ENVIRONMENTAL BENIGN MITIGATION OF MICROBIOLOGICALLY INFLUENCED CORROSION (MIC)

QUARTERLY REPORT

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ABSTRACT

Title: Environmental Benign Mitigation of Microbiologically Influenced Corrosion (MIC)

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Objective: The overall program objective is to develop and evaluate environmental 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 one or more environmental benign, a.k.a. "green" products that can be applied to maintain the structure and dependability of the natural gas infrastructure.

Approach: The technical approach for this quarter were isolation and cultivation of MIC-causing microorganisms from corroded pipeline samples, optimizing parameters in the laboratory-scale corrosion test loop system and testing the effective concentrations of *Capsicum* sp. extracts to verify the extent of corrosion on metal coupons by batch culture method.

Results: A total of 22 strains from the group of heterotrophic, acid producing, denitrifying and sulfate reducing bacteria were isolated from the gas pipeline samples obtained from Northern Indiana Public Service Company in Trenton, Indiana. They were purified and will be sent out for identification. Bacterial strains of interest were used in antimicrobial screenings and test loop experiments. Parameters for the laboratory-scale test loop system such as gas and culture medium flow rate; temperature; inoculation period; and length of incubation were established. Batch culture corrosion study against *Desulfovibrio vulgaris* showed that one (S_1M) out of the four *Capsicum* sp. extracts tested was effective in controlling the corrosion rate in metal coupons by 33.33% when compared to the untreated group.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

The overall objective of this project is to develop, test, and apply environmentally benign agent(s) to control corrosion associated with internal surfaces of metal (iron or steel) pipes used in natural gas transmission. The overall hypothesis is that agents exist in nature that inhibit some or all of the steps executed by microorganisms in the formation of biofilm. As biofilm formation is an absolute prerequisite for the initiation and production of microbially influenced corrosion (MIC), blocking biofilm formation or propagation will block or mitigate MIC.

The general approach is to evaluate natural products isolated from plants, and possibly animals or microorganisms for their abilities to block the attachment, physiology, or reproduction of microbial agents that are responsible for microbiologically influenced corrosion. The first natural product to be tested is the oil that can be extracted from the seeds and pods of pepper plants. These plants are members of the Genus *Capsicum* and the first species evaluated was *Capsicum annuum*. The effective components or constituents (isolation and identification of these constituents in a previous and ongoing project funded by Gas Technology Institute) of this product will then be tested for its environmental impact and effects, effective concentrations, modes of application, and stability against isolated MIC microorganisms under simulated field conditions. A commercially viable agent that aids in MIC control and is environmentally friendly is the ultimate target with preliminary data to determine commercialization potential and cost-benefit analysis.

EXECUTIVE SUMMARY

Presented is a summary of research activities from October 2002 to December 2002. The main goal of this project is to develop an environmentally benign compound that could prevent and/or control microbially influenced corrosion (MIC) in the interior of metal gas pipelines.

The majority of this quarter's activities were directed towards Objectives No. 1) to isolate and cultivate MIC-causing microorganisms / biofilm, 2) to design, construct and test a laboratory-scale pipeline simulation system and 5) to identify and test effective pepper oil components.

Samples from the corroded gas pipelines obtained from the Northern Indiana Public Service were used to isolate bacteria from the groups of acid-producers, heterotrophs, denitrifiers, sulfate-reducers, iron-reducers and methanogens. Twenty-two isolates were purified from the group of acid-producers, heterotrophs, denitirifiers and sulfate- reducers and were prepared for identification. Strains of denitrifying, sulfate-reducing (SRB) and acid-producing (APB) bacteria have already been used in antimicrobial screening and test loop studies. Conditions for the laboratory-scale test loop system like gas and culture medium flow rate, temperature, inoculation period and length of incubation were established using Indiana isolates APB #139 and SRB # 139-A. Total cell count and viable cell counts showed increasing numbers from Day 0 to Day 28 which is indicative that test loop parameters were optimized and can be useful for the succeeding trials. Only minor modifications will have to be made mechanically. For the batch culture corrosion experiment, the extent of corrosion on metal coupons incubated with the extracts was monitored over time by weight loss determination. The study against *Desulfovibrio vulgaris* showed that one (S_1M) out of the four *Capsicum* sp. extracts tested was effective in controlling the corrosion rate in metal coupons by 33.33% when compared to the untreated group.

