



Ennoblement of stainless steel in fresh water influenced by manganese oxidizing biofilms
by Renee Loretta LaFond

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Environmental Engineering - Chemical
Montana State University
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Abstract:

Frequently, microbially influenced metallic corrosion is either completely overlooked or goes unrecognized. Microbial corrosion of stainless steel (SS) is a serious and expensive problem of concern to petrochemical industries, drinking water distribution systems, hydropower plants and other industries who utilize SS storage tanks, pipeline, and associated parts. Corrosion can occur electrochemically and can be enhanced microbiologically. Metal ion oxidizers such as manganese bacteria are important because they generally form thick, bulky deposits, which create concentration cells or harbor other corrosive microbes. This investigation has two phases, a field study with 8 sites, and a lab component. Mn-oxidizing biofilms can colonize 316L SS in natural waters and influence the electrochemistry at the SS surface. Environmental factors, which potentially influence the dynamics of manganese oxide accumulation on passive metal surfaces in parallel with open circuit potentials, were monitored in the field. Those factors were pH, total organic carbon (TOC), temperature, dissolved oxygen (DO), water chemistry, and dissolved manganese. Measuring the open circuit potential (E_{corr}) of SS in solution is an electrochemical technique used to demonstrate the relationship between the metal oxidizing organism and metal corrosion. Monitoring for an increase in E_{corr} or ennoblement was used as a tool for detecting the initiation of corrosion. In the laboratory, *Lepthothrix discophora*, a manganese oxidizing bacterium, was grown in flow-cell bioreactors. SS coupons were exposed in growth medium along with the microorganisms.

Environmental factors, including pH, TOC, and dissolved manganese concentrations were individually manipulated to isolate their effect on ennoblement at SS surfaces. Manganese concentration and pH were determined to be the most critical, in situ and in the laboratory. In natural fresh water 0.01 ppm dissolved manganese was sufficient for biomineralization of manganese oxides by biofilms which colonized the SS surface. A pH range of 6.4 - 8.5 favors ennoblement as well. Temperature, TOC and DO had an observed effect on ennoblement, but were not limiting in the environments studied. Results from this study isolate some of the risk factors for ennoblement of 316L SS. These experimental results allow for more informed decisions on water quality requirements for industrial processes.

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INFLUENCED BY MANGANESE OXIDIZING BIOFILMS

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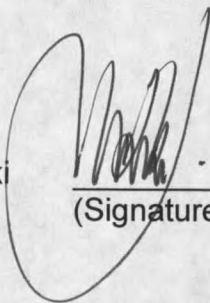
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Dr. Zbigniew Lewandowski

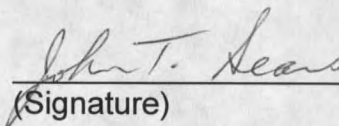


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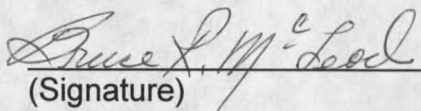


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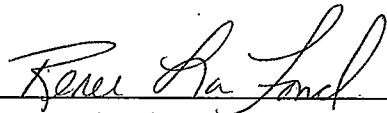
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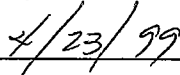
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ABSTRACT

Frequently, microbially influenced metallic corrosion is either completely overlooked or goes unrecognized. Microbial corrosion of stainless steel (SS) is a serious and expensive problem of concern to petrochemical industries, drinking water distribution systems, hydropower plants and other industries who utilize SS storage tanks, pipeline, and associated parts. Corrosion can occur electrochemically and can be enhanced microbiologically. Metal ion oxidizers such as manganese bacteria are important because they generally form thick, bulky deposits, which create concentration cells or harbor other corrosive microbes. This investigation has two phases, a field study with 8 sites, and a lab component. Mn-oxidizing biofilms can colonize 316L SS in natural waters and influence the electrochemistry at the SS surface. Environmental factors, which potentially influence the dynamics of manganese oxide accumulation on passive metal surfaces in parallel with open circuit potentials, were monitored in the field. Those factors were pH, total organic carbon (TOC), temperature, dissolved oxygen (DO), water chemistry, and dissolved manganese. Measuring the open circuit potential (E_{corr}) of SS in solution is an electrochemical technique used to demonstrate the relationship between the metal oxidizing organism and metal corrosion. Monitoring for an increase in E_{corr} or ennoblement was used as a tool for detecting the initiation of corrosion. In the laboratory, *Leptothrix discophora*, a manganese oxidizing bacterium, was grown in flow-cell bioreactors. SS coupons were exposed in growth medium along with the microorganisms. Environmental factors, including pH, TOC, and dissolved manganese concentrations were individually manipulated to isolate their effect on ennoblement at SS surfaces. Manganese concentration and pH were determined to be the most critical, *in situ* and in the laboratory. In natural fresh water 0.01 ppm dissolved manganese was sufficient for biomineralization of manganese oxides by biofilms which colonized the SS surface. A pH range of 6.4 – 8.5 favors ennoblement as well. Temperature, TOC and DO had an observed effect on ennoblement, but were not limiting in the environments studied. Results from this study isolate some of the risk factors for ennoblement of 316L SS. These experimental results allow for more informed decisions on water quality requirements for industrial processes.

CHAPTER 1

INTRODUCTION

The study of the relationship between biomineralization, microbially influenced corrosion (MIC), and ennoblement is a timely subject, brought to the forefront of corrosion science in association with biofilm research and mechanical failure of supposedly corrosion resistant metals. Ennoblement is the most recently identified phenomenon of the three, observed in the 1960's (5) and in the 1980's (30) in association with electrochemical processes that occur at the surface of metal alloys. *In situ*, ennoblement has been shown to occur in the presence of microorganisms and their associated metal oxides. (6,8,20,21) Ennoblement can be induced in the absence of microorganisms in the laboratory, through non-biological means such as addition of MnO_2 paste (6) or electrochemical application. (20) In nature, chemical oxidation of manganese, although possible, is a very slow process and the unlikely explanation for initiation of corrosion of stainless steel.

Microbes are present in virtually all soils, fresh and salt waters. Corrosive microbes include acid producers, mold growers, slime formers, sulfate reducers, hydrocarbon feeders, and metal ion concentrators/oxidizers. (19) There is considerable overlap within these classes. Metal ion oxidizers such as iron and manganese bacteria are important because they generally form thick, bulky

deposits, which create concentration cells (4,21) or harbor other corrosive microbes. (14,19,28,36) They are abundant worldwide, particularly in natural waters. This investigation will examine the role of manganese oxidizing microorganisms in natural waters, and how they influence the stainless steel surfaces they colonize.

Manganese is the 2nd most abundant metal on earth, next to iron (24), and likewise is regarded as a nuisance rather than a toxic component of drinking water. (1,39,40) The presence of manganese in raw water presents a problem, because, unlike iron, manganese is not oxidized by air at neutral pH and is not removed during water treatment processes unless a chemical oxidation step is included. (1,33) The maximum standard contaminant level (MSCL) for manganese in drinking water set by the USEPA is 0.01 mg/L.

Traditionally, pitting corrosion has been associated with iron oxidizing bacteria; however, reports by Tetnall (36), Kobrin (19) and others (4,16,17,21,28) identified manganese oxidizing bacteria associated with localized corrosion of 304 and 316 SS. These organisms modify the medium by creating strong concentration gradients and generating unexpected chemicals at the metals surfaces (i.e., metal oxides). Problems associated with manganese oxidizing microorganisms are many. They biologically oxidize manganese, forming MnO_2 deposits on pipeline surfaces and foul equipment such as cooling towers, water lines, heat exchangers, etc. Manganese dioxide is essentially insoluble over the entire pH range of drinking water treatment and industrial use. (1) These

deposits cause clogging of filters, restriction of water flow and in hydroelectric pipelines, and result in head loss at the turbines. In drinking-water distribution systems, sloughing of manganese oxide deposits results in water of poor aesthetic quality which has a brown-black color and undesirable taste. (33,39,40) These deposits cause widespread problems including staining of fixtures, equipment, swimming pools, and laundry.

Several species of fungi, algae and bacteria have the ability to oxidize Mn^{2+} or deposit manganese oxides. (2,12,25,31) In the presence of manganese oxidizing microorganisms, Mn(II) is biologically oxidized to Mn(IV) . (6,11,12,24) Most manganese oxidizing microorganisms are not motile when covered with manganese oxides but have motile stages. (2) Manganese oxides are deposited within extracellular structures: capsules, sheaths, stalks and holdfasts.

The type of oxide formed can vary according to the type of microorganism and with changes in chemical, physical and growth conditions of cultures.¹¹ Manganese oxides produced include birnessite (MnO_2), manganite (MnOOH), hausmannite (Mn_3O_4) and others. (15) Microorganisms generally form disordered manganese oxides. (15,27) These oxides have an indeterminate structure but tend to be more electrochemically active than common Mn-oxides and it is unclear why. It has been suggested that they have higher electrochemical activity as a result of; i) better proton diffusion within the oxide particles, ii) greater purity of the MnO_2 content, iii) greater particle size, porosity, and density, iv) greater chemical homogeneity, or v) greater conductivity

compared with the common oxides. (16,32,35,37) On the basis of these studies, deposits of manganese are assumed to be manganese dioxide (MnO_2).

MnO_2 is formed only very slowly by chemical reaction of dissolved Mn^{2+} and oxygen in natural waters, although it is the thermodynamically stable phase. (1,15) Linhardt reported that below $\text{pH}=8$ and $\text{Mn}^{2+} = 0.1$ ppm the chemical reaction rate can be neglected compared to the formation of higher manganese oxides and hydroxides via a biological route. (20)

Manganite (MnOOH) is possibly a precursor in microbial formation of disordered manganese oxide, which supports the theory that deposition of Mn-oxides in natural waters is a biological process and not a chemical one. Under conditions of near-neutral pH and 30°C , chemical conversion of MnOOH to MnO_2 within a period of days is highly unlikely. It would take at least a number of months and possibly years, since metastable $\gamma\text{-MnOOH}$ can exist unchanged for some time. (15)

Oxidation of Mn^{2+} with chlorine is rapid only at pH of 8.5 or higher. (1) Most natural waters generally do not have concentrations of chlorine greater than 200ppm, nor $\text{pH} > 8.5$. Removal of Mn(II) is enhanced by the presence of solid manganic dioxide and may occur within a reasonable time without oxidation. (1,24) This is an autocatalytic phenomenon exhibited by manganese oxides. They strongly adsorb other divalent metal ions, including iron (23), copper, and zinc.

Biofilms consist of a consortium of immobilized cells attached to some substratum. (3) That substratum can be any surface such as metal, plastic, concrete, etc. Microorganisms form synergistic communities that conduct combined processes that individual species cannot. In the case of manganese oxidizers, they may remove Mn^{2+} , detoxifying environments and making it habitable by other microorganisms. They also remove oxygen from the environment.(19,36) Dense deposits of cells and metal ions create oxygen concentration cells that effectively exclude oxygen from the area immediately under the deposit. Under-deposit corrosion is extremely important because it initiates a series of events that are individually or collectively extremely corrosive. In an oxygenated environment, the area immediately under the deposit becomes deprived of oxygen. That area becomes a relatively small anode compared with the large surrounding oxygenated cathode. (4,20) The critical potential for corrosion in this case is lower than E_{pit} of the free surface and under deposit corrosion occurs. (19,36)

There are several mechanisms proposed for MIC. For purposes of this paper, focus is on the mechanism that involves the redox-properties of the deposits, as the relevant process in initiating corrosion of SS.

316L SS is a passivating metal, with a chromium concentration $\geq 12\%$, molybdenum and nickel. A thin film of metal oxide protects the SS against corrosion, even at relatively high E_{corr} . This layer is electronically conductive, is spontaneously formed at the metals surface under air, and is mainly of

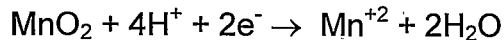
chromium. Passivity can break down under certain conditions, and subsequent corrosion may occur.

