



Great Lakes Maritime Research Institute

A University of Wisconsin - Superior and
University of Minnesota Duluth Consortium

Development and Succession of Microbial Communities Associated with Corroding Steel Pilings in the Duluth-Superior Harbor

Interim Report

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November 15, 2008

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*Research funded in part by the Great Lakes Maritime Research Institute.
This study was supported by the U.S. Maritime Administration
Grant # DTMA1-G-06-005*

Executive Summary

The overall objective of this research is to determine if corrosion of sheet steel pilings observed in the Duluth-Superior Harbor is accelerated by microbiologically influenced corrosion (MIC). The corrosion of sheet steel coupons was compared at six sites in the Duluth-Superior Harbor, ranging from a low corrosion site (Oliver Bridge), sites in the outer harbor (DSPA berth 4, Cutler Magner), to sites in the inner harbor (Midwest Energy, Hallett Docks 5 and 7) where corrosion is several times higher than expected in freshwater. Microbial biofilms that developed on the sheet steel coupons at these sites were sampled in August 2007 and again in October 2008. DNA was extracted from biofilms collected in 2007 and a preliminary comparison of the molecular diversity of these bacterial communities was completed using T-RFLP, a DNA fingerprinting method. This comparison revealed that different bacterial communities had developed on steel coupons from three sites in the inner and outer harbor during the first ten months of exposure. DNA has yet to be extracted from the samples collected in early October 2008. DNA fingerprints of the bacterial communities in these samples will be constructed, compared to each other to determine if different communities develop at various sites in the harbor affected by corrosion, and compared to samples collected from the same sites in 2007 to evaluate the development and succession of these communities. In addition, the structure of bacterial communities that develop on these steel coupons in the harbor will be compared to those that develop on identical coupons in the laboratory to help verify that a separate laboratory experiment mimic conditions that control the development of bacterial communities in the Duluth-Superior Harbor.

Introduction

Steel sheet piling material used for docks, bridges and bulkheads in the Duluth-Superior Harbor (DSH) has been reported to be corroding at an accelerated rate (Marsh et al. 2005). The increased rate of corrosion appears to have begun in the late 1970's in the DSH and will require expensive replacement if the cause and possible remedies cannot be identified. About 20 kilometers of steel sheet piling appear to be affected in the DSH, which an initial analysis in 2005 estimated may cost more than \$100 million to replace (Marsh et al. 2005) but which now may cost more than twice as much as this initial estimate (A. Ojard, Duluth Seaway Port Authority, pers. comm.).

Most of the corrosion in the DSH is confined to the first 1.5 meters below the water line and decreases from 1.5 to 3 meters below the surface. Extensive zebra mussel colonization occurs on these pilings from about 3 m to the bottom of the steel pile, where little or no corrosion is observed. The steel sheet piling reported to be corroding at an accelerated rate has an orange rusty appearance characterized by blister-like, raised tubercles on the surface. These tubercles vary in diameter from a few millimeters to several centimeters and when removed, large and often deep pits (6 to 10 mm) are revealed in the steel piling, which is sometimes perforated. This pattern of corrosion is consistent with the appearance of corrosion caused by iron-oxidizing bacteria (Hamilton 1985).

Three lines of evidence indicate that the rate of corrosion of steel materials is very fast in the DSH. First, the pit depths in steel pilings within this harbor are deeper than the average pit depths for comparable corroding materials in freshwater (Marsh et al. 2005). More recently in early September 2006, Bushman & Associates, Inc. (Bushman 2006) measured corrosion rates of 5 to 6 mils per year using a linear polarization resistance method at sites that appeared most affected by corrosion in the DSH. They indicated that these rates were considerably higher (approx. 2 to 12 times) than normally measured in potable waters and can only be explained by some factor accelerating the corrosion rate such as microbiologically influenced corrosion (MIC). Finally, orange corrosion tubercles rapidly appeared on unprotected steel sheet pilings that were replaced at the Superior Entry to the DSH during June and July 2006. By September 2006, there were already 2 to 3 mm deep pits below these corrosion tubercles on these steel sheet pilings

