to be autoclaved and to achieve an airtight seal to maintain anaerobic conditions. The microbial inoculum consisted of the CO-RD sample described above, which by now had been subcultured through 3 generations (in GCM), and the incubation time for the experiment was 48 days at 30^{o}C. The inhibitors tested in this experiment were all employed at 0.1% in duplicate reactors:

- Ammonium cerium nitrate (ACN, $(NH_4)_2Ce(NO_3)_6$), a potential inhibitor of denitrifying bacteria
- Sodium nitrate ($NaNO_3$), which inhibits SRB activity (*45*)
- 4-((pyridine-2-yl)methylamino)benzoic acid (PMBA), a recently-discovered (*32*), potent inhibitor of methanogenic bacteria.
- Pepper (hexane extract of Chile de Arbol extract #1, as above)

Each inhibitor was tested in duplicate incubation vessels (3 electrodes/vessel = 6 electrodes total); positive (inoculated, no inhibitor) and negative (non-inoculated media) controls were also included (in duplicate). All electrodes were cleaned and weighed prior to set-up. Total culture volume was 200 ml, which consisted of 190 ml fresh GCM and 10 ml of CO-RD stock culture; final concentration of inhibitors was 0.1% by weight. Because the pepper extract was added as a hexane solution (total volume 2.0 ml), an equal volume of hexane was added to each of the cultures in the other conditions as well, in order to ensure that no possible artifacts were introduced through an inconsistency of this nature.

The incubation period for this run was 48 days. All possible electrochemical data (LPR, HM, EN, and pitting factor) were collected throughout the run. At the conclusion, all electrodes were collected and cleaned, and re-weighed for weight-loss determinations. Weight-loss results from the three removable metal electrodes in each SmartCet probe are summarized in Figure 13. The amount of corrosion observed in the negative control exceeded that in the positive control. This is no doubt a reflection of the fact that the negative controls did not remain sterile. Nonetheless, it is somewhat surprising that less corrosion was observed in the positive controls that were inoculated with corrosion associated bacteria obtained from a gas pipeline. The duplicate samples for each condition tested show fairly good agreement for four of the six conditions and the standard deviations for most samples are good. These results indicate that the

pepper extract is the most effective corrosion inhibitor in these experiments and that the presence of additional nitrate appears to result in increased corrosion, at least in one of the samples. The divergent results obtained with the two PMBA samples makes it difficult to form a firm conclusion, but it appears that PMBA may inhibit MIC while ammonium cerium nitrate does not.

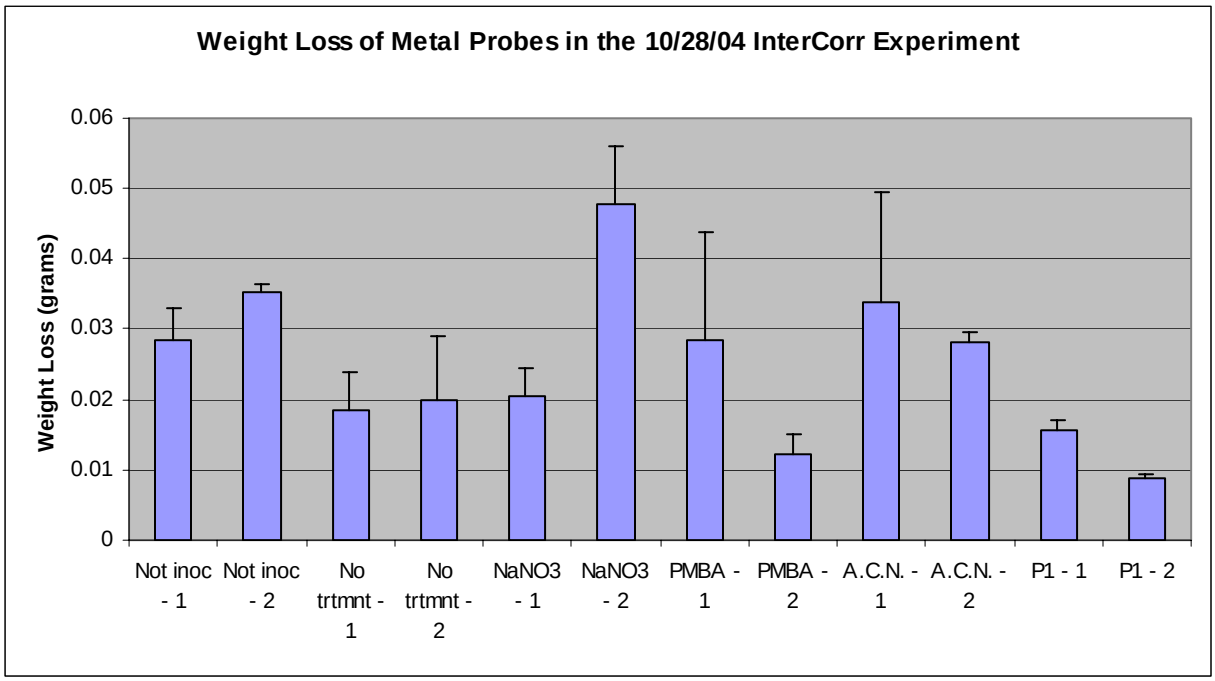
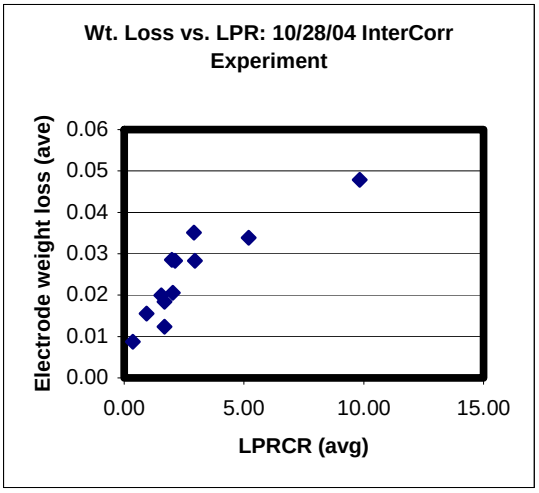


FIGURE 13. WEIGHT LOSS OF METAL PROBES IN THE 10/28/04 INTERCORR EXPERIMENT.

The electrochemical data for general corrosion (LPR) and pitting factor for all 12 samples throughout the 48-day incubation are shown in Figures attached as an appendix to this report. The Figure showing the LPR rates for the duration of the 10/28/04 InterCorr experiment illustrates that the general corrosion rate for each sample undergoes fluctuations during the course of the experiment but tend to stabilize after about 30 days of incubation for most samples. The fluctuating nature of the data in the LPR rate Figure illustrates that different conclusions would be drawn if the experiment had been incubated for shorter periods of time. In particular, it would save time in performing corrosion experiments if electrochemical results obtained after several hours or days could provide accurate predictions regarding the amount of corrosion resulting from each condition, but this is clearly not possible. The sample "Nitrate-2" shows the greatest variation of all the data in the 10/28/04 LPR Figure, with values ranging from 2 to 20, and it is difficult to know how best to interpret the significance of the corrosion rate being higher than most samples for the majority of the experiment, especially since this sample was unique in showing a sharply declining corrosion rate at the end of the experiment. If one tries to rank the 12 samples based on the amount of corrosion indicated in the 10/28/04 LPR Figure the ranking from highest to lowest corrosion would be Nitrate-2 (2 to 20 LPR), ACN-1 (8 LPR), Sterile-2 (4 LPR), ACN-2 (2.5 LPR), and all the rest of the samples which all have values of about one LPR unit. This ranking agrees reasonably well with the ranking of samples as determined by the amount of weight loss as shown in Figure 13; however, the magnitude of the differences in the samples does not agree. The LPR data in the 10/28/04 LPR Figure suggests that the magnitude of corrosion between samples varies by as much as 18-fold, while the data in Figure 13 illustrate that the magnitude of differences observed for weight loss between these same samples were only 2-fold.

