Great Lakes Maritime Research Institute

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

Determining if Microbiologically Influenced Corrosion is Responsible for the Accelerated Loss of Port Transportation Infrastructure

Final Report

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Executive Summary

Steel structures in the Duluth-Superior harbor on Lake Superior in northern Minnesota are reported to be corroding at an accelerated rate. This study examined the possible role of microbiologically influenced corrosion (MIC) in this phenomenon, specifically the roles of iron-oxidizing and sulfate-reducing bacteria. A laboratory microcosm experiment of five different treatments was designed to explore the influence of water quality and microbes on the corrosion of sheet steel coupons. The treatments included unaltered harbor water, harbor water supplemented with sodium sulfate, autoclaved harbor water, autoclaved harbor water with an iron-oxidizing bacterium inoculum, and unaltered Lake Superior water. Replicate steel coupons were placed in microcosms of the five treatments and maintained for 12 months. The instantaneous corrosion rate of steel coupons was measured semimonthly and biofilm samples were collected each month from additional coupons in each treatment. The highest instantaneous corrosion rates were observed in the harbor water treatments (with or without added sodium sulfate) and the lowest rate was observed in Lake Superior water. Cumulative corrosion parameters were measured at the end of the experiment and showed different trends than the instantaneous corrosion rate. The coupon area covered by corrosion tubercles was not different between treatments. More weight was lost from steel coupons immersed in Lake Superior water than from coupons in autoclaved harbor water. Other than this difference, the weight of steel lost from coupons was not different in the various treatments. The deepest corrosion pits formed on steel coupons in Lake Superior and unaltered harbor water. Extracellular polymers produced by microorganisms were more abundant on coupons in harbor water that was not autoclaved and by the end of the experiment, there were more iron-oxidizing bacteria on coupons immersed in harbor water (with or without added sulfate) than in any of the other treatments. The abundance of the dissimilatory sulfite reductase (*dsr*A) gene, a proxy for the abundance of sulfate-reducing bacteria, increased several orders of magnitude on coupons in harbor water from near zero to over 10^6 copies•cm^{-2}. After five months, coupons in harbor water (with or without added sulfate) had more copies of the *dsr*A gene than coupons exposed to Lake Superior water. These results indicate that the abundance of sulfate-reducing bacteria and to a lesser degree the abundance of iron-oxidizing bacteria and extracellular polymers were correlated with the instantaneous corrosion of steel coupons in the laboratory. Thus, these groups of bacteria as well as other types of bacteria within these biofilms should be investigated further in future studies because their activities may be directly or indirectly responsible for the accelerated corrosion of steel structures in the Duluth-Superior harbor.

Introduction

Sheet steel structures in the Duluth-Superior harbor (DSH) are reported to be corroding at an accelerated rate (Marsh et al. 2005, Bushman and Associates 2006). Many structures have developed large orange blister-like, raised tubercles. Corrosion tubercles are hollow mounds of corrosion products and deposits that cap localized region of metal loss. Under these tubercles, deep pits may form in the steel, and at its most severe, the corrosion can leave large holes that perforate the sheet steel of docks and bulkheads. Up to 20 km of structures may be affected by corrosion in the DSH. Many other harbors in western Lake Superior are also reported to show a similar corrosion phenomenon (Sharrow, J. and Clark, G. pers. comm.).

It is clear that corroding steel structures in the DSH are covered by complex microbial biofilms that contain bacteria of the type responsible for corrosion of steel in other environments (Hicks 2009). Several types of bacteria have been implicated in corrosion of steel in other environments, including iron-oxidizing bacteria (Xu et al. 2007). We previously isolated an iron-oxidizing bacterium tentatively identified as *Sideroxydans lithotrophicus* from corroded areas in the DSH. Also, electron microscopy has shown that large numbers of bacteria, possibly iron-oxidizers, grow on the underside of tubercles and associate with amorphous iron hydroxides. In addition to iron-oxidizing bacteria (FeOB), sulfate-reducing bacteria (SRB) have been implicated in corrosion in other environments (Hamilton 1985, Little et al. 2000). The concentration of sulfate varies with location in the DSH. Some areas with the highest dissolved sulfate concentrations also show the most aggressive corrosion and highest corrosion rates.

The pattern of corrosion in this harbor that correlates with dissolved sulfate concentration, the presence of iron-oxidizing bacteria, and the abundance of the dissimilatory sulfite reductase gene from SRBs found on the surface of corroding steel suggests that microbes are associated with this corrosion, possibly through a phenomenon known as microbiologically influenced corrosion (Little and Lee 2007, Xu et al. 2007). Microbiologically influenced corrosion (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). While data from our prior field research (Hicks 2009) lead us to suspect that MIC may be responsible for the accelerated corrosion seen in this harbor, they did not provide conclusive evidence. Thus, a laboratory experiment was designed to test the effects water quality and microbes in this corrosion process and demonstrate if the metabolism of microbial biofilms attached to these steel structures is accelerating the corrosion of the sheet steel material in the DSH.