The corrosion in the DSH appears similar to accelerated low water corrosion (ALWC) reported during the past decade on marine steel pilings in the United Kingdom and Baltic Sea (Christie 2001, Graff and Seifert 2005), which may be accelerated by the action of sulfate-reducing bacteria. MIC is rarely caused by a single microbial group, but more often by consortia of microbes including iron-oxidizing and sulfate-reducing bacteria (Hamilton 1985, Rao et al. 2000, Starosvetsky et al. 2001). Thus, we examined bacterial communities in biofilms attached to sheet steel coupons at corroded sites and areas less affected by corrosion to understand the colonization and development of these communities and determine if differences in the composition of bacterial communities at these sites indicate the participation of different bacterial species in the accelerated corrosion process ongoing in the DSH.

The specific objectives of this project were to:

1. Sample microbial biofilm communities on sheet steel coupons over 16 months at six sites that span a spectrum of corrosive environments in the Duluth-Superior Harbor
2. Extract DNA from these microbial biofilms for molecular analyses
3. Compare the molecular diversity of the bacterial communities on these steel coupons using a community DNA fingerprinting method (T-RFLP) to understand the development of these communities and determine if specific types of communities are affiliated with high corrosion areas.

Methods

Metal tray frames containing sheet steel coupons were attached 1 meter below the waterline to corroding structures at six sites in the DSH between October 3 and 17, 2006 by AMI Consulting Engineers (Fig. 1). These six sites span the spectrum of low to high corrosion environments in the DSH: Duluth Seaway Port Authority berth 4 (DSPA), Cutler Magner Co. dock (CM), Hallett Dock 5 (HD5), Hallett Dock 7 (HD7), Midwest Energy dock (MWE), and the Oliver Bridge (OB). Each tray contains eight (0.375 inch thick), rectangular steel coupons (4.5 x 7.5 inches) cut from hot rolled ASTM-A328 steel, the same material used to construct steel sheet pilings used for docks and bulkheads in many parts of this harbor. Each coupon was weighed on a certified scale to the nearest 0.1 gram before being placed in the harbor and isolated from the steel tray frame and other coupons with HDPE plastic spacers (Fig. 2).