The data for pitting factor determined for the 12 samples in the 10/228/04 InterCorr experiment are also shown in a Figure included in an appendix to this report. This graph of Pitting factor data for the course of the 10/28/04 InterCorr experiment shows no consistent and significant differences are observed between these samples. The data for LPR, ECN, HM, and pitting factor plotted versus weight loss for the 10/28/04 InterCorr samples are shown in Figures 14 through 17 respectively. Fairly good correlations between LPR vs weight loss, ECN vs weight loss, and HM vs weight loss are observed for this data set as shown in Figures 14, 15 and 16 respectively. Therefore, while the magnitude of the differences between samples is not the same

between electrochemical data and weight loss data the electrochemical data and the weight loss data obtained in the 10/28/04 InterCorr experiment show good agreement and tend to reinforce the validity of the data that indicate that hexane extract of Chile de Arbol pepper appears to be the best inhibitor of MIC and the presence of excess nitrate results in increased MIC in these experiments. A further analysis of the data from the 10/28/04 InterCorr experiment was a determination of the amount of protein, and indicator of the quantity of biofilm, present on each of the electrodes on the SmartCet probes from these experiments. Figure 18 shows a graph of weight loss vs protein for these samples, and once again a reasonable correlation is observed. It would be expected that an increased amount of biofilm would correlate with an increased amount of corrosion, however, it is possible that metabolic inhibitors may decrease the amount of corrosion without significantly decreasing the amount of biofilm/protein.



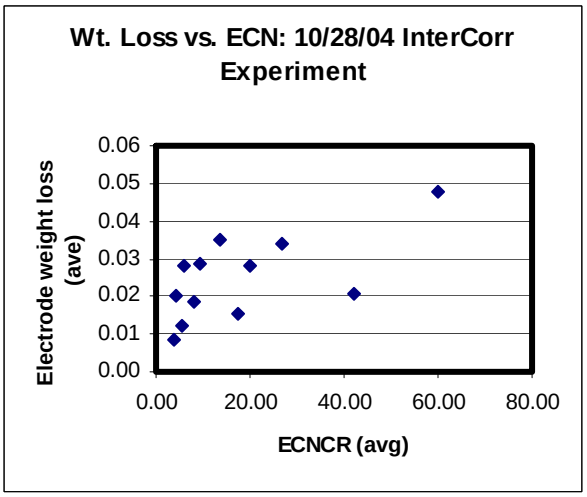
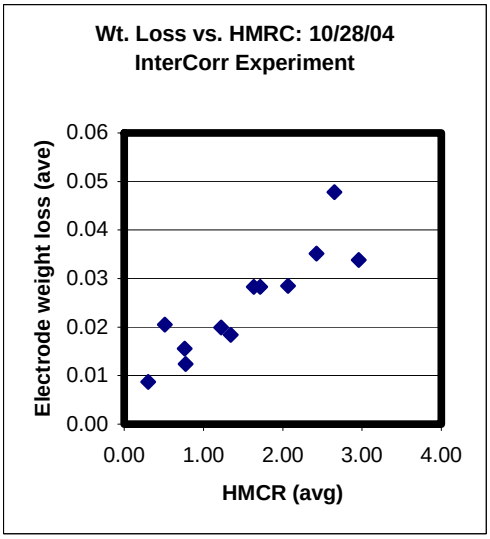


FIGURE 14. WEIGHT LOSS VERSUS LPR: 10/28/04 INTERCORR EXPERIMENT.

FIGURE 15. WEIGHT LOSS VERSUS ECN: 10/28/04 INTERCORR EXPERIMENT.



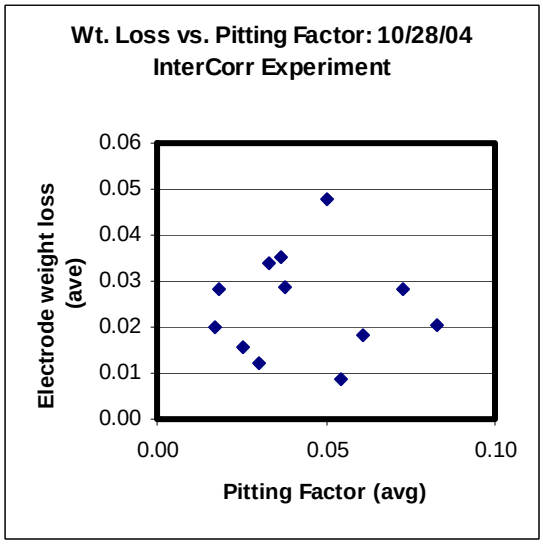


FIGURE 16. WEIGHT LOSS VERSUS HM: 10/28/04 INTERCORR EXPERIMENT.

FIGURE 17. WEIGHT LOSS VERSUS PITTING: 10/28/04 INTERCORR EXPERIMENT.

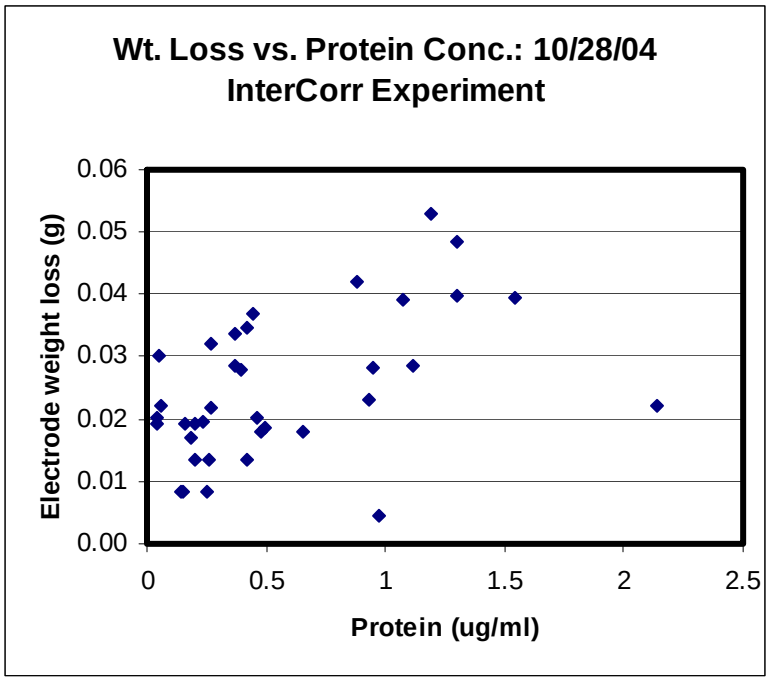


FIGURE 18. WEIGHT LOSS VERSUS PROTEIN CONCENTRATION: 10/28/04 INTERCORR EXPERIMENT.