The specific objectives of this project were to:

1. Construct and maintain a microcosm experiment to determine if microbiologically influenced corrosion is the cause of steel corrosion occurring in the DSH.
2. Measure the instantaneous rate of steel corrosion in non-biological and biological treatments to estimate the acceleration of corrosion due to microbial activities.
3. Determine if iron-oxidizing and sulfate-reducing bacteria are responsible for accelerating the corrosion of sheet steel used to construct docks and bulkheads in the Great Lakes.

Methods

We examined the effect of five experimental treatments on the corrosion of the steel coupons (Table 1). This study combined measurements of corrosion of experimental steel coupons (instantaneous corrosion rate, area covered by tubercles, and corrosion weight loss and pit depth) with an investigation of bacteria that colonize and develop on these coupons in each experimental treatment.

Table 1. Experimental treatments in aquatic microcosms.

Treatment	Description
1	Autoclaved harbor water
2	Unaltered harbor water
3	Unaltered Lake Superior water
4	Harbor water supplemented with sodium sulfate
5	Autoclaved harbor water + *S. lithotrophicus* inoculum

Microcosm construction –
Experimental microcosms constructed from 10-gallon glass aquaria (Aqueon Glass, 50.8 cm x 25.4 cm x 30.5 cm) were used to test the corrosive effects of various chemical and microbiological treatments (Table 1). Each microcosm was equipped with an aquarium pump (Aquarium Systems Mini-Jet 404) to constantly circulate the water (~ 2 L/hr), and covered with a piece of acrylic (0.2 cm x 50.8 cm x 25.4 cm) with one corner cut out to allow gas exchange. The microcosms and covers were washed with soapy water and hydrochloric acid (10% by volume), and rinsed with Milli-Q water, and the aquarium pumps were soaked in 70% ethanol before treatments were established and when these treatments were renewed. All microcosms were incubated in the dark at 13°C (e.g., average water temperature in the DSH during the ice-free season) in a variable temperature room.

Duplicate microcosms of the five treatments were constructed and filled with water (20 liters) of each treatment type. The first experimental treatment consisted of autoclaved harbor water, which served as a "reduced" biological treatment but not a sterile non-biological control. Harbor water was collected from the intake of the Great Lakes Aquarium in Duluth, MN. Water in this treatment was autoclaved for 60 min at 121°C. The second treatment contained unaltered harbor water. The third treatment used water from the nearshore zone of Lake Superior, collected northeast of Duluth from the Lakewood City Water Intake Station, which serves as the water supply for Duluth, MN. Both the harbor and lake water from these sources were available year-round, when ice prevents direct access to the harbor and Lake Superior. The fourth treatment contained unaltered harbor water that was supplemented with sodium sulfate (44 mg/L). The final treatment contained autoclaved harbor water and steel coupons that were inoculated with an iron-oxidizing bacterium, which was isolated from corroding steel in the Duluth-Superior harbor and tentatively identified as *Sideroxydans lithotrophicus* by partial sequencing of its 16S rRNA gene. This bacterial strain (HD506_02) was grown in FeS gradient culture tubes (Emerson and Moyer 1997), then the growth bands were extracted from multiple tubes (~10-12 ml) and used to inoculate the steel coupons with an atomizer bottle (~2 ml per coupon). Treatments in each

aquatic microcosm were renewed monthly (within a few days of sampling) to prevent fouling of the water that was expected because the microcosms are closed systems (Ksoll et al. 2007). After the steel coupons were temporarily removed, water was emptied from each microcosm and then the microcosms, covers, and pumps were cleaned as before. New water of the appropriate treatment was placed in each microcosm and then the steel coupons (unaltered during treatment renewal) were reintroduced. Fresh *S. lithotrophicus* inoculum were sprayed on coupons from the autoclaved harbor water + *S. lithotrophicus* treatment before these steel coupons were returned to their respective microcosms.

Steel coupons (19.1 cm x 11.7 cm x 1.25 cm) identical in composition to the sheet steel used to construct docks and bulkheads in the harbor were cut from cold rolled sheet steel (ASTM A328, a low carbon steel), which the Hallett Dock Company (Duluth, MN) donated after replacing a portion of their corroded docks in 2007. About 90% of the docks and bulkheads in the DSH are constructed from this type of steel (Chad Scott, AMI Consulting Engineers, pers. comm.). Each experimental microcosm contained three steel coupons. Each of these coupons was washed with soapy water, lightly brushed, and rinsed with Milli-Q water to remove any loose material. Each coupon was designated with a unique number, weighed (each coupon weighed about 2 kg before the experiment), and then wrapped in aluminum foil and autoclaved before being randomly assigned to a specific experimental treatment. All coupons were equipped with a high-density polyethylene (HDPE) handle (10.2 cm x 3.8 cm x 2.5 cm) to facilitate handling of the coupon. Each microcosm had an HDPE coupon holder (25 cm x 20 cm x 2.5 cm) with three grooves (25 cm x 1.3 cm) to hold three steel coupons upright and keep them electrically isolated from one another. Like the aquaria, the HDPE handles and holders were washed with soapy water, and then rinsed with dilute hydrochloric acid (10% by volume), and Milli-Q water.