Ennoblement

The ability of the medium to oxidize a metal is reflected by the electrochemical (open circuit) or corrosion potential (E_{corr}) for the metal in the medium. (20) Ennoblement is an initiation step in the corrosion of SS. (6,20,21) In ennoblement, deposition of manganese oxides coinciding with microbial colonization of passive metals shifts the corrosion potential in the positive (noble) direction. In the case of 316L SS in natural water, the corrosion potential increases dramatically by more than 500 mV, remaining fixed at approximately +350 mV vs. a standard calomel electrode (SCE). (6)

For ennoblement to occur, an electrochemical cell, comprised of an anode, cathode, a connection between them for electron transport, and an electrolyte solution are needed. The electrolyte solution conducts ions between the anode and cathode. Corrosion will not occur if any of these components is absent.

Redox couples $\text{Fe}^{2+}/\text{Fe}^{3+}$ and $\text{Mn}^{2+}/\text{Mn}^{4+}$ influence E_{corr} that may lead to pitting. (20) (Figure 1.1) Manganese oxidizing microorganisms in the bulk fluid colonize SS. Mn^{2+} dissolved in the water is biomineralized and deposited in electrical contact with the SS. Reduction of the MnO_2 , is the electron consuming cathodic reaction.



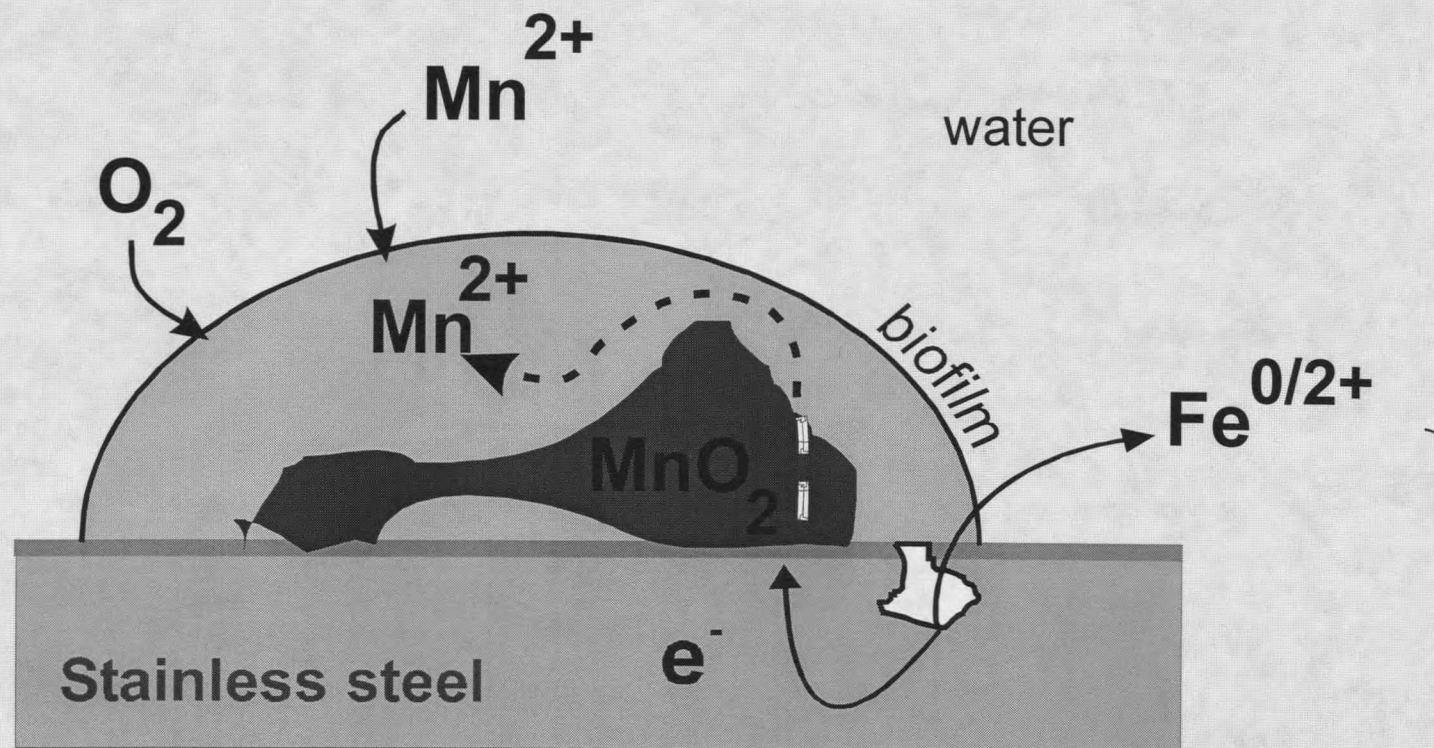


Figure 1.1 Pitting under the influence of Mn-oxidizing biofilms.

The anodic oxidation of iron, which generates electrons, balances the cathodic reaction.



At this stage an electrochemical cell has been established. The result is an increase in cathodic activity, where high currents are supplied for extended periods of time. This is the critical phase at the beginning of pit growth where E_{corr} of the stainless steel approaches the pitting potential (E_{pit}). E_{pit} is primarily determined by the alloy composition, the surface condition (type, density and size of defects), the chloride concentration and pH.

In order for ennoblement to occur, deposits containing MnO_2 must be in electrical contact with the metal. If MnO_2 mineralized by manganese oxidizing microorganisms is embedded in or on a layer of organic matter, i.e. within sheaths or slime, and no direct contact to the metal is possible, it will not influence the electrochemical process. (6,20) If the oxide is in contact with the metal cell death or lysis within biofilms does not necessarily mean cessation of the influence on electrochemical processes. Once the metal oxide is deposited on the metal surface, the electrochemical processes occur independent of the cellular viability.

Measuring ennoblement in the environment and in the laboratory is an electrochemical technique used as an indicator of potential corrosion. For example, the more noble the E_{pit} of the SS, the more corrosion resistant it is. (29)

Ennoblement is an excellent indicator of the status of the SS in situ. For instance, when elevated E_{corr} approaches E_{pit} there is potential for corrosion.

To summarize, metal oxidizing organisms within the biofilm influence metallic corrosion in fresh water, (6,20) and sea water. (21) Manganese oxides formed by manganese oxidizing biofilms on the surface of 316L stainless steel result in ennoblement. E_{corr} increases in the positive direction until under "appropriate" conditions, E_{corr} reaches the pitting potential. Ennoblement participates in the depassivation of the chromium oxide passive layer, which gives chromium alloys their corrosion resistant properties. In natural waters, in the presence of manganese oxidizing microorganisms, corrosion potentials can shift to more anodic values. (29) This means pit potential has been exceeded and as a consequence pitting can be induced. Thus, ennoblement can be a precursor to corrosion.

Pit Corrosion

Pitting occurs when portions of the metal surface lose their passivity and dissolve rapidly. (4) All metals undergo pitting corrosion under a given set of experimental conditions, although susceptibility varies widely. Impurities in the metal surface, sediment accumulations, adherent biofilms and accumulation of corrosion products are all related directly or indirectly to the development of electrode areas for corrosion circuits. There have been several cases of SS pit corrosion in freshwater hydropower plants, and cooling systems where

microbiological causes were implicated. (20,28,29) . Pitting is most often observed on steel grades AISI 304 and AISI 316. The areas often involve surface imperfections or weld seams and/or the heat-affected zones. Very often, only a hardly perceptible pit can be detected on the surface, with a cavernous hole beneath. Marked, rust-colored blisters or deposits are found in the area of the corrosion. Pitting of SS is often concentrated at porous weld seams. (4,19,29,36) Pitting at weld seams has occurred when fresh water was used to test the integrity of weld seams of tanks used for fossil fuel or nuclear power generating plants, and condenser tubes at an electric utility. (19) Pitting also depends on the presence of an aggressive chemical species in the environment and a sufficient oxidizing potential. (4)

According to the current models, certain aggressive ions such as chloride may adsorb preferentially at surface defects and may penetrate the passive layer. (4) Acidification and chloride enrichment reduce the probability of repassivation by lowering E_{pit} . If the metal surface is ennobled and E_{corr} exceeds or equals the critical potential (E_{pit}), stable pitting corrosion at relatively high penetration rates may develop. (20) This leads to localized corrosion.

Various species of bacteria were present in almost all cases where microbiological analyses were performed, (15,20) indicating increased microbial activity could be responsible for metal deterioration. Pitting of SS is often observed in the presence of slime formers and sulfate-reducing bacteria, (19,29) where as Fe-and Mn-oxidizing organisms are detected less commonly. (22,29)

This observation may be misleading since 1) scientists were not looking for manganese oxides in association with SS corrosion 10 years ago, and 2) so little oxidized manganese is required to change the electrochemistry at the surface that it goes undetected. Recently, pitting and localized corrosion have been identified in stainless steels, exposed to natural waters, in the absence of high chloride concentrations (1) and in the absence of SRB's. (1,20) implicating other corrosion mechanisms such as that influenced by biological deposition of manganese oxide.

Du Pont had problems with corrosion in SS storage tanks, 304L and 316L. Potable well water with 200 ppm chlorides (a common concentration of chloride found in natural waters) was used to test the integrity of SS tanks. (19) Water was left standing in the tanks, resulting in pit corrosion. Examination of the corrosion sites revealed a marked accumulation of manganese oxides and manganese and iron oxidizing bacteria present. Dupont's protocol for corrosion prevention then focused on the water quality used to test tanks . (19)

To actually predict the likelihood of pit propagation, more information is required, including: (a) the probability that a pit will nucleate under a given set of conditions, and (b) the probability that once a pit nucleates, it will survive (i.e., become stable). (4) Pitting can severely limit the performance of the material and information gained about ennoblement could be used to identify certain factors which would put a system utilizing natural water in association with SS components at risk for pitting corrosion.

Pit nucleates most often go undetected because superficially the pit is insignificant, and hides the extent of corrosion underneath. System failure is the result. (28,30) Shut down and material replacement is very costly to the company. Leaking of potentially caustic materials due to pitting can go undetected for long periods also, resulting in environmental hazards. Microbial corrosion is a serious and expensive problem of concern to many industries.

Background

PREVIOUS WORK ON ENNOBLEMENT BY MN-OXIDIZING BACTERIA

Leptothrix discophora, is a manganese oxidizing bacteria that has been extensively studied. (2,10,11,12) It forms a biofilm which readily oxidizes dissolved manganese. It had been shown by the Structure Function Group at Montana State University Center for Biofilm Engineering, to facilitate ennoblement in batch culture. (6,7)

W. Dickinson conducted ennoblement studies in Roskie Creek and previously reported that 316L stainless steel ennobled in this natural fresh water stream. (6) He noted in his experiments that E_{corr} for stainless steel coupons exposed in situ began to increase within 24 hours and continued to increase at varying rates until it reached steady state at approximately +400 mV. He examined the biofilm microscopically, which showed an orange-brown biofilm consisting of annular rings covering 10-20% of the SS surface. Coverage depended on duration of exposure Further analysis revealed that the biofilm

consisted of Fe (III) compounds, MnO_2 , calcium, carbon and oxygen. Total manganese in the biofilm after 14 days exposure was $1.6 \mu\text{g/cm}$. (6) Epifluorescent microscopy revealed the presence of a sheathed filamentous microorganism characteristic of iron and manganese oxidizing bacteria.