To get further information regarding the effect of metabolic inhibitors on MIC, serum bottle experiments were performed in parallel with the 10/28/04 InterCorr experiments. These serum bottle experiments utilized the same mixed culture of corrosion associated microorganisms obtained from a gas pipeline, CO-RD, to inoculate triplicate samples for each test condition. The test conditions, each in triplicate, included sterile/uninoculated controls, positive controls that were inoculated but have no inhibitor present, and various inhibitors each present at 0.1%: pepper extract #1, PMBA, ACN, nitrate glutaraldehyde, bismuth nitrate, anthraquinone, BES, molybdate, molybdenum disilicate, and hexane. The results of the weight loss determinations for the metal coupons in these experiments are summarized in Figure 19. The data in Figure 19 show very minor standard deviations and the positive controls experienced more corrosion than the sterile/negative controls. This is probably due to the fact that maintaining sterile conditions in serum bottles is easier than using reactors that must accommodate the InterCorr SmartCet probes. In any event the results shown in Figure 19 indicate that molybdate and glutaraldehyde were the most effective compounds in inhibiting MIC while the other compounds did not appear to significantly inhibit MIC. When the results in Figures 13 and 19 are compared, different trends are observed and no consistent conclusions can be drawn except that the presence of additional nitrate appears to increase the level of corrosion. The data in Figure 13 indicate that pepper extract and PMBA are potent inhibitors of MIC, but the results in Figure 19 do not confirm this. To further explore these data the amount of weight loss versus protein content was plotted for these serum bottle experiments and the results are shown in Figure 20. The extremely low protein values obtained from most of the metal coupons makes it difficult to analyze, but some evidence of a correlation appears to exist.

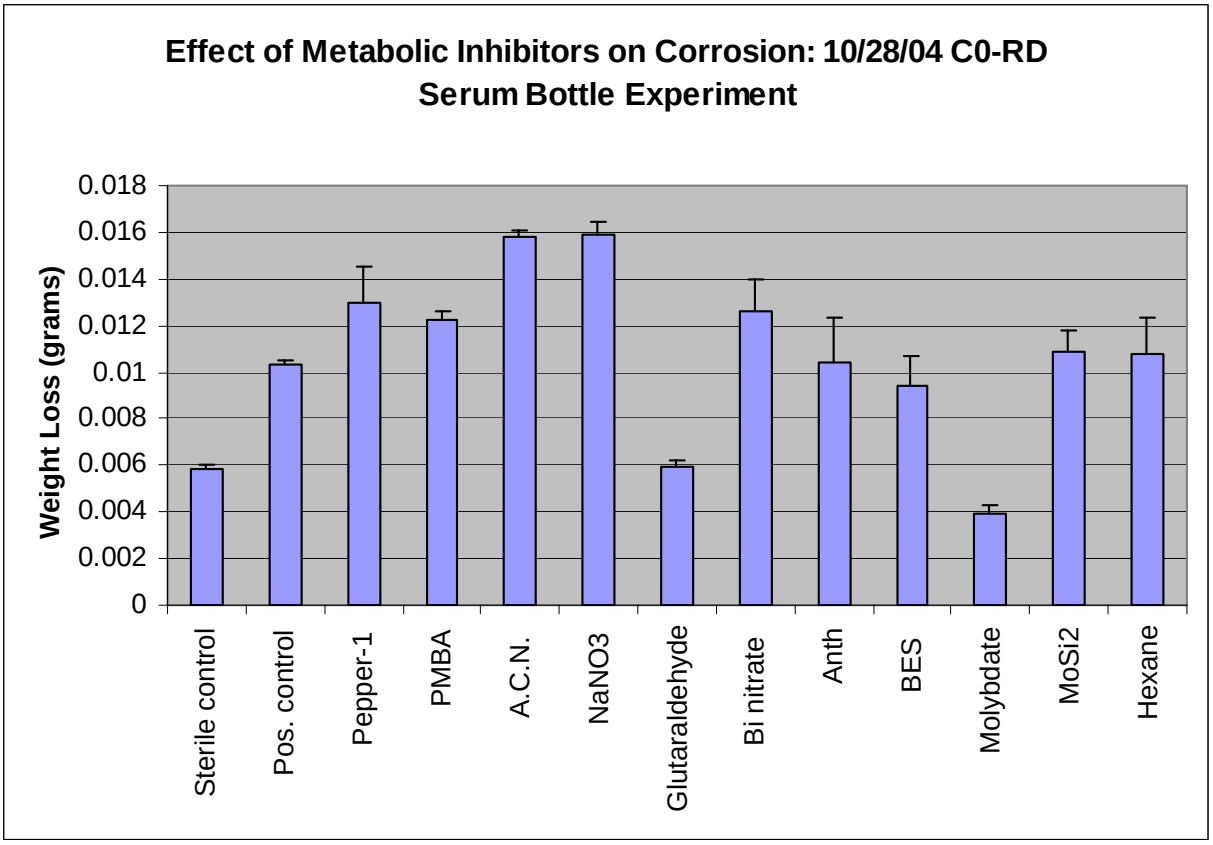


FIGURE 19. EFFECT OF METABOLIC INHIBITORS ON CORROSION: 10/28/04 CO-RD SERUM BOTTLE EXPERIMENT.

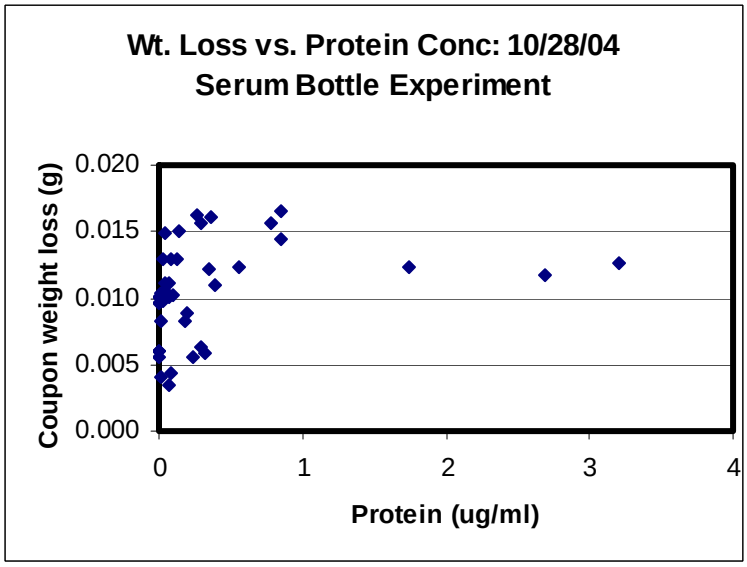


FIGURE 20. WEIGHT LOSS VERSUS PROTEIN CONCENTRATION: 10/28/04 SERUM BOTTLE EXPERIMENT.

An additional set of experiments were performed to evaluate the effects of metabolic inhibitors of MIC except the concentration of the inhibitors was 0.01% or 10-fold lower than concentrations tested in previous experiments. These serum bottle experiments were performed in triplicate and tested various metabolic inhibitors individually and in combination. These serum bottle experiments utilized the same mixed culture of corrosion associated microorganisms obtained from a gas pipeline, CO-RD, to inoculate triplicate samples for each test condition. The test conditions, each in triplicate, included sterile/uninoculated controls, positive controls that were inoculated but have no inhibitor present, and various inhibitors each present at 0.01%: pepper extract #2, PMBA, ACN, bismuth nitrate, anthraquinone, molybdate, and pairwise combinations of these inhibitors. The results of the weight loss determinations for the metal coupons in these experiments are summarized in Figure 21 which indicate that the standard deviations for almost all samples were remarkably small. The amount of corrosion observed in the positive control was rather similar to the negative control making it challenging to test for the inhibition of MIC. Nonetheless, most inhibitors and combinations of inhibitors in these experiments resulted in decreased corrosion. Anthraquinone resulted in markedly increased levels of corrosion, but the similar nature of the results obtained from almost all of the other samples makes it difficult to conclude that any of the compounds is better than another as regards inhibiting MIC.