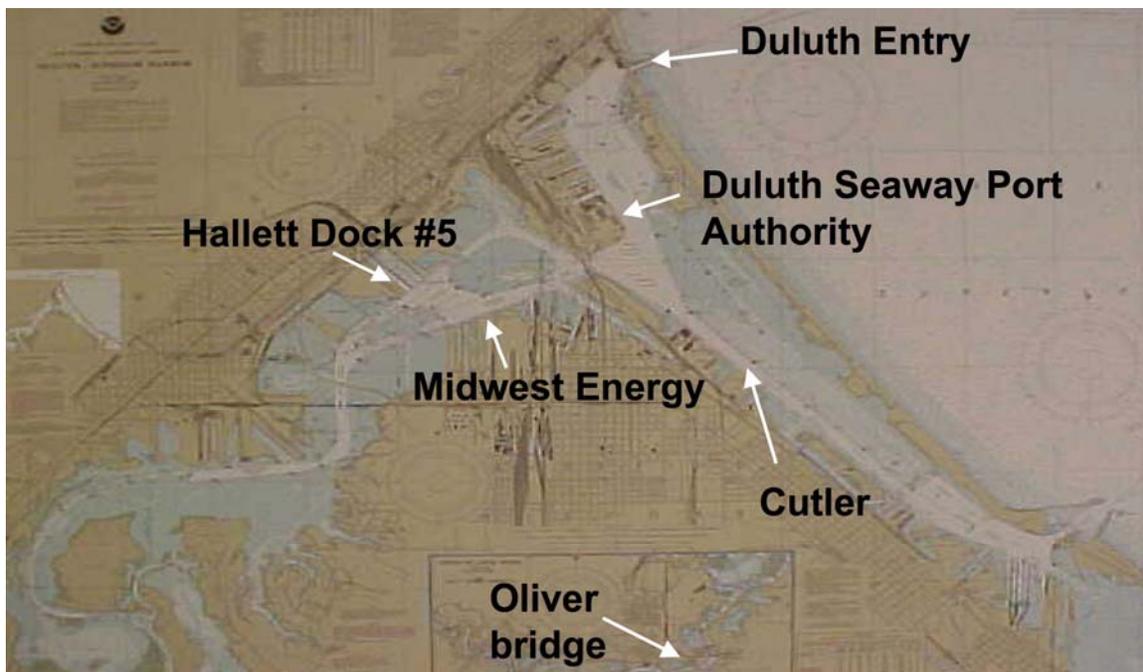


Figure 1. Map showing locations of sheet steel coupon trays in the Duluth-Superior Harbor. [Map courtesy of AMI Consulting Engineers]

A commercial diver from AMI Consulting Engineers, who installed the coupon frames for the U.S. Army Corps of Engineers, recovered two steel coupons from each site on August 14, 2007 and again on October 8, 2008. All coupons were transported to the laboratory in water from which they were collected. Photographs were taken of both sides of each coupon to visually document the extent of corrosion.



Figure 2. Photograph of metal tray frame and sheet steel coupons (separated by white HDPE spacers) before being installed in the Duluth-Superior Harbor during October 2006. [Photograph courtesy of AMI Consulting Engineers]

Sampling microbial biofilms –

Microbial biofilm material was removed from the first coupon from each site to analyze the diversity and development of bacterial communities attached to these steel sheets. On 14 August 2007, six replicate subsamples were scrapped from each coupon, three from each side. A metal weighing spatula was used to scrap material from a 6 x 11.5 cm area. Material from each replicate was placed in a sterile 50 ml plastic centrifuge tube with a rinse of Milli-Q water used to clean the scrapped replicate surface. Each subsample was frozen at -20°C until DNA was extracted. On the same day coupons were collected in October 2008, biofilm material was scrapped from both surfaces of each coupon with an acrylic scraper, and rinsed into a sterile plastic trough with Milli-Q water. Afterwards, the coupon surfaces were brushed with a toothbrush and rinsed again with Milli-Q water. The combined scrapings and rinses from each of the coupons were transferred to sterile 50 ml plastic centrifuge tubes and frozen at -20°C until DNA can be extracted.

Dr. Brenda Little (Naval Research Laboratory, Stennis Space Center, MS) is examining microbial biofilm communities and corrosion products on the second coupon from each site using an environmental scanning electron microscopy. Afterwards, both coupons from each site will be returned to AMI Consulting Engineers, scrubbed clean of all biological material, digested for 10 min. at 24°C using the ASTM C.3.5 digestion protocol for removing biological

material, corrosion pits will be counted, and then the coupons will be reweighed to estimate the weight loss of due to corrosion.

Microbial community DNA extraction –

Total DNA extracted directly from microbial biofilm samples was used for 16S rDNA-based T-RFLP analysis to identify differences in bacterial communities on coupons from all sites. Total DNA in microbial biofilms (3 replicates each) on steel coupons collected from each of the six sites sampled in 2007 (18 total samples) was extracted using an UltraClean Soil DNA Kit (MoBio Laboratories, Inc.) following the instructions of the manufacturer. Nucleic acid concentrations and purity were determined spectrophotometrically by measuring absorbance with a Nanodrop spectrophotometer. Samples removed from the coupons recovered during October 2008 are still being processed. DNA will be extracted and purified from these samples using the same procedure.

T-RFLP community DNA fingerprinting –

Terminal restriction fragment length polymorphism (T-RFLP) analysis, a community DNA fingerprinting technique, was used to distinguish bacterial communities collected from different sites based on the size of terminal fragments of 16S rRNA genes following restriction endonuclease digestion (Braker et al. 2001, Moeseneder et al. 1999). A preliminary analysis has been completed for biofilm samples recovered from coupons collected on August 14, 2007. I have requested an extension for this project, so DNA can be extracted and T-RFLP analyses can be completed for biofilm samples recovered from steel coupons collected from the six sites in the DSH on October 8, 2008.