EXPERIMENTAL

Objective 1 – Isolate and cultivate MIC-causing microorganisms / biofilm

Five samples from the corroded gas pipelines obtained from Northern Indiana Public Service (Trenton, IN) were processed anaerobically using the Most Probable Number (MPN) method (Garthright 2001) and standard anaerobic techniques (Holdeman, 1977; Balch and Wolfe, 1979) to isolate microorganisms from the groups of acid-producing bacteria (APB), heterotrophic bacteria, denitrifying bacteria, sulfate-reducing bacteria (SRB), iron-reducing bacteria and methanogenic bacteria. Salinity and pH of the six types of media that correspond to each bacterial group was adjusted according to that of the original sample. The tubes were incubated at 30^{o}C and MPN was determined after 6 weeks. 100 uL aliquot from the MPN tube was streaked onto corresponding agar plate media and incubated anerobically at 30^{o}C. Isolates were purified by repeatedly streaking on agar plates until isolated colonies were obtained.

Objective 2 – Design, construct and test a laboratory-scale pipeline simulation system

The previously reported test loop system was used in this experiment. C1018 steel coupons (Metal Samples Co., Munford, AL) with a diameter of 7/8 inch and thickness of 1/8 inch were cleaned ultrasonically in ethanol for 1 min, degreased with acetone air-dried and stored under vacuum (Jayaraman, et al, 1997). They were weighed, glued to plastic studs and inoculated with the bacteria before being attached to the test loop. A mixture of 2% (v/v) each of the overnight culture of APB isolate #139 and SRB isolate #139-A incubated at 30^{o}C were used as inoculum. Coupons were submerged to the inoculum in petri dishes and were incubated inside the anaerobic glove box for 96h prior to attachment to the loop. After studying the flow rates, growth media at room temperature was pumped into the loop allowing a gentle spray to each coupon for 2 seconds every 30 minutes and waste was collected in a separate reservoir. Wet methane gas was supplied by allowing it to pass through a flask with water at the inlet of the test loop. Duplicate coupons were collected at day 0 and every 7 days thereafter for total cell count, viable cell count, and weight loss determination. All coupons were rinsed with 0 05M phosphate buffered saline (PBS), pH 6.8 to remove any non-adherent cells. For total cell count determination, coupon surfaces were

scraped 7-10 times using a sterile scalpel blade into a 2% glutaraldehyde in cacodylate buffer pH 7.3 and sonicated for 5 mins before being fixed at room temperature for 2h or at 4^{o}C overnight. Total cell count was done by staining the cells with 0.1 mg/mL acridine orange solution in phosphate buffer after serial dilution and counting the number of fluorescent cells under epifluorescence microscopy with an emission wavelength of 488-514 nm (Stoodley, et al, 1999). For viable cell count determination, coupon surfaces were scraped using sterile scalpel blade into PBS and sonicated for 5 mins to disperse any clump of cells. Viable cell count was done by staining cells with Live /Dead *Bac*Light (Molecular Probes, OR) after serial dilution and counting the number of cells that fluoresce green under epifluorescence microscopy with an emission wavelength of 500- 635 nm. Corrosion rate was calculated in mm/y (ASTM, 1999) after being cleaned according to ASTM standard methods (ASTM, 1999).

Objective 5 – Identify and test effective concentrations of pepper oil components

The same kind of coupons as above were prepared similarly and used for this study. They were weighed and placed inside rubber –stoppered flask and autoclaved using the gravity cycle (121^{o}C, 15 mins.). The flasks were incubated at 30^{o}C with 2% inoculum (v/v) from an overnight *D. vulgaris* culture in Modified Baar's Medium and the respective test compounds such as the pepper extracts (H1E at 312.5 ug/mL; H1M, S1E, S1M at 156 ug/mL), ampicillin (6.25 ug/mL), and methanol (0.625%). These final concentrations were based on the results of the minimum inhibitory concentration assay that was done previously. Duplicate coupons were harvested every 7 days up to 28 days while the media with the test compounds was replenished. The coupons were cleaned according to ASTM standards (ASTM, 1999) and weighed. The specific mass loss was determined in mg/cm^2 (for the total surface of the coupon, 3.87 cm^2) as an indicator of the extent of corrosion (Jayaraman, et al, 1999) and the corrosion rate was calculated in mm/y (ASTM, 1999).