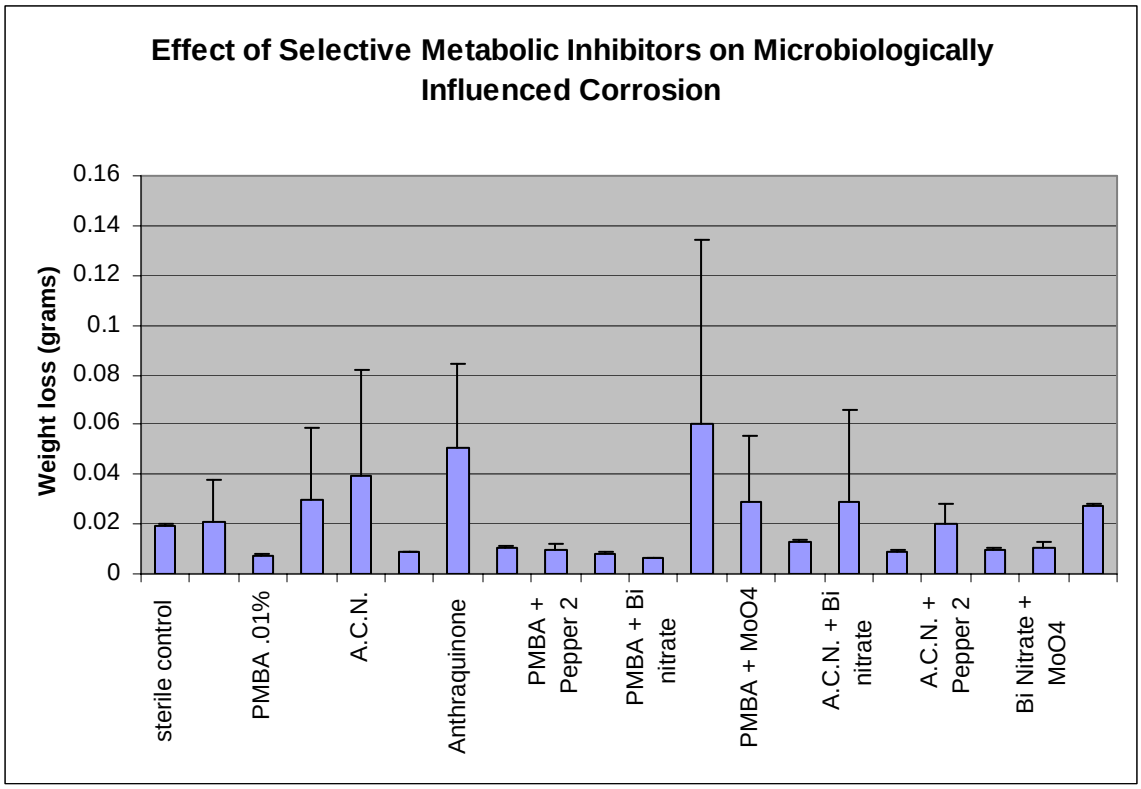


FIGURE 21. EFFECT OF SELECTIVE METABOLIC INHIBITORS ON MICROBIOLOGICALLY INFLUENCED CORROSION.

During the course of this project multiple experiments were performed using mixed cultures of corrosion associated microorganisms and conditions designed to mimic conditions in gas and oil pipelines and the results obtained in these experiments showed variable results preventing us from making firm conclusions. However, it seems that the majority of data indicate that hexane extract of Chile de Arbol pepper, and molybdate are effective inhibitors of SRB, bismuth nitrate is the most effective inhibitor of denitrification, and PMBA is the most effective inhibitor of methanogens among the compounds tested in this study. Each of these compounds was effective in minimizing MIC. Conversely, the presence of increased concentrations of nitrate result in increased levels of MIC. However, these experiments were performed under conditions in which the microbial culture and the inhibitor were added simultaneously. An alternative method of testing corrosion is to examine the effect of metabolic inhibitors on established biofilms.

Mature Biofilm Studies

It is well known that it is more difficult to kill or inhibit microbial cells within biofilms as compared with bacterial cells in solution (planktonic cells). However, problems of MIC in gas and oil pipelines involve biofilms. Accordingly, several experiments were performed to evaluate the ability of various metabolic inhibitors to minimize the corrosion caused by mature biofilms of pure and mixed cultures. The sulfate reducing bacterium *Desulfovibrio vulgaris*, and the mixed culture of corrosion-associated bacteria obtained from a natural gas pipeline, CO-RD, were used in biofilm experiments. These cultures were incubated for seven days in general corrosion media in the presence of metal coupons to allow biofilms to become established on these metal surfaces. Subsequently, individual biofilm-coated metal coupons were placed in serum bottles containing fresh GCM with and without metabolic inhibitors. These serum bottles were then incubated for 4 to 12 weeks at room temperature. Figure 22 shows the results of weight loss determinations from an experiment with biofilms formed with *Desulfovibrio vulgaris* and the effect of various inhibitors present at concentrations ranging from 0.05% to 0.2% and incubated for four weeks. The conditions tested included no treatment/inhibitor, hexane extract of Chile de Arbol peppers (P1), glutaraldehyde (Glut), nitrate (Nit), anthraquinone (Anth), and hexane. Each condition was tested in triplicate.