One steel coupon in each microcosm was modified to measure polarization resistance and subsequently estimate the instantaneous corrosion rate. Biofilm on the surface of these coupons was not sampled each month from these coupons, but all biofilm material was removed for possible analysis at the termination of the experiment. A copper wire (12-gauge) was brazed to the top edge of these coupons next to the HDPE handle so as not to interfere with the formation of corrosion products on the face of the coupons. The plastic insulation was stripped from the top 5 cm of the wire, and the bare wire and brazed surface was covered with an epoxy glue to protect it from corrosion. Thus, the steel coupon and not the wire served as the working electrode.

Sampling –

The experiment was started on May 15, 2008 and maintained for 12 months. The polarization resistance of modified steel coupons was measured semimonthly. Each month, biofilm and corrosion products were collected from the surface (about 20-30 cm^2) of two steel coupons in each microcosm (those not used for polarization resistance measurements). Biofilm and corrosion products from each coupon were scraped into a sterile 50 ml plastic tray using an acrylic scraper. After scraping, this area on each coupon was rinsed into the same tray with Milli-Q water. This scraped area was then scrubbed with a toothbrush, and rinsed again with Milli-Q water into the tray. This complete sample was then transferred to a sterile 15 ml plastic centrifuge tube and centrifuged at 2000 x *g* for 5 min. Afterwards, water overlying the sample was decanted leaving the biofilm and corrosion products which were stored briefly at 4°C.

Subsamples of this material then distributed and used for counting total prokaryotic cells, culture-based estimates of iron-oxidizing bacterial abundance, and the remainder was frozen (-20°C) to extract DNA for molecular analyses.

Corrosion Analyses –

Linear Polarization Resistance. The polarization resistance (Scully 2000) was measured in one steel coupon in each experimental microcosm biweekly (i.e., duplicate measurements per treatment). A mercury/mercury sulfate saturated calomel electrode was the reference electrode. A platinum-niobium mesh screen was the counter electrode. The reference electrode was placed 5 cm into the water near the coupon, while the counter electrode was immersed in the water immediately adjacent to the steel coupon. Polarization resistance was determined by connecting these electrodes to a PC computer with the Gamry Framework software. Electrical potential was fixed at 80 interval points between -10 mV and +10 mV of the open-circuit potential, and the current density passing through the circuit was measured at each of these points. The inverse of polarization resistance ($1/R_p$) is proportional to the instantaneous corrosion rate (Scully 2000).

Corrosion Tubercle Area. Each month, digital photographs were made of both sides of the steel coupons used for polarization resistance measurements. Prints of these images were used to measure the area covered by orange corrosion tubercles. The image of the coupon was cut out and weighed. Afterwards, areas that were covered by corrosion tubercles were removed with an X-Acto knife, and the image was reweighed to estimate the percentage of the coupon area that was covered by orange corrosion tubercles in each treatment. The average area covered by tubercles was determined by subtracting weight of the coupon image minus the tubercles from the weight of the original image of the coupon. Values were averaged from both sides of the same coupon in each replicate microcosm from each treatment.

Coupon Mass Loss and Corrosion Pit Depths. After the experiment was terminated in May 2009, the steel coupons were cleaned by washing them in 6N HCl with 0.35% hexamethylenetetramine (ASTM International 2003). After 20 minutes, the coupons were rinsed in tap water and lightly scrubbed with a test tube brush. If corrosion products remained, then a coupon was placed in the solution for another 5 min, rinsed and brushed again. The steel coupons from each treatment (n=6) were then air dried and reweighed to determine the weight loss due to corrosion, a measure of uniform corrosion. Two coupons from each treatment were shipped to the Naval Research Laboratory (Stennis Space Center, MS), where pit profiles and the maximum depth of five pits (a measure of localized corrosion) were measured on both sides of each steel coupon using a Microphotonics Nanovea PS50 non-contact optical profiler and a 3.5 mm optical laser pen.

Microbiological Analyses –

Total Prokaryotic Cell Abundance. Subsamples (0.02 g each) of biofilm and corrosion product collected each month from all steel coupon were preserved with formaldehyde (2% final concentration) and stored at 4°C until cells were stained with DAPI and counted using epifluorescence microscopy and UV illumination (Porter and Feig, 1980). Aliquots were stained for 5 minutes and then filtered onto black polycarbonate filters (Poretics, 0.22 µm-pore). Fluorescent prokaryotic cells in at least 10 fields were counted (1,000 x total magnification) for

each subsample (n=4 per treatment) and these counts were converted to prokaryotic cells•cm^{-2} of steel surface.

FeOB Dilution Cultures. Bimonthly, a biofilm subsample (0.02 g) from one steel coupon in each replicate microcosm was diluted with 300 µl of sterile modified Wolfe's mineral medium (MWMM) in a sterile 96-well microplate. Then, ten-fold serial dilutions were made of this solution up to a 10^{-6} dilution. Iron gradient culture tubes containing a soft agarose gel of MWMM overlying a FeS plug (Emerson and Moyer 1997) were inoculated with a portion of these ten-fold serial dilutions to estimate the abundance of iron-oxidizing bacteria by a dilution-to-extinction technique. These gradient culture tubes were incubated at room temperature in the dark. After 4 weeks, the tubes were examined for growth of iron-oxidizers indicated by the formation of distinct rust-colored bands in the overlying agarose gel. The presence of cells in the rust-colored bands, which indicated the growth of iron-oxidizing bacteria, was verified by epifluorescence microscopy. Tubes with the highest dilution that showed rust-colored bands (and cells) were used to estimate the abundance of iron-oxidizing bacteria (i.e., iron-oxidizer CFU•cm^{-2} of steel surface).