Dickinson described several processes which may influence ennoblement of metal surfaces and utilized techniques to elucidate the manganic oxide mechanism of ennoblement. (9) Microbial processes which potentially influence redox potentials include photosynthesis, extracellular hydrogen peroxide, and other active oxygen species. Organic acids, NAD^+ , FAD, and quinones which accumulate within biofilms may be reduced if the reduction potential is greater than E_{corr} . Respiratory macrocycles may elevate E_{corr} by electrocatalyzing oxygen reduction. Superoxide formation near the metal surface may increase the rate of cathodic oxygen reduction on iron at neutral pH. High levels of extracellular electrocatalytic enzymes within biofilms may increase oxygen reduction rates. Using microelectrodes Dickinson demonstrated that dissolved oxidants did not accumulate in the bulk biofilm during ennoblement. (8) Using cathodic polarization techniques he showed that cathodic current density over a specified range was independent of oxygen concentration confirming that dissolved oxygen was not the cause of the increased cathodic current density observed in ennoblement. (6)

Dickinson measured capacitance during ennoblement and amounts of reducible surface material before and after ennoblement in order to study the

relationship between ennoblement and changes in surface oxide properties of the metal. (8) Capacitance studies confirmed that surface properties were modified during the ennoblement process. Coulametric titration indicated that a new reducible surface phase, more strongly oxidizing than iron oxides, developed during ennoblement. (8) To confirm ennoblement was dependent on the presence of manganic oxides, biofilms on ennobled coupons were reductively dissolved. Decreases in E_{corr} corresponded with release of Mn(II) into solution. (6) Dickinson also conducted experiments in which MnO_2 paste applied to the SS coupons caused an increase in E_{corr} in the same range as the in situ experiments. (6) Further experimentation showed that dissolution of biomineralized MnO_2 from the stainless steel surface correlated a decrease in E_{corr} . Linhardt's studies showed that 1% surface coverage with MnO_2 is enough to increase E_{corr} of SS to steady state at approximately +400 mV vs. SCE. (20)

Utilizing electrochemical tools Dickinson was able to discriminate between solution and surface effects. Polarization measurements provided information on the identity and abundance of electroactive species produced by microbial processes. His results clearly demonstrated that biogenic manganese dioxide induces SS ennoblement.

RELEVANT CONDITIONS FOR ENNOBLEMENT BY MnO_2

Environmental factors have pronounced effects on the corrosion of SS by influencing constituents of the biofilm, interfacial chemistry, and resulting E_{corr} .

(22) It was the goal of this work to examine selected "exposure conditions" and monitor the effects on SS electrochemistry. Environmental parameters including TOC, flow velocities, temperature, pH, DO, and the potential presence of phototrophic organisms were studied.

TOC was chosen as a study parameter because it supports biofilm growth. TOC is present in all natural and processed waters, but its concentration can vary widely. Little et. al. (22) generalized that increasing TOC increases the substrate or carbon source available to the biofilm. Mn-oxidizers use TOC to grow and form biofilms. At high organic loadings, the substrate flux into the biofilm will reach a constant value as a result of one of the following: (a) the growth rate of the microbial population in the biofilm reaches a maximum, (b) the thickness of the biofilm exceeds the penetration depth of the substrate into the biofilm, or (c) the electron acceptor or another nutrient becomes nutrient limiting. In some cases, large amounts of carbon may inhibit manganese oxidation. (1)

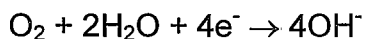
Dissolved manganese concentrations in fresh water varies among different water systems depending on hydrogeology, seasonal precipitation, and mixing dynamics, for example. Mn concentrations can be heavily impacted by mining, farming and ranching also. The amount of dissolved manganese available for oxidation may effect ennoblement.

Flow velocities became an important parameter after the first season's results. Analysis of results indicated that increased flow increased the rate of ennoblement of SS. Renner reported that the level of pit potential depends on

flow velocities. (29) This makes sense when considering that hydrodynamic shear stress which is related to flow, influences transport, transfer, and reaction rates within the biofilm, as well as detachment. (22)

Temperature was chosen as a viable parameter because bulk water temperature influences the rate of most chemical and biochemical reaction and transport processes within the biofilm. Biofilm formation is generally considered to be more of a problem in the summer months because higher temperatures increase the rate of biological processes, (3,22) which would indirectly effect biological manganese oxidation. The influence of temperature on biofilm growth processes is much greater when cells are nutrient saturated, i.e. growing at their maximum specific growth rate. (3) Increased temperature has been shown to elicit increases in open circuit potentials (E_{corr}) of SS over long term monitoring. (29)

pH plays a major role in biofilm attachment, dissolution of metals, and controls the chemical species present in the water. (1,4,22) In association with corrosion of metal surfaces and corrosion products, pH in the microenvironment is affected as well. Cathodic reduction of oxygen may result in an increase in pH of the solution in the vicinity of the metal.



The metal will form metal cations at the anodic sites. If the metal hydroxide is the thermodynamically stable phase in the solution, the metal ions will be hydrolyzed by water with the formation of H^+ ions. If the cathodic and anodic sites are

separated from one another, the pH at the anode will decrease and that at the cathode will increase. The pH at the anode depends on specific hydrolysis reactions. (20)

It was important to include dissolved oxygen (DO) as a study parameter because DO is usually the predominant oxidizing agent in natural waters, traditionally attributed to driving the cathodic reaction. DO and dissolved manganese in the bulk fluid are necessary for the biological oxidation of manganese, which effects the electrochemistry at the SS surface.

Algae and photosynthetic bacteria utilize light to produce oxygen that can accumulate within a biofilm. Many of these organisms are also capable of oxidizing manganese and reside within the consortium of microorganisms in a natural fresh water biofilm. Therefore visible light can be an important environmental parameter to study.

Purpose of Study

The purpose of this study was to determine whether ennoblement, in correlation with deposition of manganese oxides on 316L stainless steel, occurs in all natural fresh water systems. In particular this work examined the environmental factors influencing the dynamics of manganese oxide accumulation on passive metal surfaces. The working hypothesis was that ennoblement of 316L SS would not occur at all fresh water exposure sites, depending on the environmental conditions in different water systems. In

addition to *in situ* studies, laboratory experiments were conducted to isolate those parameters favoring ennoblement.

Open circuit potentials of exposed SS coupons in 8 fresh water sites in Southern Montana were monitored over several weeks *in situ*. Potentials were analyzed in parallel with environmental factors, including temperature, dissolved oxygen, pH, conductivity, manganese oxide deposition and water chemistry. Results from this study gave insight as to how particular characteristics of the water affect the electrochemistry at the SS surface.

CHAPTER 2

MATERIALS AND METHODS

Experimental Methodology for Field Studies

Field studies encompassed two ice-free seasons in Montana. 316L SS coupons were exposed in eight fresh water locations. Suitable sites were selected based on location, impact factors and some basic limnological factors such as depth and stratification. Parameters measured at approximately 2 week intervals included dissolved oxygen, (DO) dissolved manganese and 15 other metals concentration in the source water, pH, temperature, conductivity, and total organic carbon (TOC). Two to three SS coupons were collected on sampling dates and manganese oxides were dissolved from the surface. Dissolved manganese was then measured by inductively coupled plasma emission spectrophotometry (ICP). The open circuit potential (E_{corr}) of each SS coupon was measured *in situ*, on each sampling date.

Dickinson, et al. (6,7) demonstrated that ennoblement of stainless steel coupons placed in a freshwater stream in Bozeman, MT was caused by the deposition of manganese-rich material on the coupon surface. This same stream, Roskie Creek, was used as the control stream for all *in situ* experiments performed throughout this study. Water chemistry, temperature profiles, DO profiles and assorted stream parameters were obtained to further characterize

this stream. Line graphs demonstrating ennoblement of 316L SS were likewise generated as control experiments during two ice-free seasons.

Observations during the first season of field studies (1997) did in fact reveal that ennoblement does not occur at the same rate in all natural fresh water systems. Electrochemistry at the SS surfaces exhibited a wide range of open circuit potentials, depending on the exposure site. Ennoblement occurred at varied rates, if at all, depending on the water chemistry and other characteristics, of the exposure sites.

Based on results from the 1997 field sites, alternative study sites were chosen for the 1998 field studies. Flow and duration of exposure, two factors not considered in initial selection of *in situ* sites, turned out to be important parameters in the ennoblement phenomenon.

***IN SITU* STUDY LOCATIONS**

Cliff Lake is a naturally formed lake, one of several remote lakes in the southern portion of Madison County, Montana, located on National Forest Service land. It is very pristine, impacted minimally by recreational use. Elevation of Cliff Lake is 6300 ft. The maximum depth of the lake is 120 ft. Depth at the sampling site was 13 m. The lake was oxygen and temperature stratified for most of the year. Turnover occurs twice a year, during spring and mid-fall, where the lake is completely mixed. Snow melt, run-off, and ground water comprise the majority of lake water.

Roskie Creek is located on the outskirts of Bozeman, Montana in Gallatin County. The elevation is 4810 ft. At the sampling site the creek is approximately 8-12 inches deep during normal flow periods. Flow is steady and moderate for the majority of the year with increased flow and water volumes during spring run-off. The creek is a farm ditch fed by run-off from the surrounding mountains south of Bozeman. The creek is relatively clean, somewhat impacted by farming, ranching and rural development upstream.

Hebgen Dam and Hebgen Lake are man-made. The lake was formed by damming of the Madison River in the early 1900's. It is located in the southern part of Gallatin County, 8 miles north of West Yellowstone, Montana. An earthen dam at the west end of the lake spills into the Madison River. Elevation at the dam is 6548 ft. Farming and ranching in the near vicinity, recreational use and a recent boom in residential development have affected the lake. The lake is classified as clean with a healthy fish population, little pollution, high dissolved oxygen and nutrient concentrations. Hebgen Lake does have a history of cyanobacterial blooms in recent years. (38) Depth varies throughout the lake. Twenty meters from the dam, at the boat barrier sampling site, the water was 6 meters deep. The lake is oxygen and temperature stratified, with turnover occurring twice a year. At the dam, the water was unstratified for the entire length of the study, due to shallower water and constant flow conditions caused by drawing of water through the spillway. Coupons were exposed at the dam during the 1997 season and in a stratified portion of the lake in 1998. The 1998

sampling site, located approximately 2 kilometers upstream of the dam, exposed coupons in up to 15 meters of water.

Metro Storm Drain is located in Silver Bow County, in Butte, Montana. The elevation is 5548 ft. The water source is municipal run-off, from underneath Butte, and flow rate fluctuates depending on amounts of precipitation, snowmelt and municipal run-off. Water chemistry is unique for this study in that the water is extremely metal laden, the result of shallow mining beneath Butte. In fact, in the 1950's Butte was the number one supplier of manganese for steel production in the United States. It is not surprising then, that the MSD stream contains up to 10 ppm dissolved manganese as well as high concentrations of other metals. The depth of the stream at the sampling site is 10-14 inches. A pump diverts run-off water into a constructed wetland pond, and this was the original 1997, study site. When it was observed that flow might be an important parameter in ennoblement rates, the exposure site was moved to the stream in 1998.

Weasel Creek is located in Gallatin County, Montana, in the Bangtail Mountain Range. The elevation is 5586 ft. More specifically the study site is at the base of Weasel Creek drainage in Bracket Creek. Depth of the water at the exposure site is 8-12 in. Source water is run-off from the U.S. National Forest Service land. The water is very pristine, and impacted only by minimal recreational fishing.

FIELD SAMPLING DEVICES

Canoe. A 17 ft. Coleman Canoe was used to reach the buoy and pontoon on the lakes. It required two people and a good deal of equipment to install, monitor and remove lake experiments.

Buoy, boat barrier pontoon. Three frames were suspended from either a buoy or a pontoon at each deep-water lake site, and anchored to the bottom of the lake. The frames were connected in series on one rope at depths that coincided with the epilimnion, thermocline and hyperlimnion of the lake.

SS Coupons and Holders. Austenitic AISI 316L stainless steel (Fe-18Cr-12Ni—2.5Mo) coupons were initially obtained from Metal Samples Inc., part No. EL 405. Prior to initiation of field studies, a 316L SS sheet was purchased and coupons were punched "in house". Before exposure, 316L SS coupons were rinsed and sonicated in distilled water, rinsed in ethanol and air dried to remove oils before inserting them into holders. Coupon holders for field studies are constructed from a modified ½ inch PVC threaded plug. Coupons were sealed into the holder with epoxy, allowed to dry, then sanded systematically using wet 120 to 600 grit paper. Nickels et. al. (26) demonstrated that microbial colonization of a substratum appears to increase with increasing surface roughness and that the absence of surface cracks and crevices resulted in a marked diminution of total biomass. Rigorous polishing assured repeatability of experiments, uniformity between coupons, and reduced imperfections in order to

minimize chances of corrosion at surface flaws, yet observe ennoblement. The epoxy is sanded away from the coupon and the coupon lies flush in the holder. Again the coupons were rinsed and sonicated in distilled water to remove stainless steel shavings, rinsed with ethanol and air dried. They were allowed to stand at least 24 hours to restore the passive layer, then mounted on frames.