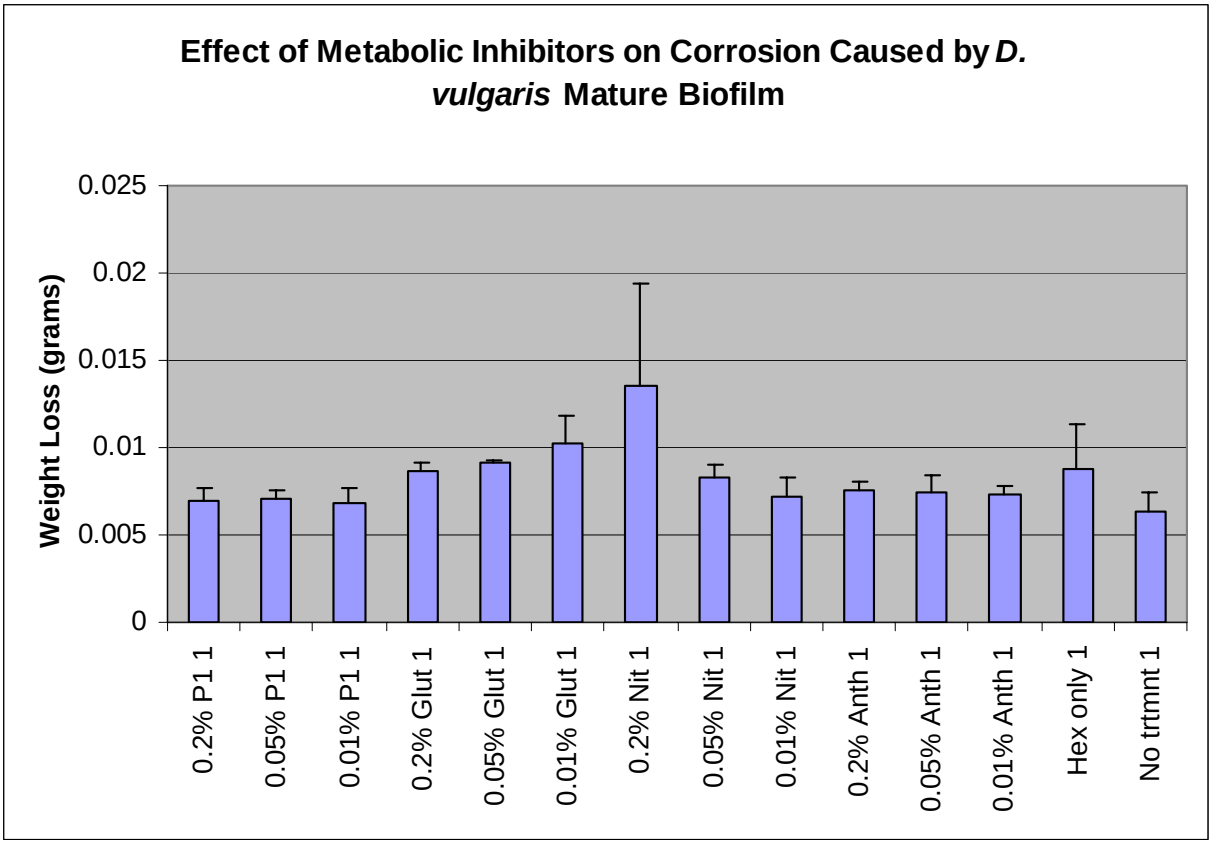


FIGURE 22. EFFECT OF METABOLIC INHIBITORS ON CORROSION BY *DESULFOVIBRIO VULGARIS* MATURE BIOFILM.

The results shown in Figure 22, and the results obtained from coupons after the 7-day biofilm formation period (data not shown), indicate that the majority of corrosion occurred as a consequence of the week-long incubation required to establish biofilm on the coupons. The no-treatment control that was incubated in the absence of inhibitor did not show appreciably more corrosion than the other test conditions. This lack of significant amounts of corrosion during the four-week incubation made it difficult to determine the effectiveness of various compounds to inhibit corrosion, but increased concentrations of nitrate resulted in increased levels of corrosion. It is not clear if the reason that nitrate stimulates MIC is due directly to nitrate reduction, which has been demonstrated with pure cultures of denitrifying bacteria, or due to an indirect effect of stimulating sulfate reduction. Many SRB are known to be capable of reducing nitrate as well as sulfate. The reduction of nitrate can be associated with the reoxidation of sulfide forming sulfate, which in turn may be reduced back to sulfide.

The effects of nitrate on corrosion rates/MIC are particularly important, as the gas and oil industry is increasingly adopting the practice of using nitrate injection into sour gas and oil deposits to decrease the hydrogen sulfide concentration. As explained above, the reduction of nitrate can be coupled with the oxidation of sulfide to sulfate, and the use of nitrate injection in production operations has been shown to be successful in decreasing the hydrogen sulfide content of gas and oil. However, the widespread practice of nitrate injection will increasingly result in increased concentrations of nitrate present in produced water resulting from gas and oil production. While nitrate is useful for the minimization of hydrogen sulfide concentrations in gas and oil, the presence of nitrate in the water can, based on our results, be expected to impact the levels of MIC experienced by gas and oil production operations and pipelines.

Two experiments involving mature biofilms of mixed cultures of corrosion associated microorganisms were performed that incubated for 4 weeks or 12 weeks, and the results are shown in Figures 23 and 24 respectively. The biofilms were established on metal coupons incubated for 7 days in GCM; then triplicate samples were used to test various metabolic inhibitors. The conditions tested in the experiments shown in Figure 23 were: No inhibitor, glutaraldehyde, BES, anthraquinone, ammonium cerium nitrate (ACN), hexane extract of Chile de Arbol peppers (Pep1), hexane extract of Habanero peppers (Pep 3), and hexane. All the inhibitors yielded equivalent results, showing somewhat less corrosion than the no-inhibitor control. Again the 7-day preincubation needed to establish the biofilm was responsible for the majority of corrosion (data not shown) and all of the inhibitors arrested MIC at that level and prevented further corrosion.

The data shown in Figure 24 comes from mature mixed culture biofilm experiments incubated for 12 weeks. The 7-day preincubation required to establish biofilm on the coupons was responsible for the majority of corrosion (data not shown) but the longer incubation period, as compared with Figure 23, resulted in higher corrosion levels in no-inhibitor controls. The compounds tested included hexane extract of Habanero peppers (Pepper 3), anthraquinone, nitrate, ammonium cerium nitrate (ACN), hexane and glutaraldehyde. All compounds were present at 0.1% and all of the compounds yielded similar results, indicating that the inhibitors arrested MIC preventing further corrosion. These data indicate that the most effective inhibitor was glutaraldehyde, while nitrate was the least effective.

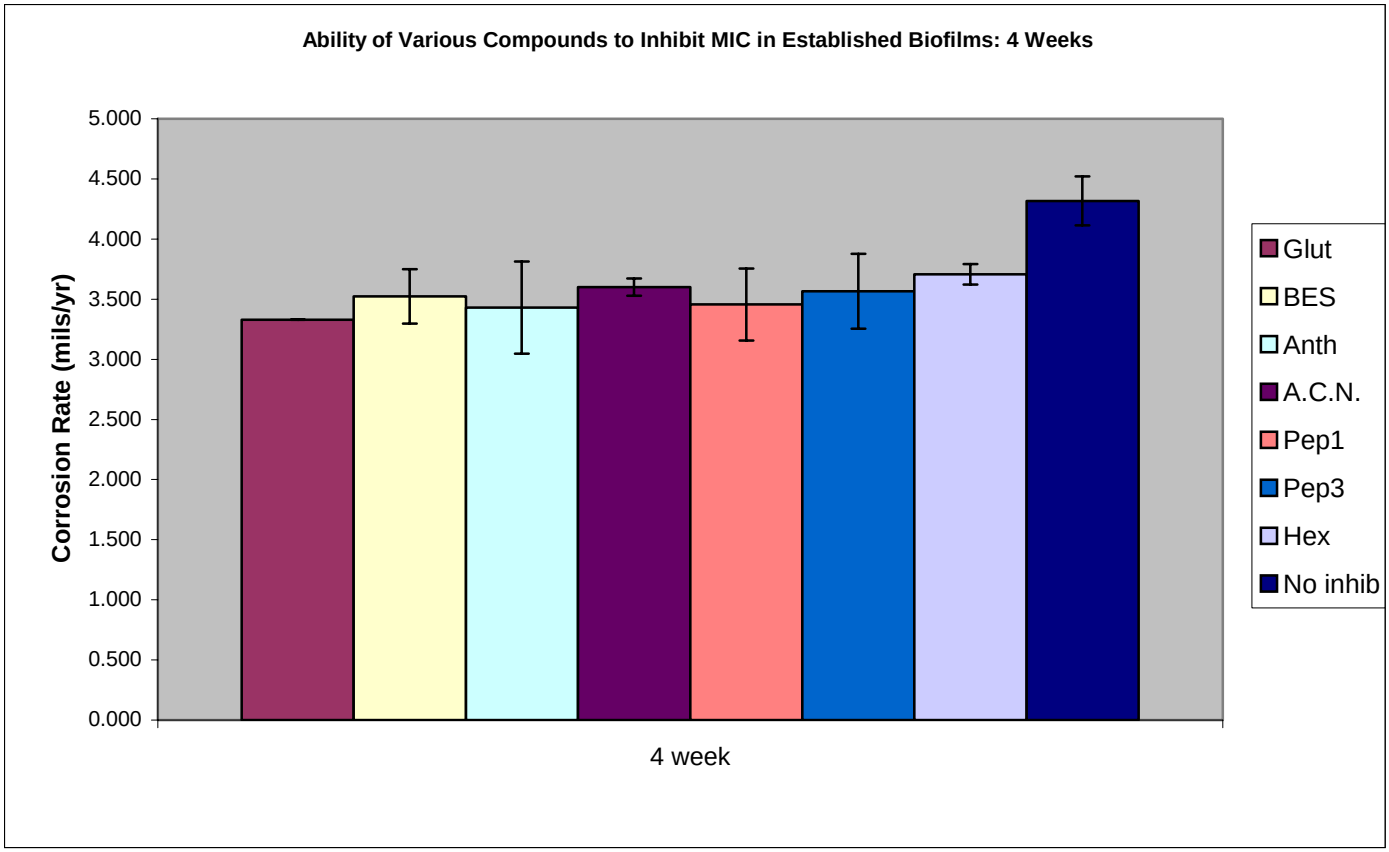


FIGURE 23. ABILITY OF VARIOUS COMPOUNDS TO INHIBIT MIC IN ESTABLISHED BIOFILMS: 4 WEEKS.