Quantitative PCR of Dissimilatory Sulfite Reductase (*dsr*A) Gene. Each month, DNA was extracted from a third subsample (approx. 0.5 g) of each steel coupon biofilm sample using a PowerSoil DNA kit (MoBio Laboratories), which uses bead beating to help lyse cells. The DNA from these subsamples was frozen (-80°C) until it was used to quantitatively amplify the dissimilatory sulfite reductase (*dsr*A) gene to estimate the abundance of sulfate-reducing bacteria. Quantitative PCR (qPCR) was used to determine the number of copies of the *dsr*A gene on steel coupons in the different treatments. The *dsr*A-specific forward (DSR-1F+) and reverse (DSR-R) PCR primers (10 µM) developed by Kondo et al. (2004) were used to amplify a 221 bp PCR product. Reaction mixtures (25 µl) contained 12.5 µl Brilliant II SYBR Master Mix (Stratagene), 0.5 µl of each forward and reverse PCR primer (400 nM final concentrations), 20 µg of BSA, 10 ng of DNA template, and nuclease-free sterile water. Schippers and Neretin's (2006) qPCR protocol was followed using a Corbett Research RotorGene 3000 thermal cycler: 95°C for 10 min, and 40 cycles of 95°C for 15 sec, 60°C for 1 min, and a data acquisition step at 85°C for 15 sec. Accumulation of newly amplified DNA was followed by the increase in fluorescence due to the binding of the SYBR green fluorescent dye to double-stranded PCR products. A melting curve analysis was performed between 72ºC and 95ºC at the end of the PCR reactions to check for PCR specificity and primer dimer formation. Standard curves ranging from 8 to 8x10^{9} copies of the *dsr*A gene (2 ag to 2 pg of genomic DNA) constructed with *Desulfovibrio vulgaris* genomic DNA (ATCC 29579D-5) were used to calculate the Ct value and subsequently the number of *dsr*A gene copies in biofilm DNA samples. No template control (blank) samples were used to check for contamination of the qPCR reactions.

Results and Discussion

Corrosion Measurements –

The instantaneous corrosion rate (1/Rp; units=ohms^{-1}) varied from month to month (Fig. 1). When instantaneous corrosion rates were averaged over the experiment for each treatment, the highest average rate ($6.7•10^{-5}$ ohms^{-1}) was observed in steel coupons from the unaltered harbor water supplemented with sodium sulfate treatment ($p<0.05$). The second highest average rate

was seen in the unaltered harbor water treatment ($4.9 \bullet 10^{-5}$ ohms^{-1}). The instantaneous corrosion rates of coupons in the autoclaved harbor water treatments (with or without the *S. lithotrophicus* inoculum were similar (4.1 to $4.3 \bullet 10^{-5}$ ohms^{-1}; $p>0.05$) and lower than the rates observed in the other harbor water treatments, but higher ($p<0.05$) that the average instantaneous corrosion rate of steel coupons measured in Lake Superior water ($1.6 \bullet 10^{-5}$ ohms^{-1}).

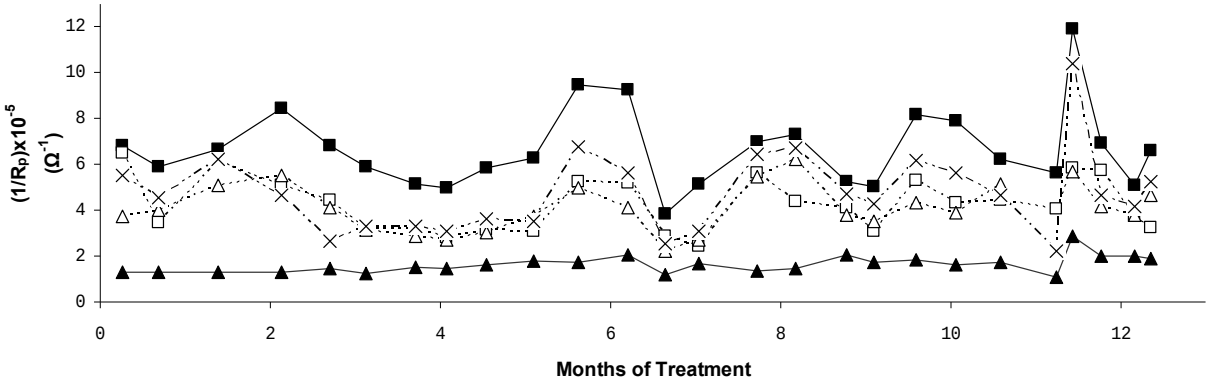

Fig. 1. Instantaneous corrosion rates ($1/R_p$) of steel coupons in the five experimental treatments estimated from measuring polarization resistance. Each point is an average of measurements from duplicate coupons in different microcosms (n=2). Key: ■ = Harbor water supplemented with sodium sulfate, x = unaltered harbor water, □ = autoclaved harbor water, Δ = autoclaved harbor water + *S. lithotrophicus*, ▲= Lake Superior water.