RESULTS AND DISCUSSION

A total of 22 isolates from the groups of acid producing, sulfate reducing, denitrifying, and heterotrophic bacteria were obtained from 5 samples from Northern Indiana Public Service. They were prepared and will be sent out for identification using the 16S rRNA gene sequence. Isolates SRB #139 A, B,C, and denitrifier #139 were used in antimicrobial screenings of some biocide components for inhibiting microbially-influenced corrosion (data not shown). A mixture of isolates APB #139 and SRB #139-A were used to grow biofilms on metal coupons that were used in the laboratory-scale test loop simulation system. Figure 1 shows an increasing trend of the total number of cells and viable cells on the metal coupons from Day 0 to Day 28. This indicates that conditions in the test loop system were optimal, thus, favoring the growth of both strains. Corrosion monitoring showed increasing weight loss over the 28 day period and decreasing trend in the corrosion rate from 0.318 mm/y on Day 0 to 0.131 mm/y on Day 28 (Figure 2). Day 0 samples were those that were collected after the 96h inoculation period and not attached to the test loop to serve as the baseline for corrosion determination. Some minor modifications need to be done on the media sprinklers inside the pipeline to make sure that the coupons will not dry up during the whole course of the experiment.

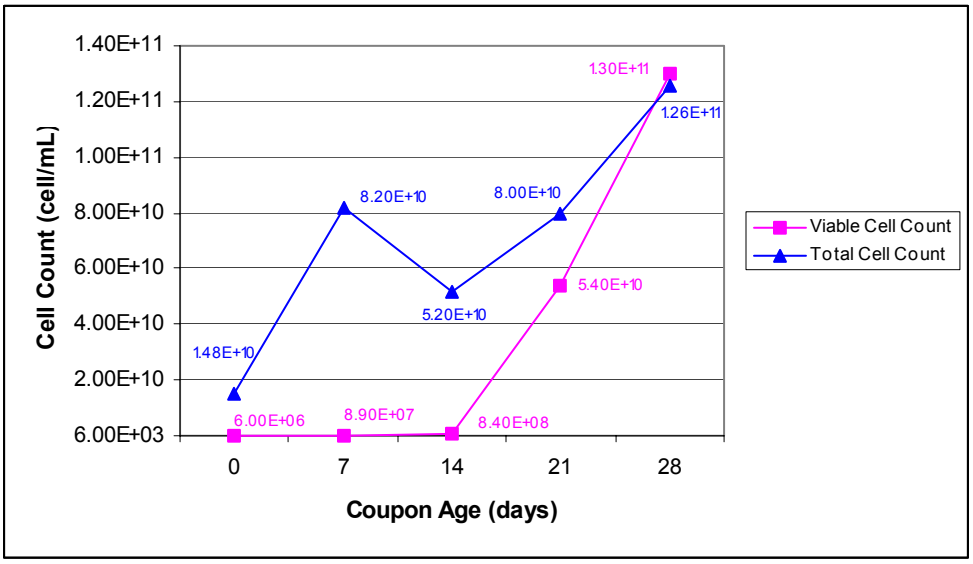

Figure 1. Total cell count and viable cell count of APB #139 and SRB #139-A on metal coupons used in the laboratory-scale test loop system.

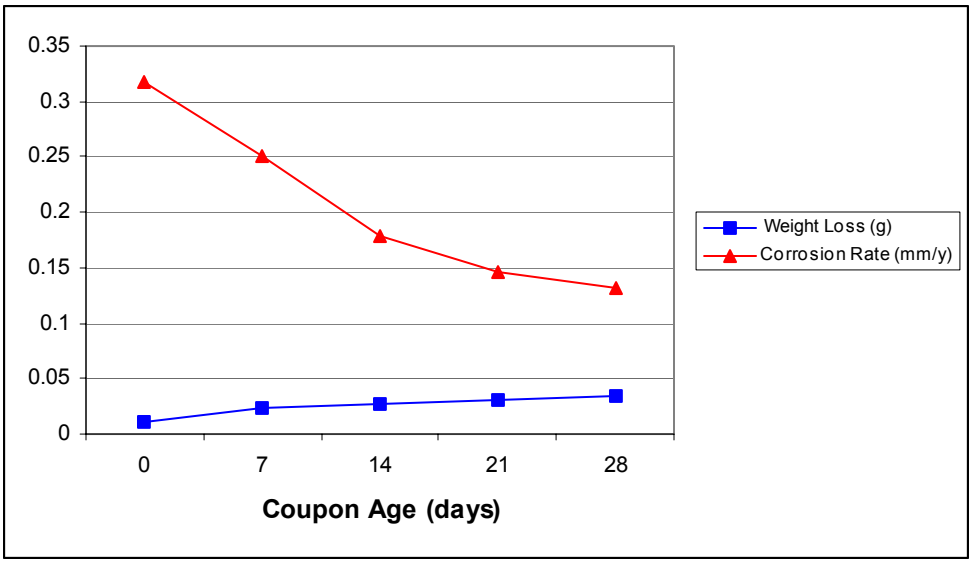

Figure 2. Weight loss and corrosion rate determination on metal coupons collected from the test loop system. Day 0 coupons were inoculated by the bacteria but were harvested before the system started running.