A PCR primer set (27F, 1492R) specific for the Bacteria domain (Lane 1991, Reysenbach et al. 1994) was used to directly amplify the 16S rDNA gene in DNA extracted from microbial biofilms. The forward primer was labeled on the 5' end with 6-carboxyfluorescein (6-FAM). Integrated DNA Technologies (IDT) synthesized all oligonucleotide primers. DNA from coupon biofilms in 2007 was amplified using puReTaq Ready-To-Go PCR beads and 25 pmol of each primer with a BioRad DNA Engine thermal cycler. The reaction conditions were 94°C for 3 min followed by 35 cycles of: 94°C for 1.5 min, 60°C for 1.5 min, and 72 °C for 2 min. After 35 cycles, extension was continued at 72°C for 7 min and then the samples were cooled to 4°C until further analysis. DNA from *E. coli* was used as a positive bacterial control. In addition to blanks (containing no DNA template), DNA from *Sulfolobus solfataricus* (ATCC 35091), an archaeal microorganism, was used as a negative control. PCR reaction mixtures containing bacterial rDNA products of the expected size (~ 1,500 base pairs, visualized on 1.5% agarose gels) were cleaned with a MoBio UltraClean PCR Clean-Up Kit.

Cleaned PCR products (200 ng) were then digested at 37°C for at least 3 h with *HaeIII* or *RsaI*. The restriction fragments were precipitated, dried, and redissolved in 10 µl of sterile, nuclease free water. The sizes of the terminal restriction fragments in these digests were determined on an ABI DNA sequencer in the GeneScan mode at the University of Minnesota BioMedical Genomics Center. The pattern of terminal restriction fragments from each sample was imported into the BioNumerics statistical software package (Anonymous 2005). The molecular weights (in base pairs) of peaks in individual samples were normalized to internal molecular weight standards (MapMarker®-1000 standard).

Afterwards, a preliminary analysis was conducted for samples collected in August 2007 to compare differences in the bacterial biofilm communities at three sites in the harbor (DSPA, Cutler Magner, Hallett Dock 7). Dendrograms showing the similarity of bacterial communities in these samples were constructed with the BioNumerics statistical software package using Pearson correlations and the UPGMA clustering method. Similarities between different samples were calculated based on Pearson correlations of terminal restriction fragment patterns between 50 and 1,000 base pairs in size.

Results and Discussion

Community DNA fingerprints based on a preliminary T-RFLP analysis (Fig. 3) indicated that bacterial biofilm communities collected from steel coupons in the inner harbor (DSPA, Cutler Magner) were different than the bacterial community composition of biofilm collected at one site in the inner DSH (Hallett Dock 7) during August 2007.