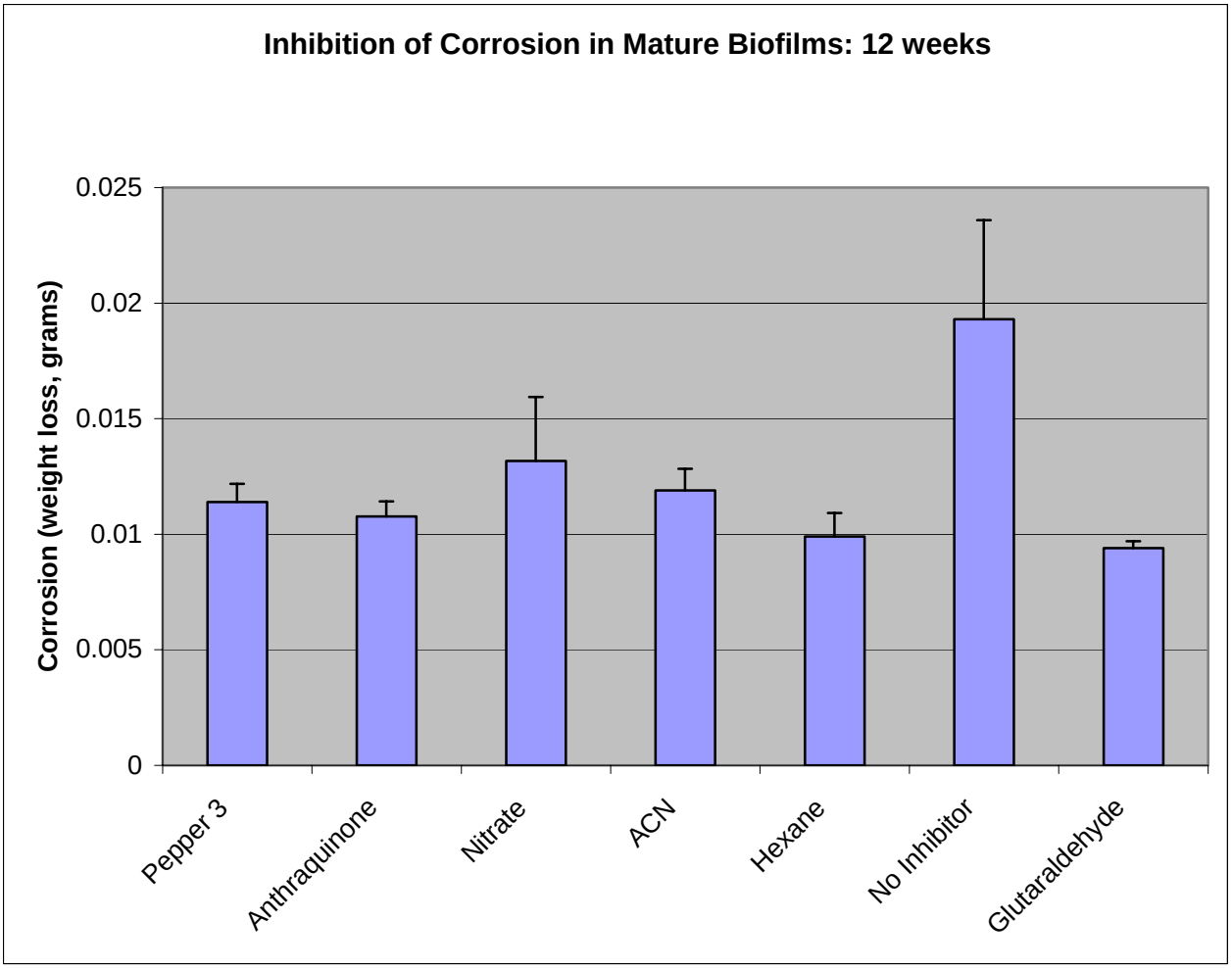


FIGURE 24. ABILITY OF VARIOUS COMPOUNDS TO INHIBIT MIC IN ESTABLISHED BIOFILMS: 12 WEEKS.

SUMMARY AND CONCLUSIONS

Experiments performed during the project included an examination of a 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, anthraquinone and extracts of pepper plants that selectively inhibit sulfate-reducing bacteria, some structural analogues of nitrate (ammonium cerium nitrate and bismuth nitrate) that may serve as selective inhibitors of denitrifying bacteria, 2-bromoethanesulfonic (BES) acid and 4-((pyridine-2-yl) methylamino)benzoic acid (PMBA) that selectively inhibit methanogenic bacteria, and glutaraldehyde a general biocide. Growth tests were performed with pure cultures of sulfate reducing bacteria and denitrifying bacteria as well as mixed microbial cultures of corrosion associated bacteria obtained from gas pipelines which demonstrated that nearly all molybdenum-containing compounds and hexane extracts of some pepper plants inhibit the growth of most sulfate reducing bacteria, but do not inhibit the growth of other types of microorganisms. Anthraquinone was not as effective in inhibiting SRB in these experiments. Bismuth nitrate was shown to be capable of inhibiting the growth of denitrifying bacteria and reducing the amount of corrosion due to MIC, while ammonium cerium nitrate (ACN) was not as effective. PMBA was found to be a more effective inhibitor of methanogenesis than BES in these experiments.

Some corrosion testing performed in this project, and indeed most of the corrosion testing reported in the literature 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, other experiments 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 kill/inhibit 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.

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, the data presented here are consistent with the hypothesis 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.