The cumulative corrosion measurements showed different trends than the instantaneous corrosion rate. There was no difference between the various treatments when the area of steel coupons covered by orange corrosion tubercles was compared (Table 2). At the end of the experiment, more weight had been lost from steel coupons immersed in Lake Superior water than from coupons in autoclaved harbor water ($p<0.05$). Other than this difference, the weight of steel lost from coupons was not different in the various treatments (Table 2). The depth of pits in the steel coupons, a measure of localized corrosion, was different in the various treatments by the end of the experiment ($p<0.05$, Table 2). Steel coupons in Lake Superior and unaltered harbor water had the deepest pits (558 and 526 μm, respectively) when compared to the other treatments ($p<0.05$). However, the pit depths in these two treatments were indistinguishable ($p>0.05$). Pit depths were different ($p<0.05$) and progressively shallower on steel coupons incubated in unaltered harbor water supplemented with sodium sulfate (458 μm) and autoclaved harbor water (411 μm).

The different corrosion parameters measured different aspects of the corrosion process and thus might be expected to yield different types of information. Polarization resistance was used to gain an instantaneous estimate of the corrosion of the steel coupons – a snapshot in time. The loss of coupon weight by the end of the experiment probably gives the best estimate of uniform corrosion across the entire surface of the steel coupons. This fact may explain why more weight was lost from coupons in Lake Superior water than in autoclaved harbor water. Lake Superior is an oligotrophic lake with very low concentrations of dissolved nutrients and metals. It is possible that more iron and other metals dissolved or diffused from the surface of the steel

coupons in Lake Superior water than in the autoclaved harbor water treatment. Measuring pit depths gives better insight about localized corrosion, which is characteristic of many types of microbiologically influenced corrosion. However, measuring polarization resistance may not always give an accurate measure of localized, pitting corrosion (Little and Lee 2007). It is interesting that the deepest corrosion pits were found on coupons in the Lake Superior and unaltered harbor water treatments. We expected the deepest corrosion pits to form on coupons in the harbor water treatment because previous field studies have demonstrated deep corrosion pits on steel structures in several areas of the DSH. We also expected to see the shallowest pits on coupons in Lake Superior water because fewer and shallower pits have been observed on steel structures at the entrance to the DSH. At times, water at this harbor entrance is almost pure lake water because of the periodic seiche of water in the Lake Superior basin.

Table 2. Cumulative corrosion measurements from steel coupons in the five experimental treatments. The area covered by tubercles is an average of measurements made on two coupons in each treatment (n=2) and the weight loss is an average for three coupons in duplicate treatment microcosms (n=6). The pit depth is an average of five independent measurements made on both sides of two experimental coupons from each treatment (n=2). Means with the same letter superscript were not significantly different. The standard error of the mean is shown in parentheses.

Treatment	**Area covered by tubercles (%)**		**Weight Loss (g)**	**Pit Depth (µm)**
	Month 2	**End of experiment**		
Lake Superior water	46^{a} (3)	45^{a} (11)	50^{a} (1)	558^{a} (32)
Unaltered harbor water	42^{a} (4)	48^{a} (7)	$46^{a,b}$ (2)	$526^{a,b}$ (19)
Autoclaved harbor water + *S. lithotrophicus*	41^{a} (4)	51^{a} (5)	$46^{a,b}$ (2)	512^{b} (36)
Harbor water + sodium sulfate	37^{a} (2)	54^{a} (4)	$46^{a,b}$ (1)	458^{c} (10)
Autoclaved harbor water	48^{a} (6)	46^{a} (3)	44^{b} (2)	411^{d} (13)

Microbiological Data –

Extracellular polymer production by microorganisms was clearly higher on coupons in both treatments containing harbor water that was not autoclaved (i.e., the unaltered harbor water and harbor water supplemented with sodium sulfate treatments) than in the other experimental treatments. Mucus-like material often dripped from coupons in these treatments when they were sampled. Less extracellular polymer was evident on the surfaces of coupons from the other treatments. These observations are important because microbial biofilm formation on surfaces is a key element in conceptual models of microbiologically influenced corrosion (Little and Lee 2007, Roberge 2007). Unfortunately, we cannot determine from the data collected in this study if there were more exopolymer-producing species or cells in harbor water or if chemical or physical characteristics of unaltered harbor water just promoted more extracellular polymer production than in the types of water used in the other experimental treatments.

The number of total prokaryotic cells on the surfaces of steel coupons ranged from $1 \cdot 10^7$ cells$\cdot$cm^{-2} to almost $7 \cdot 10^7$ cells$\cdot$cm^{-2}, increased in all treatments during the first six months and then decreased in several treatments by the end of the experiment (Fig. 2). In most months, there was a difference ($p>0.05$) in the abundance of total prokaryotic cells on the steel coupons in at least two treatments but over the entire experiment there was little difference between the treatments. When cells abundances were averaged over the entire experiment, the average number of prokaryotic cells on coupons (per cm^{-2}) in the harbor water supplemented with sodium sulfate was higher than the number of cells on coupons in the autoclaved harbor water + *S. lithotrophicus* treatment. Otherwise, there were no differences between the treatments. This was not an unexpected result. Bacteria rapidly colonize surfaces, none of the treatments were truly sterile, and water in each treatment had enough dissolved organic matter to support the growth of bacteria.