Mass loss determination was done on metal coupons that were inoculated with *D. vulgaris* and treated with the pepper extracts to check the extent of corrosion. Corrosion rates were calculated in millimeter. Results showed that out of the 4 *Capsicum sp*. extracts tested only S1M at 156 ug/mL reduced the corrosion rate by 33.33% when compared to the untreated group (Figure 3). Other varieties of *Capsicum sp*. are currently being subjected to extraction processes to produce more extracts that can be tested alone or in combination for their biofilm and corrosion inhibiting capabilities.

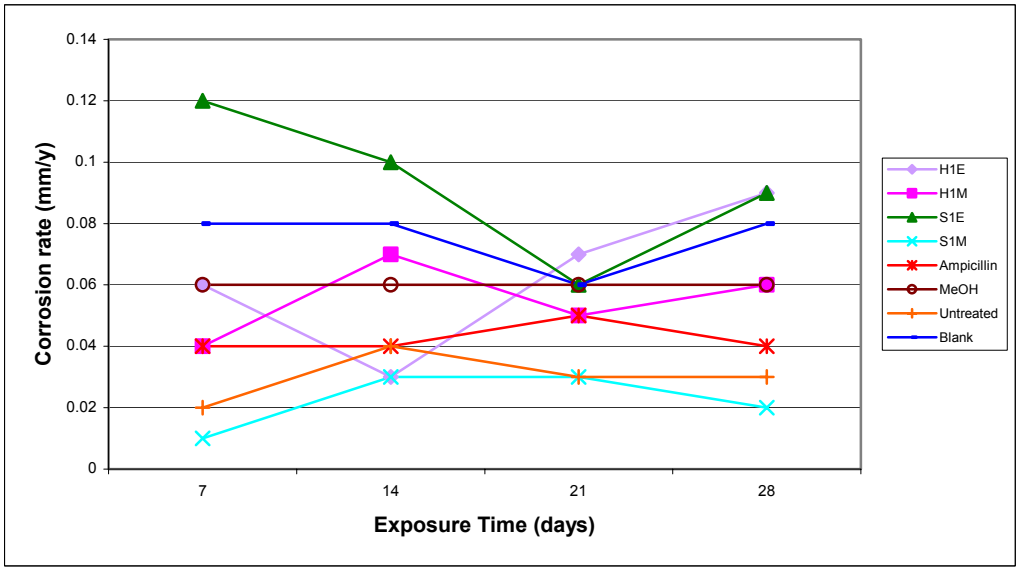

Figure 3. Corrosion rates in metal coupons that were treated with *Capsicum* sp. extracts for 4 weeks

CITATIONS

American Society for Testing and Materials. 1999. Standard practice for preparing, cleaning and evaluating corrosion test specimens. Designation: G1-90. ASTM, West Conshohocken, PA. pp. 1-8.

Garthright, W.E. 2001. Bacteriological Analytical Manual Online. U.S. Food and Drug Administration, Center for Food Safety and Nutrition.

Holdeman, L.V., Cato, E.P. and Moore, W.E.C. eds. 1977. Anaerobe Laboratory Manual, 4th ed. Southern Printing Company, Blackburg , VA.

Jayaraman, A., J.C. Earthman and T.K. Wood. 1997. Corrosion inhibition by aerobic biofilms on SAE 1018 steel. *Appl. Microbiol. Biotechnol*. **47**:62-68.

Jayaraman, A., P.J. Hallock, R.M. Carson, et al. 1999. Inhibiting sulfate-reducing bacteria in biofilms on steel with antimicrobial peptides generated in situ. *Appl. Microbiol. Biotechnol*. **52**: 267-275.

Stoodley, L.H., Rayner, J.C., Stoodley, P., Lappin-Scott, H. M. 1999. Methods in Biotechnology Vol. 12: Environmental Monitoring of Bacteria. Humana Press, NJ. pp. 307-319.