Frame Apparatus. The frames were constructed of $\frac{1}{4}$ inch PVC sheets, 3x12 inches. (Figure 2.1) Twelve $\frac{3}{4}$ inch holes were drilled through each frame, through which coupons screw in and out for easy removal. For the 1997 field study, three coupons per frame were modified with nylon plugs, which housed an 18 gauge copper wire soldered to a spring, which maintained constant contact with the back surface of the exposed SS coupon. At the lake sites, the insulated copper wire extended outside of the plug to a watertight box (Newark Electronics, Type A – 633PC7 Nema type) housed at the water surface on the buoy or pontoon. At the shallow sites, one end of the insulated copper wire remained attached to the submerged coupon holder and the other end was secured above water level and exposed to air. The purpose of these wiring methods was to be able to read the open circuit potential *in situ* without having to bring the coupons to the surface.

The above described configuration had some problems so I changed the set-up for the second year. The distance between the reference electrode and the coupons provided unstable potential measurements. The ennoblement trends were still evident when data was assimilated but to avoid the erratic

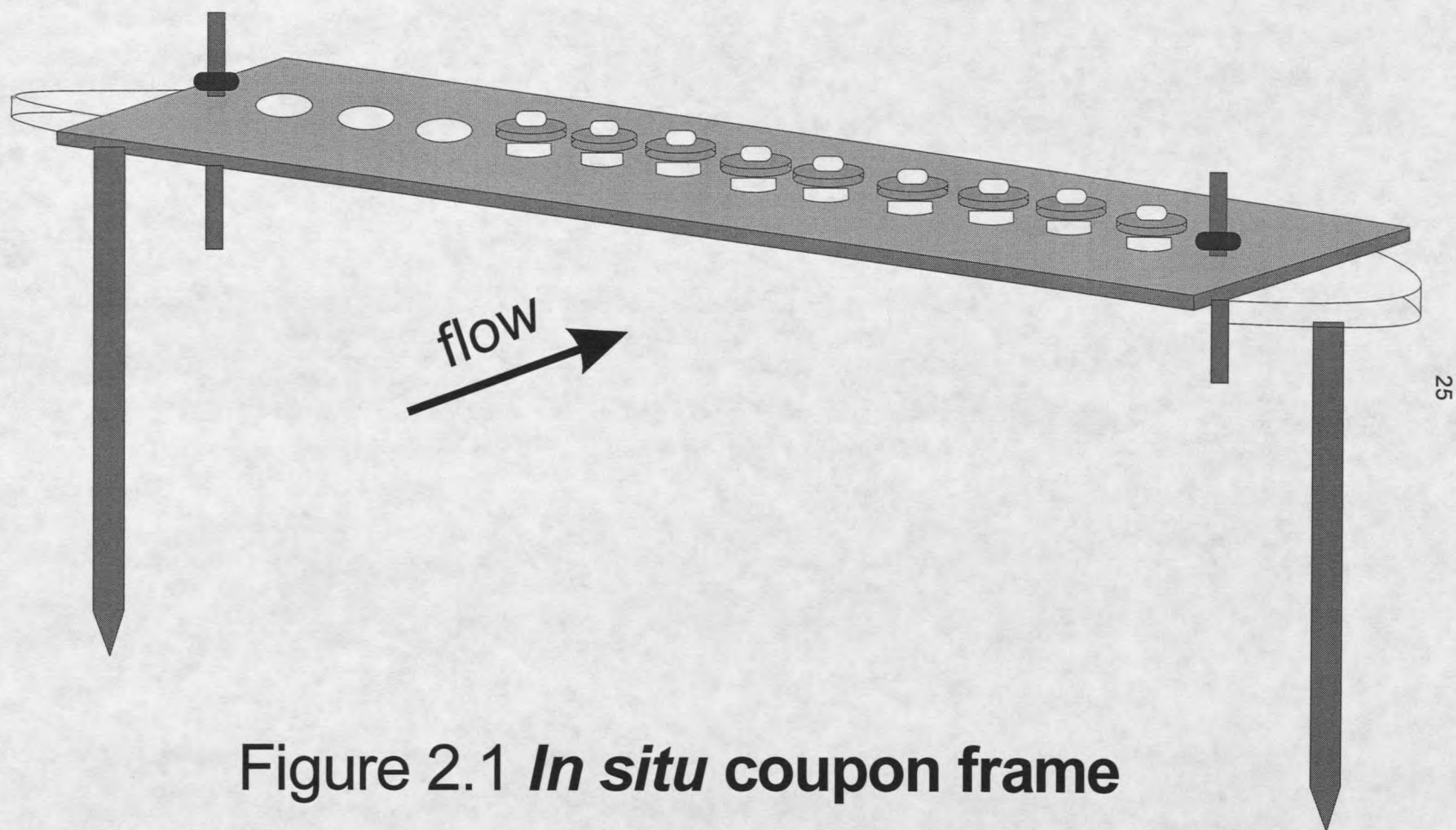


Figure 2.1 *In situ* coupon frame

measurements, the apparatus was changed. Post 1997, all coupon holders were fitted with nylon plugs. Frames were brought to the water surface, keeping SS surfaces emmersed in water. Nylon plugs were carefully removed and E_{corr} was measured vs. a standard calomel reference electrode (SCE) within 6 inch proximity to the coupon.

At the shallow field sites, mild steel or plastic stakes were attached on each end of the frame and pounded into the creek bed. The frames remained approximately 6 inches clear of any rocks or sediments.

Standard calomel electrode. The reference electrode for laboratory and field studies was the Accumet standard calomel electrode, model No. 13-620-51.

Water sampler. A deep-water sampler with a weighted trigger was constructed for collecting samples at various depths in the lakes. The deep-water sampler constructed from 3-inch polycarbonate tube was specifically designed for this project. All water samples were transported in 60-ml wide mouth plastic bottles and stored at 4°C. If water was not to be analyzed within one week a preservative was added. Surface water was collected directly into the plastic bottles, by holding the bottle 6-8 inches beneath the surface and allowing the water to fill.

Dissolved Oxygen Meter. YSI 57 and YSI 58 meters were used with a YSI 5739 dissolved oxygen probe for water analysis. Surface water DO measurements were collected at each site visit. A 50 meter cable was used for collecting DO (mg/L) and temperature (C°) profiles in the lakes.

pH meter. An Orion (Model 265) field meter was used to measure pH.

Conductivity meter. A hand held conductivity meter (Fisher Scientific) with automatic temperature compensation, was used to measure surface conductivity in the field. A YSI 33 meter was used to measure a conductivity profile in Cliff Lake and Hebgen Dam at the time of set up only during the 1997 season.

Multimeter. A hand held multimeter, Wavetech DM 23XT, was used to measure the open circuit potentials (E_{corr}) in the field.

Secchi disk. A common limnological method for determining visible light penetration in deep water is by using a secci disc. The 20 cm disc with alternated black and white quadrants is lowered into the water. When the disc is no longer visible to the naked eye, the depth is recorded.

DATA COLLECTION AND ANALYSIS OF FIELD SAMPLES

SS coupons were placed in native water for transport back to the laboratory. Water samples were stored at 4°C and processed within 1 week.

Water chemistry. Water samples were collected from all locations performed at initiation of each field study for both seasons. At the lakes, samples were taken at the three study depths within the water column. The samples were delivered to the MSU Plant and Soils testing laboratory for 16 panel metal analysis.

Ennoblement curves. Ennoblement curves were generated from E_{corr} (mV vs. SCE) data collected from each coupon at each site visit. Curves were generated by graphing E_{corr} vs. time (days).

Dissolved oxygen and temperature profiles. Profiles for the lakes were generated in graphs denoting DO vs. depth. One profile was generated per site visit. In contrast profiles for the streams denote DO vs. time of each site visit and encompass an entire study period. Temperature profiles were generated in the same manner.

pH profiles. pH measurements in the lakes were obtained at coupon exposure depths and at the surface. pH values were obtained from the streams at the time of each site visit also.

Inductively Coupled Plasma Emission Spectrophotometer (ICP). A Fison's Instruments, Accuris Model, Inductively Coupled Plasma Emission Spectrophotometer (ICP) was used to quantitate manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu), solublized from the surfaces of the SS coupons exposed *in situ*.

Manganese accumulation (also Fe, Zn, and Cu). Mn, Fe, Zn, and Cu accumulation were determined by dissolution of the biofilm on the SS in sulfuric acid using the following method. Manganese was dissolved from exposed coupons for ICP analysis. The coupons were transported in water collected from the site to the laboratory and stored at 4°C, for a maximum of 1 week. Coupons were sonicated for 1.5 minutes in seven mls of 0.5% H_2SO_4 , at room temperature

(RT) to dissolve the manganese from the SS surface. The supernatant was transported to MSU Plant and Soils Lab for ICP analysis. Controls were polished, unexposed coupons (blanks), processed simultaneously with the exposed SS coupons. Originally, the colorimetric formaldoxime method was used to quantify Mn concentrations, however this technique was not sensitive enough for manganese concentrations less than 1 mg/L. described above. Background ICP values for unexposed coupons were subtracted from values of exposed coupons. Analysis was conducted by ICP on at least one coupon per frame per site visit, and recorded as ppm manganese recovered per cm² of SS exposed.

TOC Analysis. A Dohrman DC-80 Carbon Analyzer was used to periodically quantify carbon concentrations at all field sites. Water samples were collected in duplicate from all sample locations and stored at 4°C until analysis. On the day of analysis, a fresh stock of 10 ppm calibration standard solution was diluted in nanopure water. Each sample was run in duplicate.

Manganese oxide test. Fiegel's spot test (13) was used for all 1998 *in situ* and laboratory studies to confirm the formation of manganese oxides at the surface of the SS.

Experimental Methodology for Laboratory Studies

In laboratory studies, sheath forming *L. discophora* were used to form biofilms on 316L SS coupons exposed in the flow cell bioreactor. The bacteria oxidize manganese dissolved in the bulk fluid and subsequently ennobles the SS.

Variables for these experiments included pH, dissolved manganese and pyruvate concentrations.

LABORATORY MATERIALS

Flow-cell bioreactor. A flow-cell bioreactor was constructed of $\frac{1}{4}$ inch polycarbonate for growing a single species manganese oxidizing biofilm. (Figure 2.2) The bioreactor was made with a glass bottom for easy visibility of the microorganisms, through an inverted microscope. The lid has two ports at each end, one for gas exchange and one for inoculating the reactor. There are twelve $\frac{3}{4}$ inch holes, evenly spaced between the ports for insertion of 11 coupon holders and one salt bridge. The feed well of the bioreactor is baffled to allow uniform flow through the body of the reactor. The materials and adhesives used in construction of these bioreactors could not withstand repeated autoclaving. Cleaning consisted of physically removing the biofilm with brushes and dissolution of residual oxides by soaking the reactor in 0.05% NaSO_3 . Reactors were washed in warm soapy water, followed by a detergent disinfectant, ConFlikt, Fisher Sci., (3ml/gal water for approximately 10 minutes), then thoroughly rinsed with deionized water (5 minutes each for the lid and reactor) and 90% ethanol. Finally they were placed in a laminar flow hood for 4-12 hours under ultraviolet light. The entire reactor system was assembled in the hood. All other parts to the reactor system except coupons, were autoclaved.