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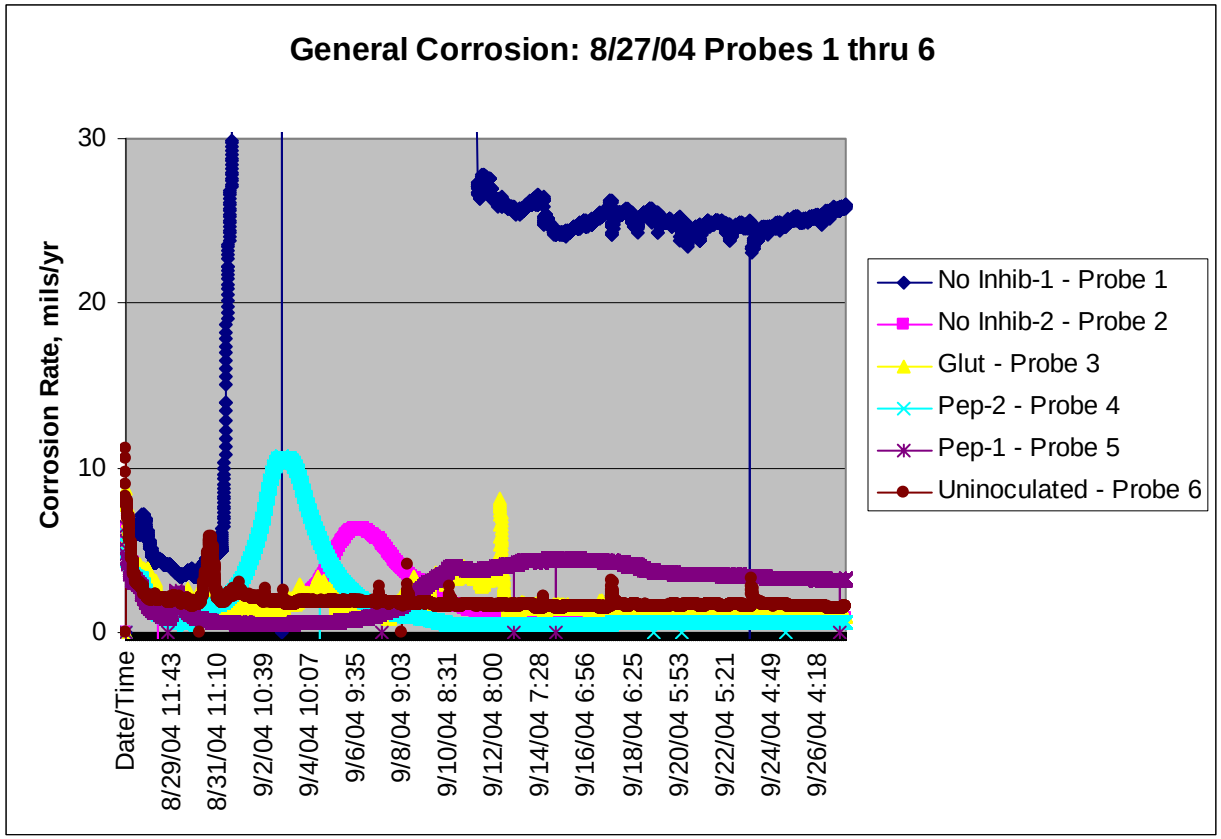
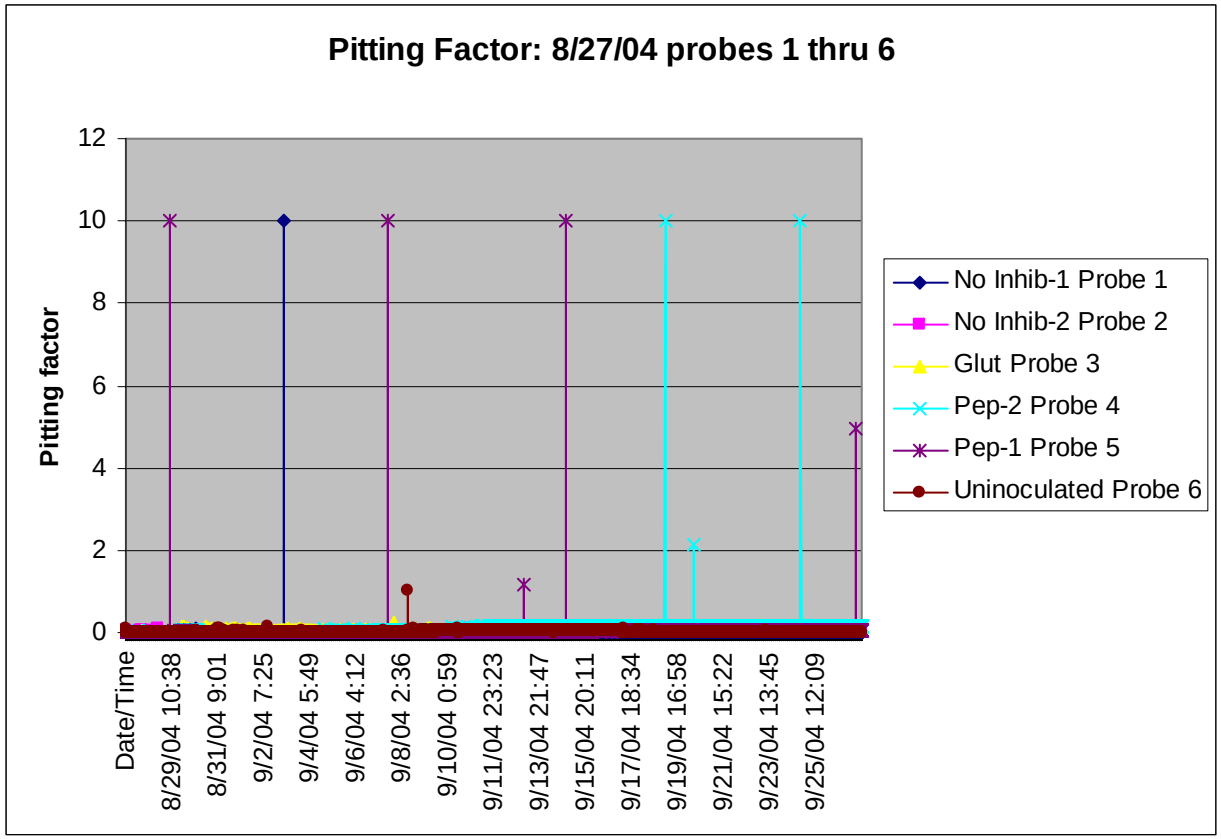
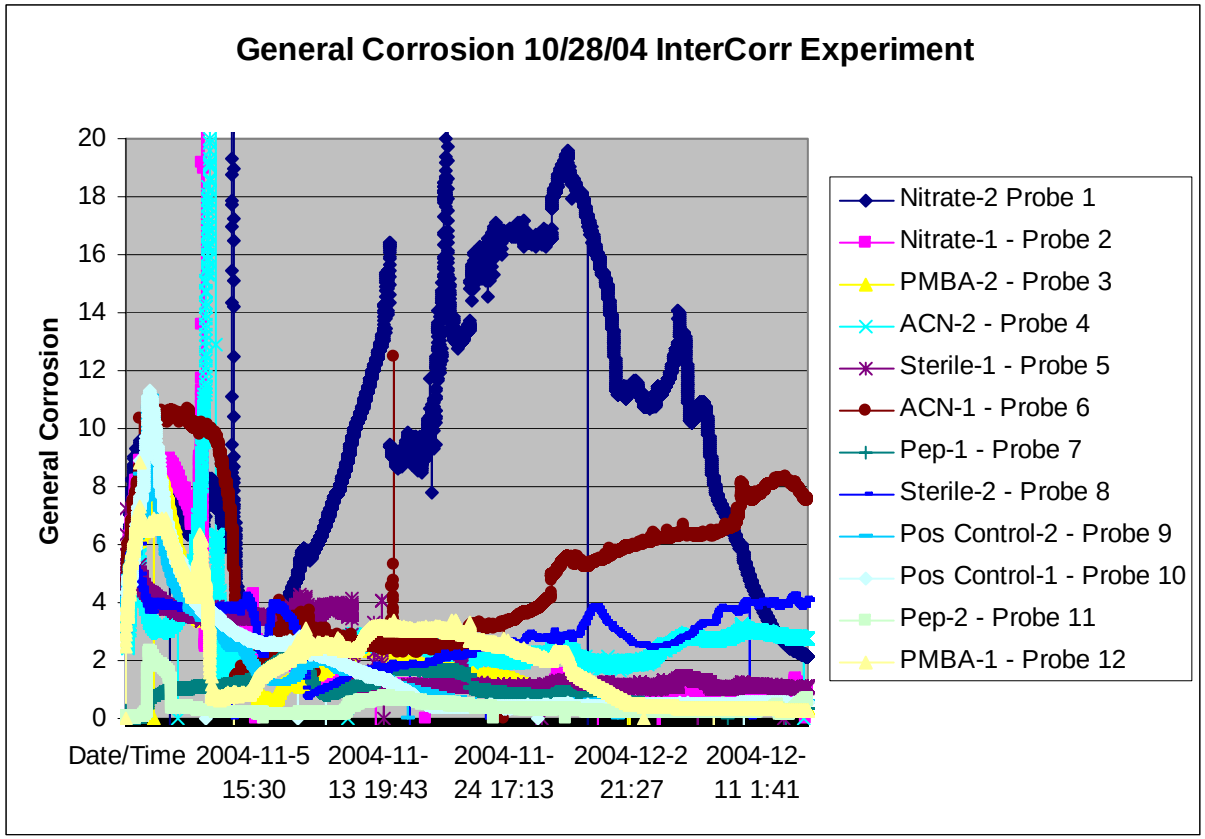
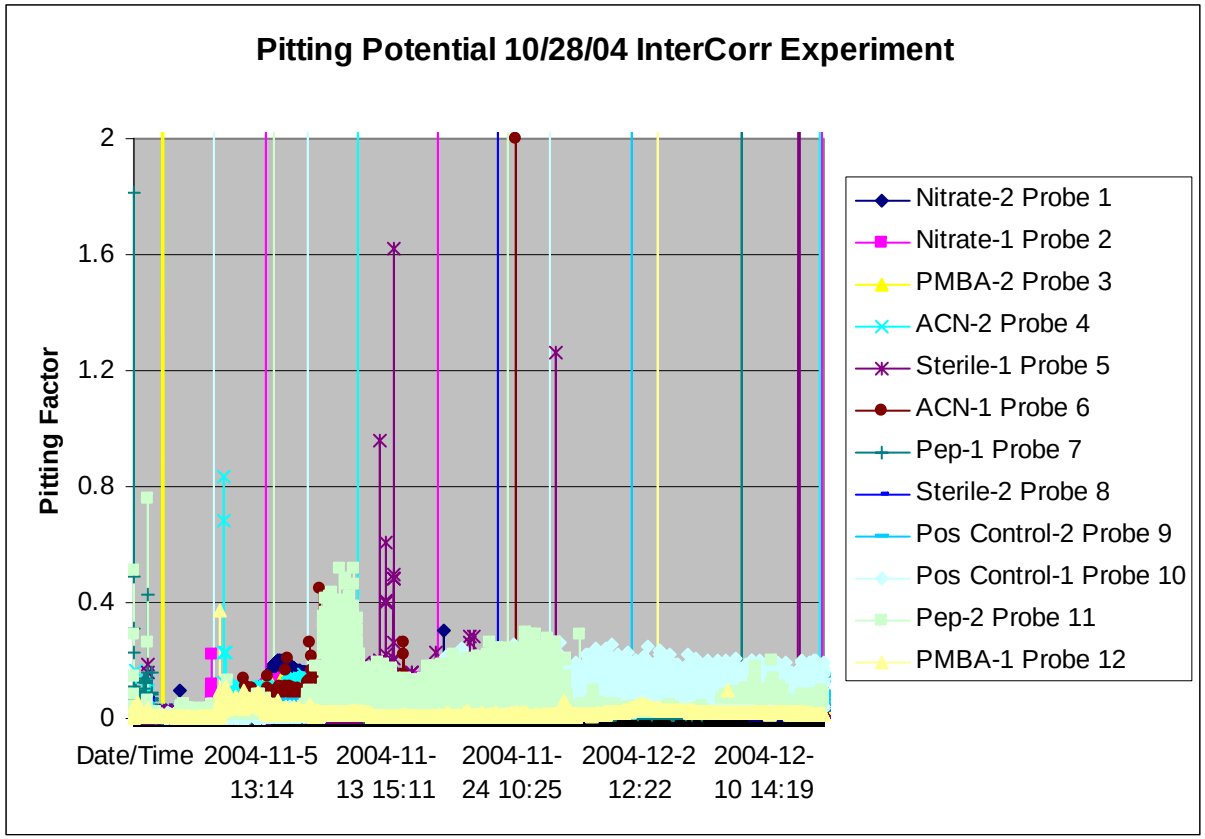
APPENDIX: GRAPHS OF LPR AND PITTING FACTORS THROUGHOUT INTERCORR EXPERIMENTS