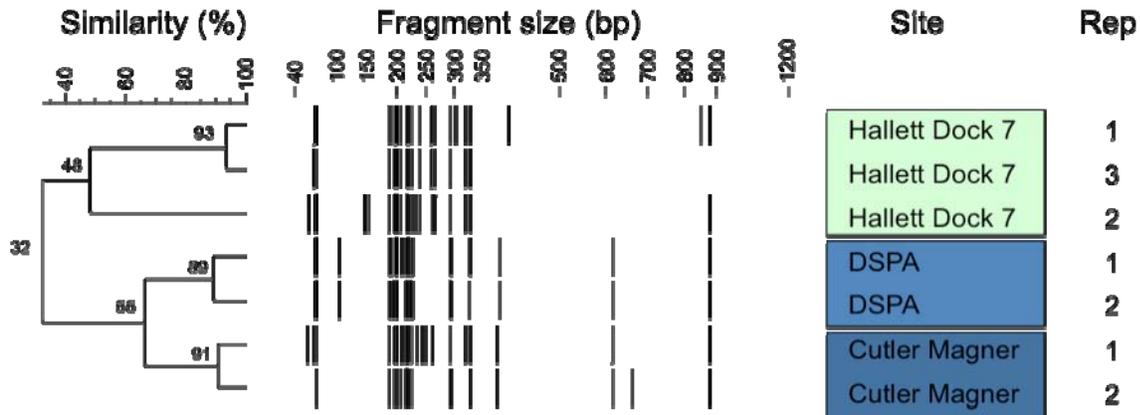


Figure 3. Similarity of bacterial biofilm communities on steel coupons after exposure for 10 months (on August 14, 2007) at inner harbor (Hallett Dock 7) and outer harbor (DSPA, Cutler Magner) sites in the Duluth-Superior harbor. T-RFLP analysis of *Hae*III digests of bacterial 16S rDNA PCR products. Similarities are based on Pearson correlations of terminal restriction fragment sizes between 50 and 1,000 base pairs.

After DNA is extracted from steel coupon biofilm samples recovered in October 2008, similar DNA fingerprint comparisons will be completed for bacterial biofilms attached to steel coupons at various sites in the harbor. Comparisons of the composition of bacterial communities on steel coupons in 2007 and 2008 will also be completed to investigate the development and succession of bacterial biofilms at each site in the DSH. Finally, the composition of bacterial biofilm communities on steel coupons placed in the DSH will be compared to the composition of bacterial communities that develop on identical coupons in a separate laboratory study, which is investigating factors that may control corrosion of sheet steel pilings in the DSH.

Preliminary Conclusions –

- The surfaces of sheet steel coupons were covered by complex microbial biofilms and

corrosion tubercles appeared within 10 months after exposure to harbor water.

- After 10 months of exposure, bacterial biofilm communities appear to be different on steel coupons incubated at outer (DSPA, Cutler-Magner) and inner harbor sites (Hallett Dock #7) in the Duluth-Superior harbor.
- Future analyses will evaluate how bacterial biofilm communities develop on sheet steel in the DSH and determine if the composition of attached bacterial communities affiliated with low and high corrosion areas in this harbor are different.

Dissemination of Study Results

1. Publications

None to date

2. Presentations

Hicks, R. E. 2008. Microbial Aspects of Corrosion in the Duluth-Superior Harbor. Natural Resources Research Institute Muffin Meeting, Duluth, MN. April 15, 2008 (oral presentation)

Hicks, R. E. 2008. Microbial Aspects of Corrosion in the Duluth-Superior Harbor. Propeller Club, Superior, WI. April 25, 2008 (oral presentation)

Bostrom, J. 2008. Microcosm Experiment. US Army Corps of Engineers Harbor Technical Advisory Committee, Duluth, MN. June 3, 2008 (oral presentation)

Little, B. J., J. S. Lee, R. I. Ray, J. Bostrom, J. M. Bergin, and R. E. Hicks. 2008. The Potential role of Iron-Oxidizing Bacteria in the Corrosion of Carbon Steel Pilings in a Freshwater Harbor. EUROCORR 2008, Edinburgh, Scotland. Sept. 11-17, 2008 (oral presentation)

Hicks, R. E. 2008. Development and Succession of Microbial Communities Associated with Corroding Steel Pilings in the Duluth-Superior Harbor. GLMRI Annual Affiliate Meeting, Superior, WI. Sept. 26, 2008 (oral presentation)

Hicks, R. E., J. M. Bergin, J. Bostrom, R. I. Ray, and B. J. Little. 2008. Structure of Bacterial Communities Associated with Accelerated Corrosion of Port Transportation Infrastructure. 17th International Corrosion Congress, Las Vegas, NV. October 6-10, 2008 (oral presentation)

3. Other

Bostrom, J. Microbial Corrosion of Steel Structures in the Duluth-Superior Harbor (tentative title). M.S. Thesis, Integrated Biosciences Graduate Program, University of Minnesota Duluth (in progress)

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Acknowledgments

We thank Chad Scott (AMI Consulting Engineers) for collecting steel coupons from frames at different sites in the Duluth-Superior Harbor during August 2007 and October 2008. This work was supported by grants from the Great Lakes Maritime Research Institute, The US Army Corps of Engineers (USACE), and the Minnesota Sea Grant College Program, NOAA Office of Sea Grant, United States Department of Commerce.