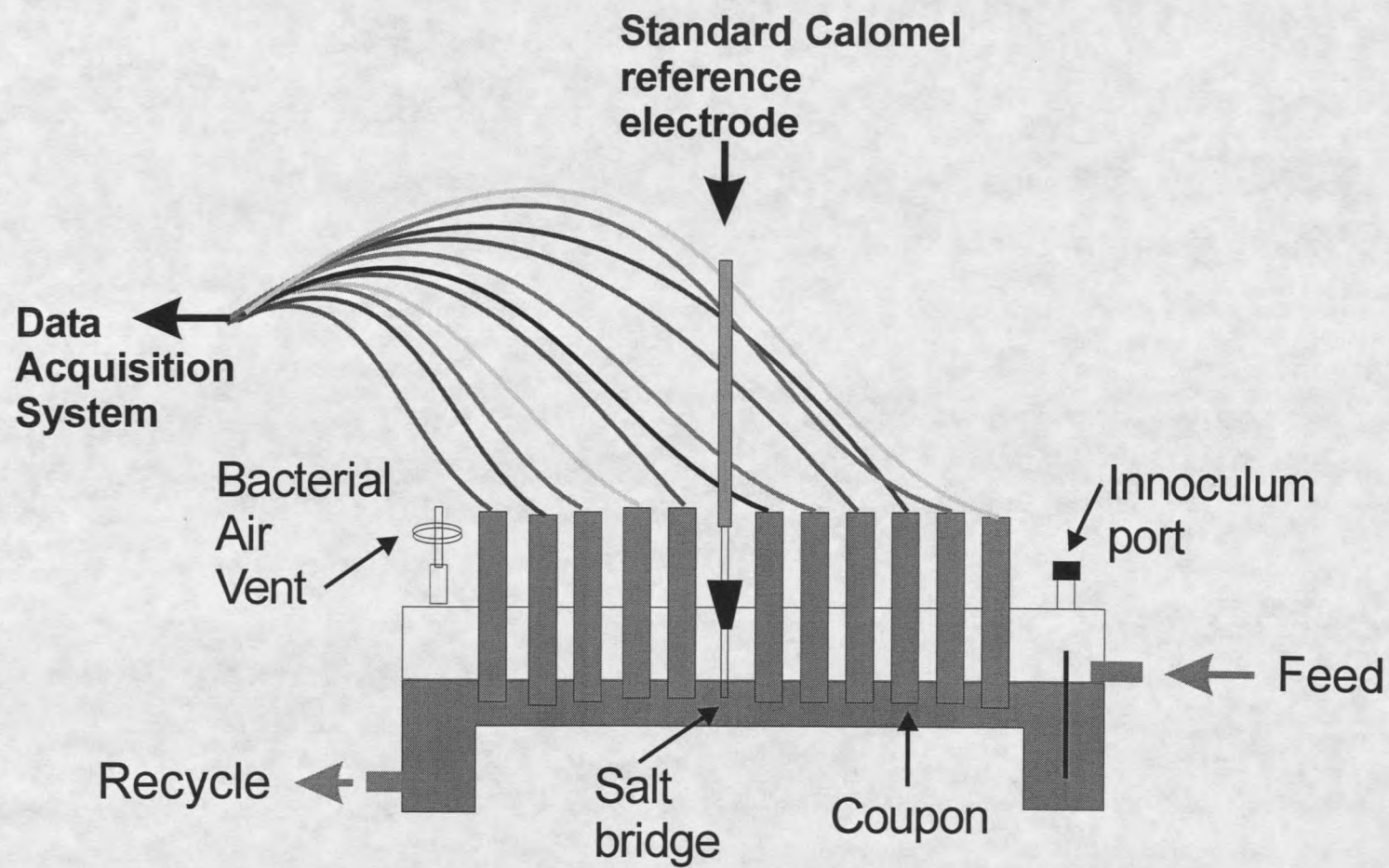


Figure 2.2 Flow-cell bioreactor

The reactor volume, including the mixing chamber, all tubing, and the bioreactor was 1.5 L. MSVP media (see below) was autoclaved and cooled to room temperature before adding sodium pyruvate, vitamins and manganese. This complete media was pumped into the mixing chamber, to the reactor and then recycled back to the mixing chamber. When the reactor was filled, fresh feed was shut down and media continued to cycle throughout the system. Bacterial cells were added via the port at the inflow of the reactor and left to cycle for 24 hours at a volumetric flow rate of 5.2 L/hr. From 24 hours on, fresh media was pumped into the mixing chamber at a rate of 0.2 L/hr. The volumetric flow rate remained constant through the reactor system at 5.2 L/hr.

Laboratory Coupon holders. Laboratory coupon holders were constructed from $\frac{3}{4}$ inch polycarbonate tubes, approximately 6 inches long. The inner wall of the tube was recessed to fit the $\frac{1}{2}$ inch diameter SS coupon. SS coupons were rinsed and sonicated in deionized water then ethanol to remove oils. Similar to field coupons holders, coupons were sealed into the holder with epoxy, allowed to dry, then sanded systematically using wet 120 to 600 grit paper. The epoxy was sanded away from the coupons leaving coupons embedded flush in the holder. Again the coupons were rinsed and sonicated in deionized water to remove stainless steel shavings, rinsed with ethanol and air dried. They were allowed to stand at least 24 hours to restore the passive layer. Each holder was fitted with a No. 1 black stopper. A steel spring was soldered to a 6 inch length of 18 gauge insulated copper wire. The copper wire protruded through the

stopper and the spring was held in constant tension against the back surface of the SS coupon when the stopper apparatus was placed in the opposite end of the coupon holder. Coupons were transferred to the laminar flow hood and dipped in 90% ethanol, then left to stand inverted for at least 1 hour under UV light before inserting into reactor. After the entire reactor was assembled and returned the bench, wires were attached to the copper wire protruding from the stopper, extending to the data acquisition system where E_{corr} was recorded every 30 minutes.

Salt bridge. A salt bridge between two reactors was designed to facilitate collection of E_{corr} data using one reference electrode. The bridge consisted of a 4-inch long glass shaft with a porous plug inserted in one end and sealed with shrink tubing. The glass tube was filled with 0.1M Na_2SO_4 agar solution. Clear rubber tubing was connected to the glass tube filled with agar and extended to the reference electrode. The rubber tubing was filled with 0.1M Na_2SO_4 .

Standard calomel electrode. Same as for field study.

Computer data acquisition. A computer program, ECORR, designed by W. Dickinson, was used in laboratory studies in conjunction with a data acquisition apparatus to continuously monitor E_{corr} at the SS coupon surfaces. (6) Data was accumulated from coupons in two reactors running simultaneously, every 30 minutes, for a period of 5-14 days.

Pumps and tubing. Peristaltic pumps from Cole Palmer Instrument Company, model no. 7553-80, 1-100 rpm, were used to maintain constant flow

through the reactor system. Two sizes of pump heads and tubing were used. Pump head 7017-20 was used with size 17 tubing for reactor feed and recycle. Pump head 7016 was used with size 16 tubing for reactor fresh feed and effluent.

Carboys. Medium was mixed and autoclaved in 20L carboys, either glass or polypropylene.

Filters. Whatman Polycap 75 AS filter devices were used to filter the growth media before it entered the continuous stir mixing chamber. This filter prevented backflow of cells and subsequent contamination of the growth media.

Bacterial air vents. Bacterial air vents from Gelman Sciences, part no. 4210, were autoclaved and mounted on reactors and media-filled carboys to allow for gas exchange, without compromising sterility in the system.

Mixing chambers. A 500 ml flask, modified with three glass ports, served as the mixing chamber. Ports were mounted at different elevations for feed, recycle and effluent. A stopper at the mouth of the flask had one port for dripping fresh feed. Effluent overflow from the mixing chamber drained into a 10 L carboy with a small amount of chlorine bleach added to kill organisms before disposal.

Culture Media. MSVP medium consists of 10mM HEPES buffer, pH 7.2, plus a mineral salts mixture, filter-sterilized vitamins and 0.1% sodium pyruvate as a carbon energy source, supplemented with various concentrations of filtered 0.1M MnSO_4 . (10) When required, MSVP medium was solidified by adding 1.5% Noble agar to the buffered solution before autoclaving.

Innoculum. Sheath forming, *Leptothrix discophora* SP-6, was obtained from American Type Culture Collection (ATCC). This microorganism was selected for laboratory studies because of its ability to form a biofilm on surfaces and oxidize manganese supplied in the culture media. The manganese oxides accumulate in the sheath of the microorganism.

DATA COLLECTION FOR LABORATORY STUDIES

Enumeration of organisms. Enumeration of organisms in the laboratory were done by AODC, acridine orange direct counts. Serial dilutions of cells were stained with 0.05% acridine orange (AO) onto a black polycarbonate filter membrane, then counted using an fluorescent light microscope.

Ennoblement curves. Ennoblement curves were generated from E_{corr} (mV vs. SCE) data collected from each coupon every 30 minutes. Curves were generated by graphing E_{corr} vs. time (days).

pH. Effluent from the mixing chamber was sampled daily and pH was measured.

CHAPTER 3

RESULTS

Field Results

The duration of individual field studies (Table 2.1) varied depending on the time required to ennobel (if at all), inclement weather, seasonal limitations, and collection of all coupons. At the conclusion of the 1997 field studies, site selection was re-evaluated based on the results. Frames from MSD pond were placed upstream, where there was some flow. Frames from Hebgen Dam were relocated to a stratified part of the lake where we could hopefully observe effects of lower oxygen and temperatures on ennoblement without traveling to the more remote Cliff Lake. Weasel Creek was added to the study because it contributed another site with flow velocities, yet had a higher pH and lower dissolved manganese concentrations than the control stream, Roskie Creek.

Dissolved Manganese. Water chemistry results by ICP indicated variable dissolved manganese concentrations among the field study sites. (Table 2.2) Water was analyzed for 15 additional minerals. MSD had by far the highest amount of dissolved manganese of all the study sites, between 6.4-8 ppm, as expected. Water chemistry results indicated MSD was significantly high in B, Ca, Cu, Fe, Mg, and Zn also. (Tables 2.2, 2.3) Cliff Lake had the lowest concentrations of dissolved manganese, less than 0.01 ppm. Manganese concentrations at Weasel Creek (Table 2.3), Hebgen Lake and Hebgen Dam

TABLE 2.1 Duration of Individual Field Studies

Location	Date Started	Date Ended	Total (weeks)
1997			
Roskie Creek	5/23/97	6/19/97	4
Cliff Lake	7/4/97	10/17/97	15
Hebgen Dam	7/8/97	9/19/97	11
MSD Pond	8/21/97	10/16/97	8
1998			
Roskie Creek	5/18/98	8/20/98	14
Hebgen Lake	5/20/98	8/28/98	15
MSD Stream	5/19/98	8/21/98	14
Weasel Creek	5/15/98	8/21/98	14

TABLE 2.2 Water Chemistry for 1997 Field Study

Metals	Cliff Lake 10/17/97 mg/L	Hebgen Dam 9/19/97 mg/L	MSD Pond 10/16/97 mg/L	Roskie Creek 10/20/97 mg/L	Detection Limit mg/L
B	<.01	0.20	0.80	0.02	0.01
Ca	27.80	11.20	128.80	81.60	
Cu	<.01	<.01	0.82	<.01	0.01
Fe	0.02	0.04	4.47	0.42	
K	3.10	4.20	9.70	7.10	
Mg	13.60	2.40	31.40	20.20	
Mn	<.01	0.02	8.05	0.09	0.01
Na	6.00	30.70	46.50	11.80	
P*	<.1	<.1	<.1	<.1	0.1
Zn	<.01	<.01	11.97	0.03	
Pb	<.5	<.5	<.5	<.5	0.01
Al	<.05	<.05	0.44	0.40	0.05
Cd	<.01	<.01	0.06	<.01	0.01
Cr	<.01	<.01	<.01	<.01	0.01
Ni	<.01	<.01	0.02	<.01	0.01

*Problem with Standard for P

Table 2.3 Water Chemistry for Streams - 1998 Field Study

Metals	Weasel Creek 5/15/98 mg/L	MSD Stream 5/19/98 mg/L	Roskie Creek 5/18/98 mg/L	Detection Limit mg/L
B	0.02	0.59	0.02	0.01
Ca	36.40	118.60	68.00	
Cu	0.03	0.32	0.02	0.01
Fe	0.62	1.96	0.08	
K	0.80	8.70	5.10	
Mg	7.40	27.50	18.10	
Mn	0.02	6.46	0.04	0.01
Na	3.20	42.20	9.00	
P*	<.1	<.1	0.10	0.1
S	1.20	120.20	2.60	
Zn	0.01	7.61	<.01	
Pb	<.5	<.5	<.5	0.01
Al	0.79	0.13	0.08	0.05
Cd	<.01	0.03	<.01	0.01
Cr	<.01	<.01	<.01	0.01
Ni	<.01	0.03	<.01	0.01

Table 2.4 Water Chemistry for Hebgen Lake - 1998 Field Study

Metals	Hebgen (3m) 6/19/98 mg/L	Hebgen (7m) 6/19/98 mg/L	Hebgen (15m) 6/19/98 mg/L	Detection Limit mg/L
B	0.23	0.23	0.24	0.01
Ca	9.20	9.20	9.30	
Cu	0.01	<.01	0.02	0.01
Fe	0.07	0.07	0.08	
K	4.20	3.90	5.00	
Mg	1.90	1.90	2.00	
Mn	<.01	<.01	0.01	0.01
Na	29.00	28.90	29.40	
P*	<.1	<.1	<.1	0.1
S	2.50	2.50	2.60	
Zn	0.02	0.01	0.03	
Pb	<.5	<.5	<.5	0.01
Al	<.05	<.05	<.05	0.05
Cd	<.01	<.01	<.01	0.01
Cr	<.01	<.01	<.01	0.01
Ni	<.02	<.02	<.02	0.01

(Tables 2.2, 2.4) fluctuated between 0.01 and 0.02 ppm. Roskie Creek (Tables 2.2, 2.3) had dissolved manganese concentrations ranging from 0.04-0.09 ppm. This is fairly high according to drinking water standards and exceeds USEPA's recommended limit for dissolved manganese in drinking water of 0.01 ppm.