General Corrosion: 8/27/04 Probes 1 thru 6
Corrosion Rate, mils/yr
30
20
10
0
Date/Time
8/29/04 11:43
8/31/04 11:10
9/2/04 10:39
9/4/04 10:07
9/6/04 9:35
9/8/04 9:03
9/10/04 8:31
9/12/04 8:00
9/14/04 7:28
9/16/04 6:56
9/18/04 6:25
9/20/04 5:53
9/22/04 5:21
9/24/04 4:49
9/26/04 4:18
No Inhib-1 - Probe 1
No Inhib-2 - Probe 2
Glut - Probe 3
Pep-2 - Probe 4
Pep-1 - Probe 5
Uninoculated - Probe 6


Pitting Factor: 8/27/04 probes 1 thru 6
Pitting factor
12
10
8
6
4
2
0
Date/Time
8/29/04 10:38
8/31/04 9:01
9/2/04 7:25
9/4/04 5:49
9/6/04 4:12
9/8/04 2:36
9/10/04 0:59
9/11/04 23:23
9/13/04 21:47
9/15/04 20:11
9/17/04 18:34
9/19/04 16:58
9/21/04 15:22
9/23/04 13:45
9/25/04 12:09
No Inhib-1 Probe 1
No Inhib-2 Probe 2
Glut Probe 3
Pep-2 Probe 4
Pep-1 Probe 5
Uninoculated Probe 6


General Corrosion 10/28/04 InterCorr Experiment
General Corrosion
20
18
16
14
12
10
8
6
4
2
0
Date/Time
2004-11-5 15:30
2004-11-13 19:43
2004-11-24 17:13
2004-12-2 21:27
2004-12-11 1:41
Nitrate-2 Probe 1
Nitrate-1 - Probe 2
PMBA-2 - Probe 3
ACN-2 - Probe 4
Sterile-1 - Probe 5
ACN-1 - Probe 6
Pep-1 - Probe 7
Sterile-2 - Probe 8
Pos Control-2 - Probe 9
Pos Control-1 - Probe 10
Pep-2 - Probe 11
PMBA-1 - Probe 12


Pitting Potential 10/28/04 InterCorr Experiment
Pitting Factor
2
1.6
1.2
0.8
0.4
0
Date/Time
2004-11-5 13:14
2004-11-13 15:11
2004-11-24 10:25
2004-12-2 12:22
2004-12-10 14:19
Nitrate-2 Probe 1
Nitrate-1 Probe 2
PMBA-2 Probe 3
ACN-2 Probe 4
Sterile-1 Probe 5
ACN-1 Probe 6
Pep-1 Probe 7
Sterile-2 Probe 8
Pos Control-2 Probe 9
Pos Control-1 Probe 10
Pep-2 Probe 11
PMBA-1 Probe 12