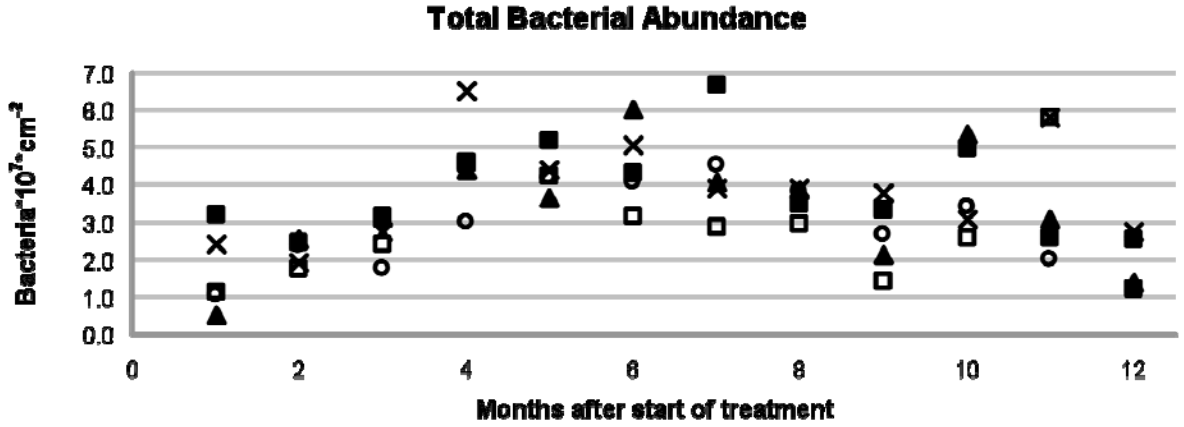

Fig. 2. Abundance of total prokaryotic cells in the five experimental treatments over the duration of the microcosm experiment. Each point represents the average of measurements from replicate steel coupons (n=2) in duplicate microcosms of each treatment. Key: ■ = harbor water supplemented with sodium sulfate, x = unaltered harbor water, ▲ = autoclaved harbor water, □ = autoclaved harbor water + *S. lithotrophicus*, ○ = Lake Superior water.

There were differences in the abundance of iron-oxidizing bacteria and sulfate-reducing bacteria on steel coupons in the different experimental treatments. Depending on the treatment, the number of iron-oxidizing bacteria increased from near zero to over 100 to 100,000 CFU$\cdot$ cm^{-2} of steel coupon during the first four to six months of the experiment (Fig. 3). After eight months, there were more iron-oxidizing bacteria on coupons in the unaltered harbor water and Lake Superior water treatments than in the treatments containing autoclaved harbor water (either with or without *S. lithotrophicus*). This fact indicates that iron-oxidizing bacteria are present in both harbor water and Lake Superior water. By the end of the experiment, there were more iron-oxidizing bacteria on coupons immersed in harbor water (unaltered or supplemented with sodium sulfate) than in any of the other treatments ($p<0.05$). Thus, it appears that chemical or other biological factors may promote the development of iron-oxidizing bacterial populations on steel surfaces in harbor water. Iron-oxidizing bacteria have been isolated from corroding steel surfaces in the Duluth-Superior harbor and have been demonstrated to cause corrosion of steel in

other environments (Hamilton 1985, Starosvetsky et al. 2001, Xu et al. 2007), so it is intriguing that bacteria with this physiology were more abundant on coupons immersed in harbor water at the end of the experiment.