Corrosion Potentials (E_{corr}) For the 1997 field studies, recall that three coupons per frame were wired to the surface atmosphere and E_{corr} for each coupon was measured at each visit. E_{corr} of three coupons per frame and standard deviation were calculated and plotted. In the case of Cliff Lake and Hebgen Dam, all nine coupons were averaged because there was no significant difference between the 3, 7, and 13 meter exposed coupons in Cliff or the 1, 3, and 6 meter exposed coupons in Hebgen Dam. Corrosion potentials were measured for the same three coupons per frame at each visit.

In the 1998 field studies, initially each frame held 12 coupons. At the time of the first site visit, corrosion potentials for all 12 coupons were measured, averaged, and the standard deviation was calculated. At around day 50, two coupons were removed for further analysis. At the following site visit, corrosion potentials for 10 coupons were measured, averaged, and standard deviations calculated based on the number of coupons remaining, and so on, until no coupons remained. Ennoblement curves for the 1997 (Figure 2.3) and 1998 field studies (Figure 2.4) are shown.

SS coupons exposed in Roskie Creek consistently required the least amount of time (approximately 2-3 weeks) to fully enoble and remain stable at

+350 mV vs. SCE. In one experiment, these elevated potentials were monitored periodically for 6 months and remained stable, at which time the coupons were removed.

Neither the MSD pond nor MSD stream SS coupons ennobled during either of the field studies. (Figures 2.3, 2.4) E_{corr} consistently fluctuated near zero mV vs. SCE in this metal laden, low pH water.

Coupons exposed at Hebgen Dam in 1997 (Figure 2.3), where the water was only 6 meters deep and unstratified, did not significantly enoble during the 80 day study period. However, at the final site visit, the corrosion potential of one coupon was +242 mV vs. SCE. There appeared to be a trend toward ennoblement. During the 1998 field study (Figure 2.5), where coupons were exposed at three depths in the water column, the trend toward ennoblement was observed at 50 days. Seven of the original twelve coupons exposed in the epilimnion had corrosion potentials above 80 mV vs. SCE. Those potentials remained stable for two weeks. Four defective coupon holders from the 3 m frame were leaking water to the inside. Inadvertently, we had collected all the intact coupons early on in the study for analysis, and were not able to observe fully ennobled coupons at 3 m. By day 65 coupons from the 7-meter frame had begun to enoble. Five of the nine remaining coupons had corrosion potentials above 100 mV vs. SCE. At the end of the study the average corrosion potentials for these coupons had increased slightly to approximately +150 mV vs. SCE. At the lowest frame, coupons did not show signs of ennoblement until about day 90.

Figure 2.3 Ennoblement of 316L SS Coupons *in situ* 1997

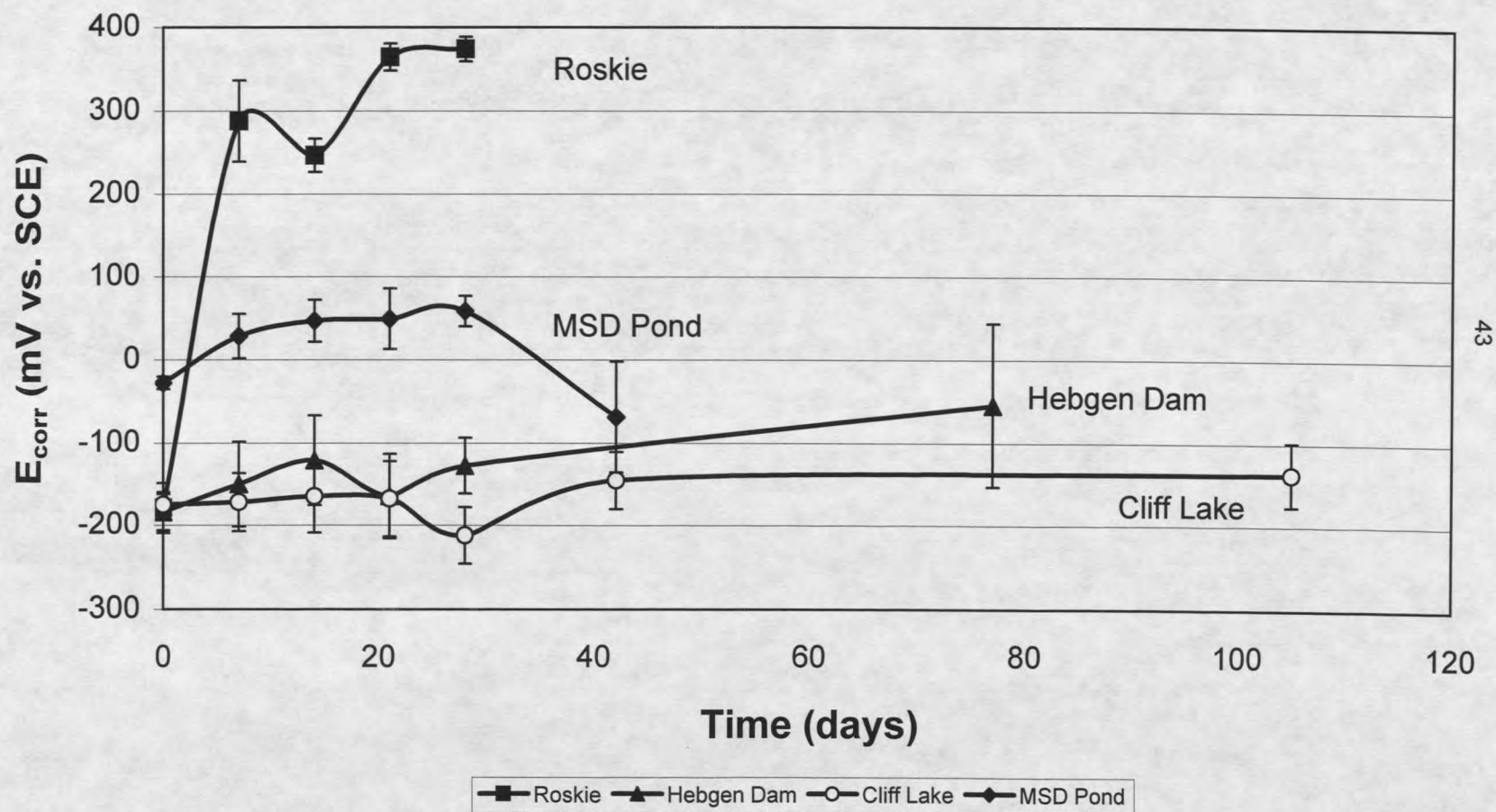


Figure 2.4 Ennoblement of 316L SS Coupons *in situ* 1998

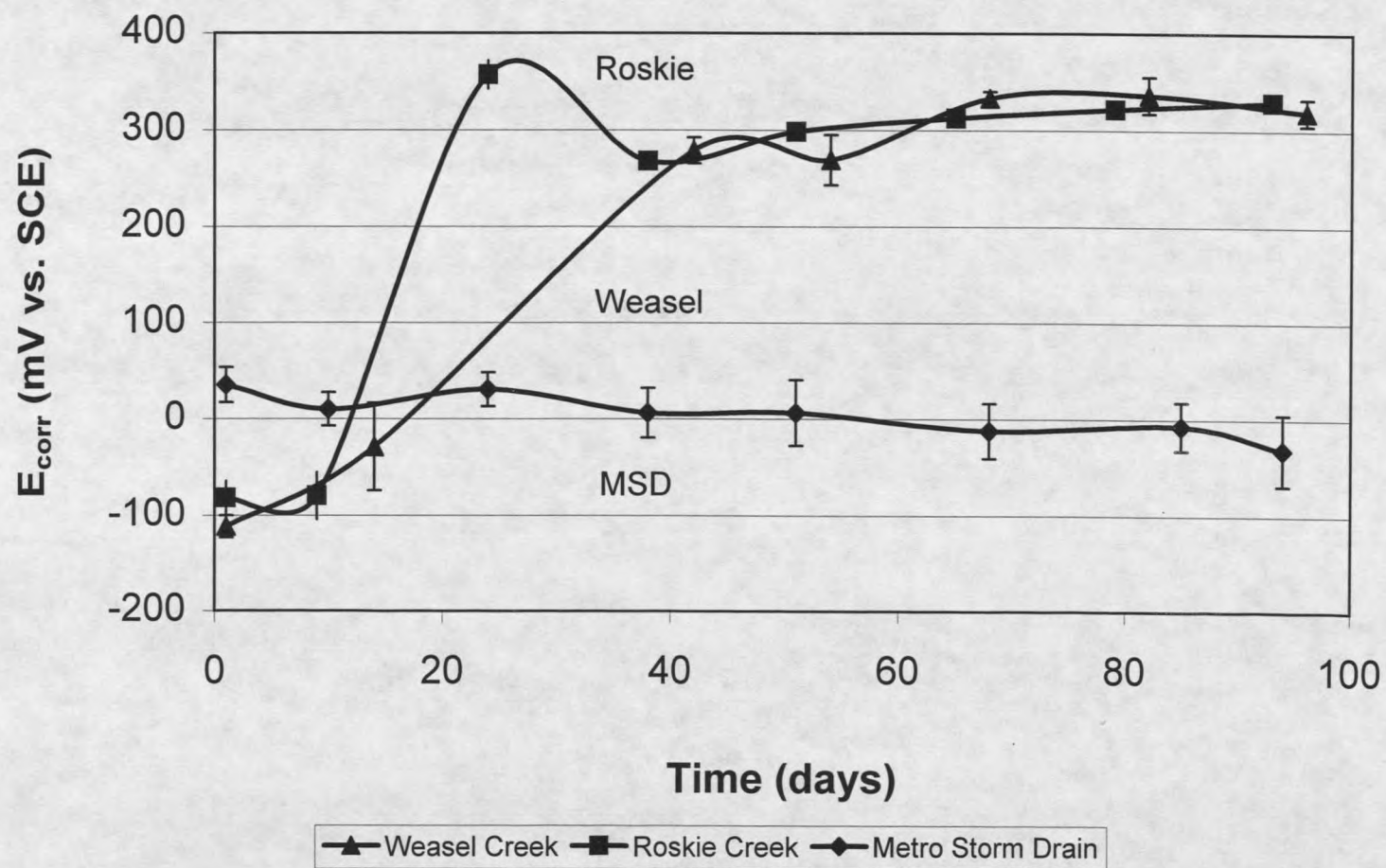
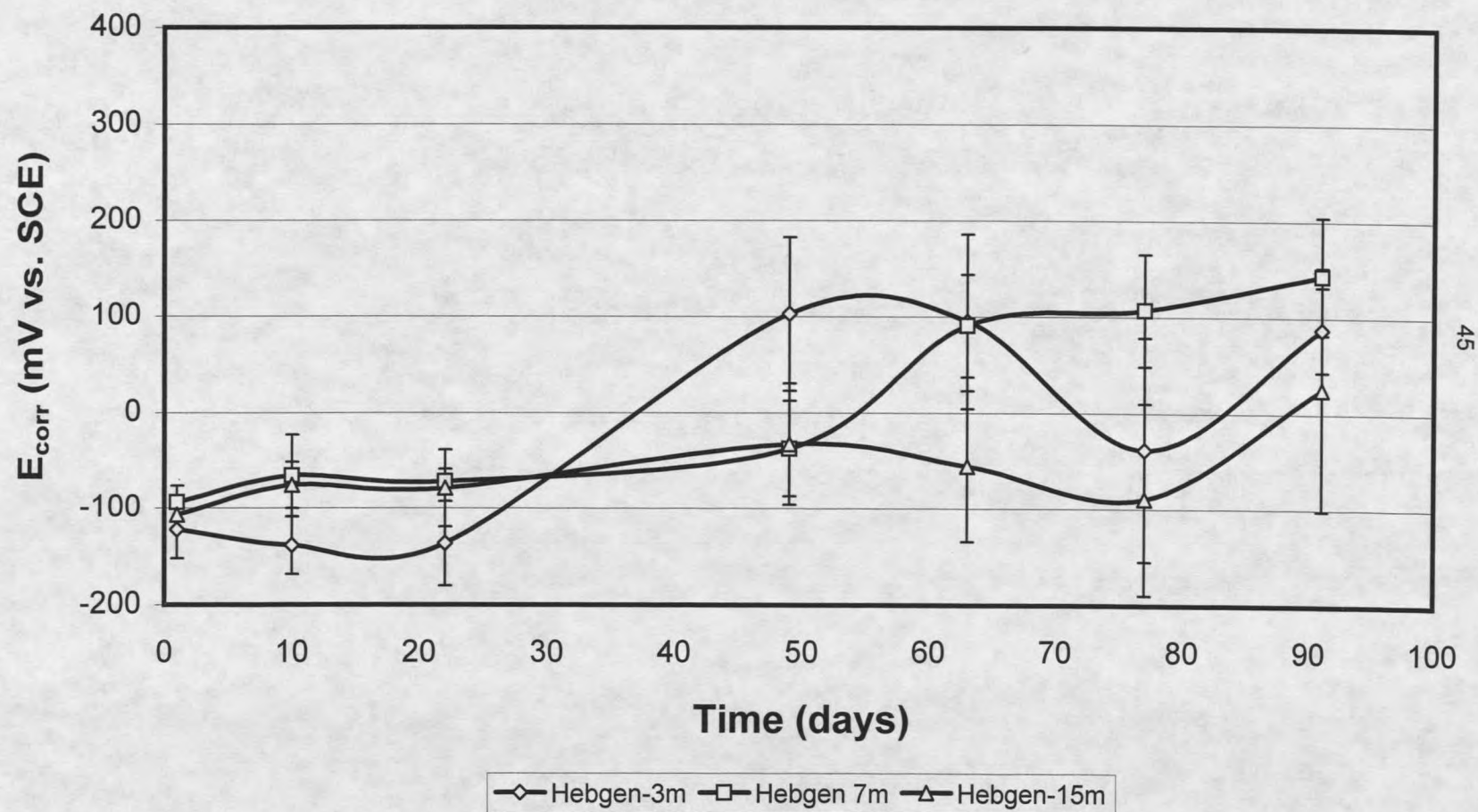


Figure 2.5

**Ennoblement of 316L SS *In Situ*
Hebgen Lake 1998**



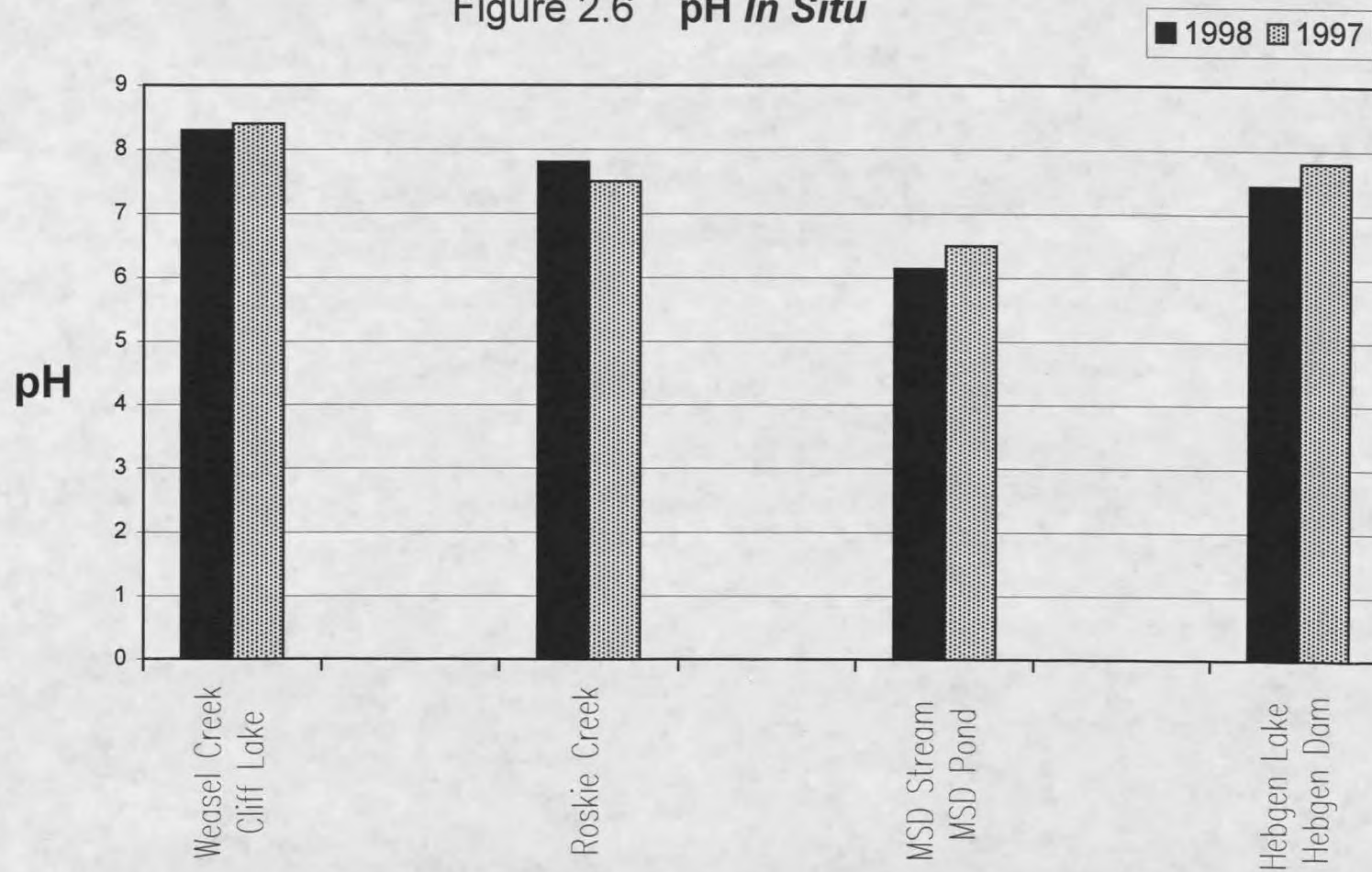
Two of the remaining three coupons had corrosion potentials between 80 and 90 mV vs. SCE.

Coupons exposed in Cliff Lake did not enoble (Figure 2.3). A maximum potential of -30 mV vs. SCE was achieved after 110 days by one coupon at the 3 meter depth.

Corrosion potentials from coupons exposed at Weasel Creek seemed to parallel Roskie Creek (Figure 2.4). The graph indicates that SS took longer to enoble at this site. However this is misleading because the multimeter was not functioning properly at the 30 day site visit to Weasel Creek and E_{corr} was not measured.

In situ pH. Average pH values were calculated from all pH data collected and results are shown in Figure 2.6. Highest pH values were observed at Cliff Lake and Weasel Creek, where pH ranged between 8 and 8.4 consistently. The site having the lowest pH was MSD. Both the pond and the stream gave pH values of 6.3 to 6.7. Roskie Creek, Hebgen Dam and Hebgen Lake had pH values just above neutral. In the lakes, pH was measured at the surface, epilimnion, thermocline, and hypolimnion. The pH varied by 0-0.3 pH points throughout the water column. This was not considered significant for this study and all pH measurements were included in the average as described above.

Figure 2.6 pH *In Situ*



Dissolved Oxygen and Temperature. Three study sites had the potential for stratification in the water column; Cliff Lake, Hebgen Lake and Hebgen Dam. Dissolved oxygen and temperature profiles were generated at each site visit. In stratified lakes, the epilimnion is the well-mixed layer at the top of the water column. It exhibits higher temperatures and dissolved oxygen concentrations. The thermocline is the transitional layer between the epilimnion and the low oxygen, low temperature layer at the bottom of the lake. In general, no mixing occurs in the hypolimnion, nor between the epilimnion and the hypolimnion until turnover in the fall.

Cliff Lake was temperature stratified at the onset of the study in July 1997, and just starting to oxygen stratify. (Figure 2.7a) Frames were suspended from a buoy to 3, 7 and 13 meter depths in the water. Turnover occurred before the last site visit in October, when profiles indicated the water was well mixed. (Figure 2.7c) Figure 2.7 shows representative profiles of DO and temperature data collected on a) 7/18/97, b) 8/20/97 and c) 10/17/97.

Water remained well mixed at the Hebgen Dam site throughout the 1997 field study. Frames were suspended from a boat barrier pontoon at 1, 3 and 5 meters. Figure 2.8 shows representative profiles of DO and temperature data collected on a) 7/8/97, b) 8/1/97 and c) 9/19/97.

Hebgen Lake was well mixed at the first site visit in May 1998 (Figure 2.9a). By July, the lake was stratified and the epilimnion was shallow, only 4 meters deep. (Figure 2.9b) Frames were suspended from a buoy to 3, 7 and 15-meter