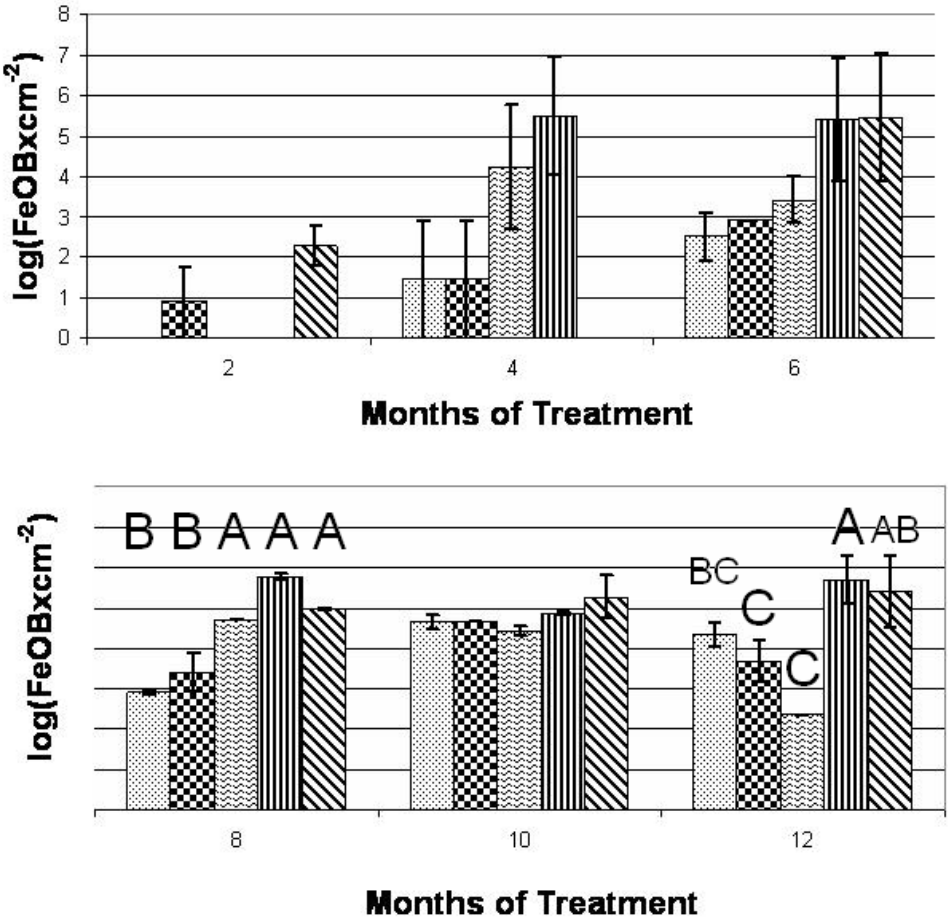

Fig. 3. Iron-oxidizing bacterial abundances on the surface of steel coupons in the five experimental treatments during the experiment. Mean values for duplicate measurements are shown. Treatments with the same letters were not statistically different. Error bars represent one standard error of the mean. Note the logarithmic scale and differences between treatments by the end of the experiment. Key: vertical lines = Harbor water supplemented with sodium sulfate; diagonal lines = unaltered harbor water, checkerboard pattern = autoclaved harbor water, stippled bar = autoclaved harbor water + *S. lithotrophicus*, wavy horizontal line = Lake Superior water.

The abundance of the dissimilatory sulfite reductase gene (*dsr*A) was used as a proxy for the abundance of sulfate reducing bacteria (Fig. 4). The abundance of this gene increased several orders of magnitude on coupons in some treatments during the course of the experiment, from near zero to over 10^6 copies•cm^{-2}. After five months, coupons in harbor water (with or without added sulfate) had more copies of the *dsr*A gene than coupons exposed to Lake Superior water

($p<0.05$). Usually, there were 100 to 1,000 fewer copies of this gene per cm^{-2} on coupons immersed in Lake Superior water than in water from the DSH (Fig. 4).

Even in oxic environments like water in Lake Superior and the DSH, the possible effects of sulfate-reducing bacteria on corrosion cannot be neglected because there are large variations in oxygen within microzones on particles or surfaces. In unaerated zones like those deep within biofilms, corrosion has often been attributed to the influence of sulfate-reducing bacteria (Hamilton 1985, Little and Lee 2007). The mechanism is believed to be a combination of direct attack of the steel by hydrogen sulfide produced by SRBs and cathodic depolarization aided by the presence of bacteria (Roberge 2007).

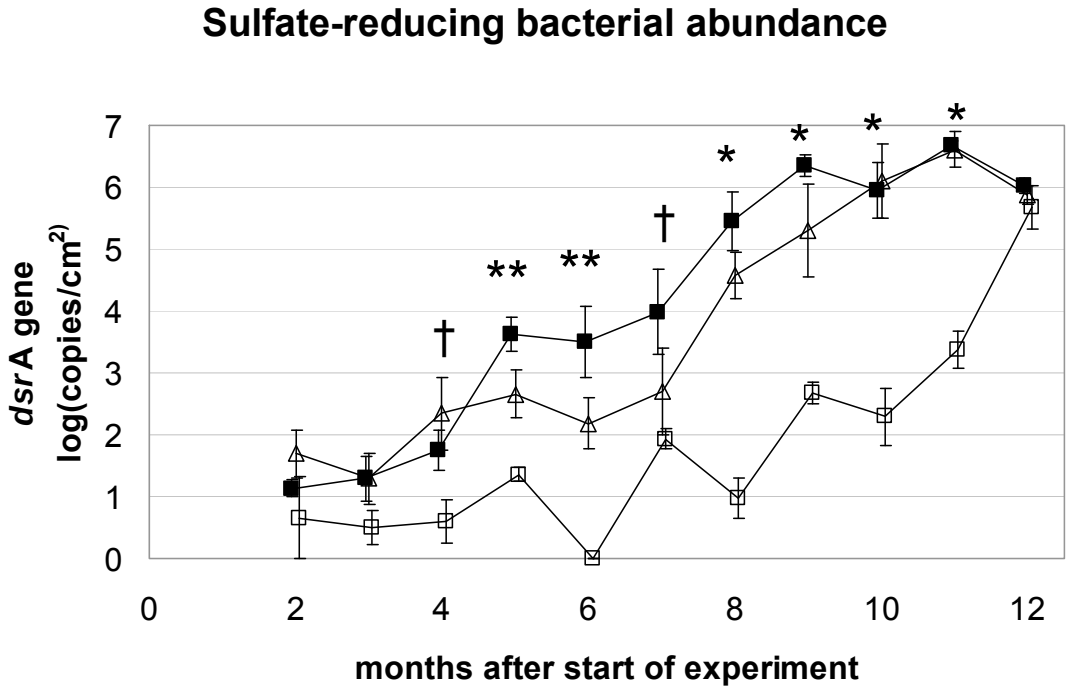

Fig. 4. Abundance of the dissimilatory sulfite reductase gene (*dsr*A), which was used to estimate the abundance of sulfate-reducing bacteria (SRB) on steel coupons in the microcosms. Mean values for three experimental treatments are shown (n=4; duplicate coupons in duplicate treatment microcosms). Symbols above months signify a significant difference between all three treatments (**), between harbor and Lake Superior treatments (*), or between one harbor treatment and the Lake Superior treatment (†). Error bars represent one standard error of the mean. Key: ■ = Harbor water supplemented with sodium sulfate, Δ = unaltered harbor water, □ = Lake Superior water.

It is clear from this study that the instantaneous corrosion rates were higher and the abundance of sulfate-reducing bacteria (and to a lesser degree iron-oxidizing bacteria) was greater on coupons incubated in water from DSH than in Lake Superior water. Interestingly, we have also found more copies of the *dsr*A gene on identical steel coupons incubated at sites in the DSH that are experiencing the most severe corrosion than at other sites with less severe corrosion (data not

shown). These facts indicate that sulfate-reducing and iron-oxidizing bacteria are correlated with the type of corrosion occurring in the DSH, even though their abundances did not correlate well with the cumulative corrosion parameters measured in this experiment. Thus, these groups of bacteria as well as other types of bacteria within these biofilms should be investigated further in future studies because their activities may directly or indirectly be responsible for the accelerated corrosion of steel structures in the DSH.

Potential Economic Impacts of the Research Results

Sheet steel piling 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 sheet steel piling appear to be affected in the DSH, which may cost more than $100 million to replace (Marsh et al. 2005). Shipping through the DSH, the largest port by total cargo volume in the Great Lakes, has a $200 million annual impact on Minnesota's economy. Solving this accelerated corrosion issue has important policy implications like the economic vitality of many companies whose 15 major cargo terminals ship ore, coal, and grain from this port.

The corrosion problem in the DSH is not only attracting local attention but also national and international attention. Other ports and businesses started to report similar steel corrosion problems once the accelerated corrosion in the DSH was discovered, reported and investigations began. A more thorough understanding of this accelerated corrosion problem in Minnesota will be invaluable because similar corrosion has now been seen in other harbors, including the port at Thunder Bay, Ontario, the second largest port on Lake Superior.

It is important to understand the mechanisms and agents responsible for this accelerated corrosion. Understanding the cause should be helpful in designing and testing mitigation practices, which could lead to improved control and remediation efforts. The results of the research experiment described here indicate that the abundance of sulfate-reducing bacteria and to a lesser degree the abundance of iron-oxidizing bacteria and extracellular polymers correlate with the instantaneous corrosion of steel coupons in the laboratory. These data as well as other recent field data and laboratory investigations (Ray et al. 2009) confirm suspicions that the activities of bacteria in biofilm communities may directly or indirectly accelerate the corrosion of steel structures in the DSH. Thus, controlling microbial biofilm formation and the activities of iron-oxidizing and sulfate-reducing bacteria may lead to success in preventing the accelerated corrosion of steel structures in the DSH.

Dissemination of Study Results

1. Publications

   Hicks, R. E. 2009. Assessing the Role of Microorganisms in the Accelerated Corrosion of Port Transportation Infrastructure in the Duluth-Superior Harbor. (University of Minnesota Center for Urban and Regional Affairs) CURA Reporter 39(1-2):4-10.

2. Presentations

   Little, B. J., 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. European Federation of Corrosion meeting (EUROCORR 2008), September 11-17, 2008, Edinburgh, Scotland. (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, National Association of Corrosion Engineers (NACE) International. October 6-10, 2008. Flamingo Hotel, Las Vegas, NV. (oral presentation)

   Bostrom, J. R. 2008. Update of Laboratory Corrosion Experiment. Corrosion Team Meeting-U.S. Army Corps of Engineers, Duluth, MN. October 9, 2008 (oral presentation)

   Bostrom, J. R., J. S. Lee, B. J. Little, and R. E. Hicks. 2009. Microbiologically Influenced Corrosion of Sheet Steel in a Lake Superior Harbor. American Society for Microbiology-109th General Meeting. May 17-22, 2009. Philadelphia, PA. (poster presentation)

   Hicks, R. E., and J. R. Bostrom. 2009. Microbial Communities Associated with Corroding Steel Pilings in the Duluth-Superior Harbor. Corrosion Team Meeting-U.S. Army Corps of Engineers, Duluth, MN. June 2, 2009 (oral presentation)

   Hicks, R. E, J. R. Bostrom, J. Lee, and B. J. Little. 2009. Determining if Microbiologically Influenced Corrosion is Responsible for the Accelerated Corrosive Loss of Port Transportation Infrastructure. GLMRI University Affiliates Meeting, Duluth, MN. September 24, 2009 (oral presentation)

3. Graduate Theses and Dissertations

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

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