Figure 2.7
Dissolved Oxygen and Temperature Profiles
for Cliff Lake 1997

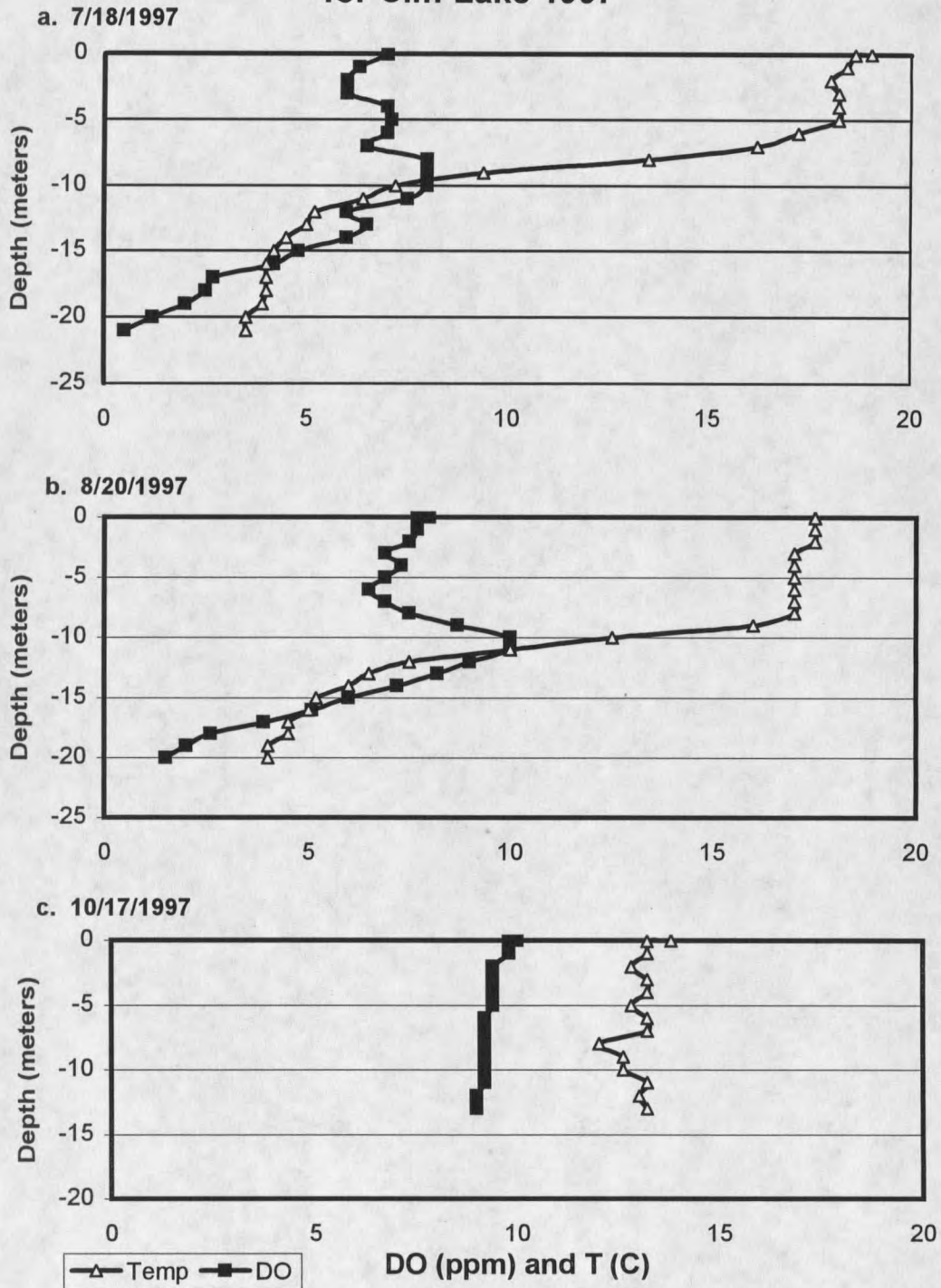
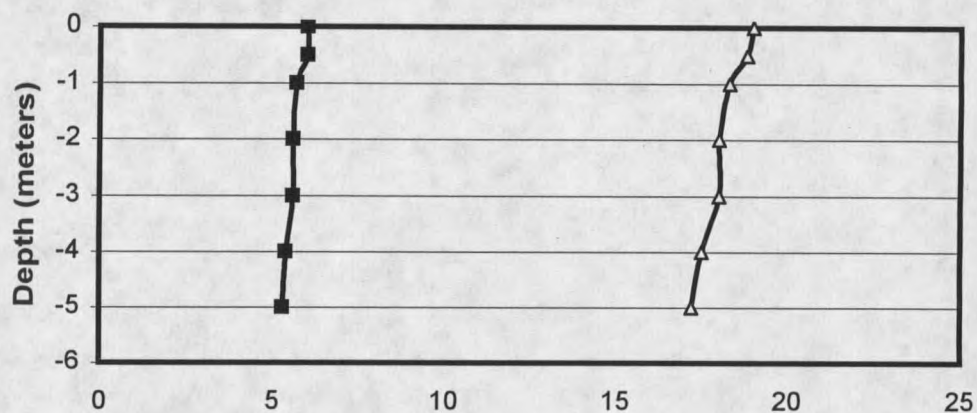
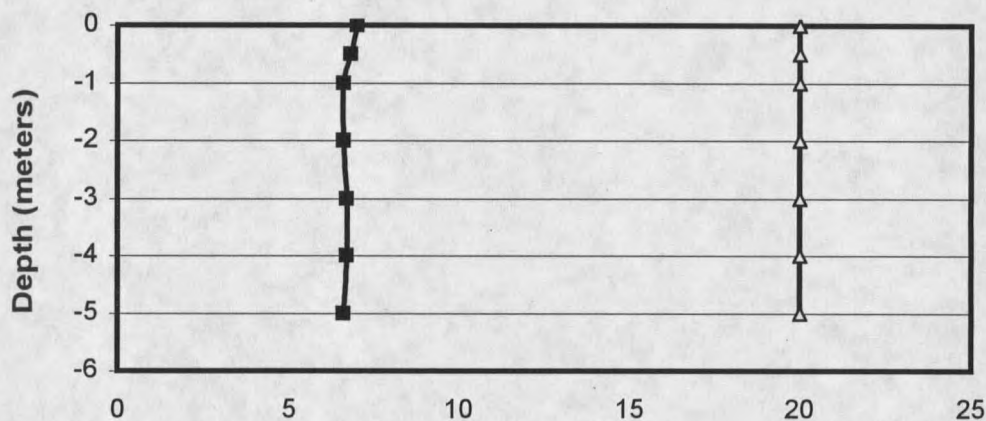


Figure 2.8
Dissolved Oxygen and Temperature Profiles
for Hebgen Dam 1997

a. 7/8/1997



b. 8/1/1997



c. 9/19/1997

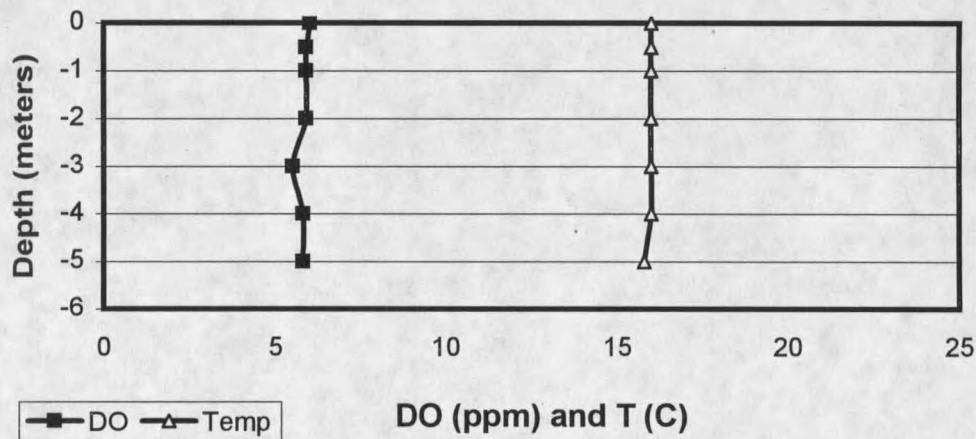
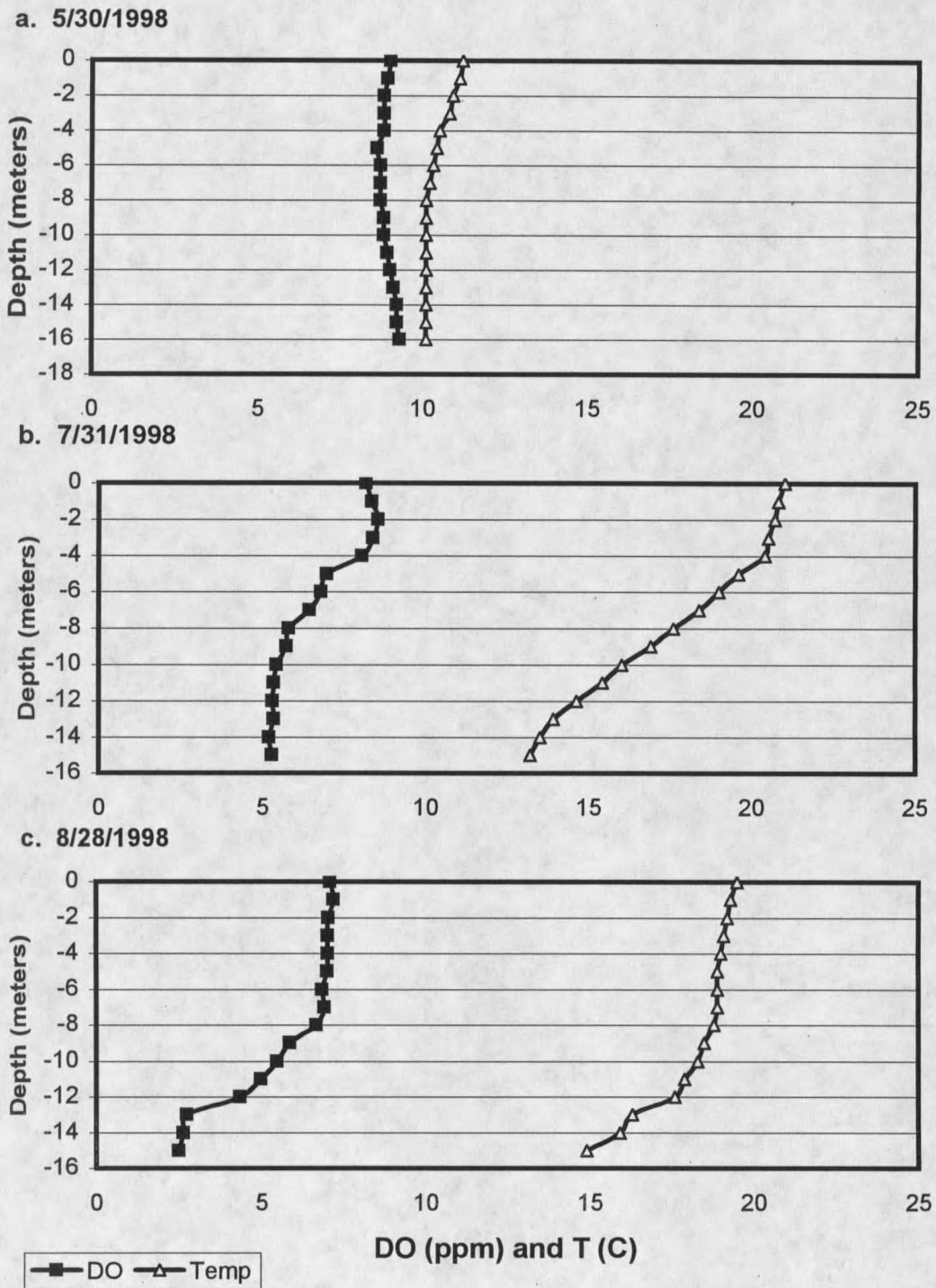


Figure 2.9
Dissolved Oxygen and Temperature Profiles
for Hebgen Lake 1998



depths, representing sites in the epilimnion, thermocline, and hypolimnion, respectively. In late August, the lake was still stratified but the epilimnion was approximately 8 meters deep (Figure 2.9c). At this point two frames were in the oxygenated zone of the water column, where DO was 7.5 ppm and the temperature was 20°C. DO was 2.5 ppm and the temperature 15°C in the low oxygen zone (hypolimnion), where the lower frame was suspended.

MSD pond (Figure 2.10) and MSD stream (Figure 2.11c) showed very similar DO and temperature profiles. DO was consistently under 5.0 ppm. This is low compared to the other sites, with the exception of the deep-water sites at Cliff and Hebgen Lake. Temperature varied, depending on precipitation and run-off, but generally fluctuated between 15-25°C for both MSD sites.

In the spring of 1998, temperatures at Roskie Creek were comparable to those at Weasel Creek, but they gradually increased and remained in the low 20's°C during the summer. (Figure 2.11a) Inversely, DO decreased as temperatures increased to 6 ppm by the end of the study period. Profiles for Roskie Creek during the 1997 were similar to 1998 (not shown).

Weasel Creek is a mountain stream and is colder than the other streams (Figure 2.11b). Temperatures ranged between 6-13°C for the study period. DO here was generally higher than all other streams, near 10 ppm over the entire study period.

Manganese dioxide spot test. Fiegle's spot test (13) for MnO_2 was incorporated into the protocol for examining coupons from the field and lab

Figure 2.10 Dissolved Oxygen and Temperature Profile for MSD Pond 1997

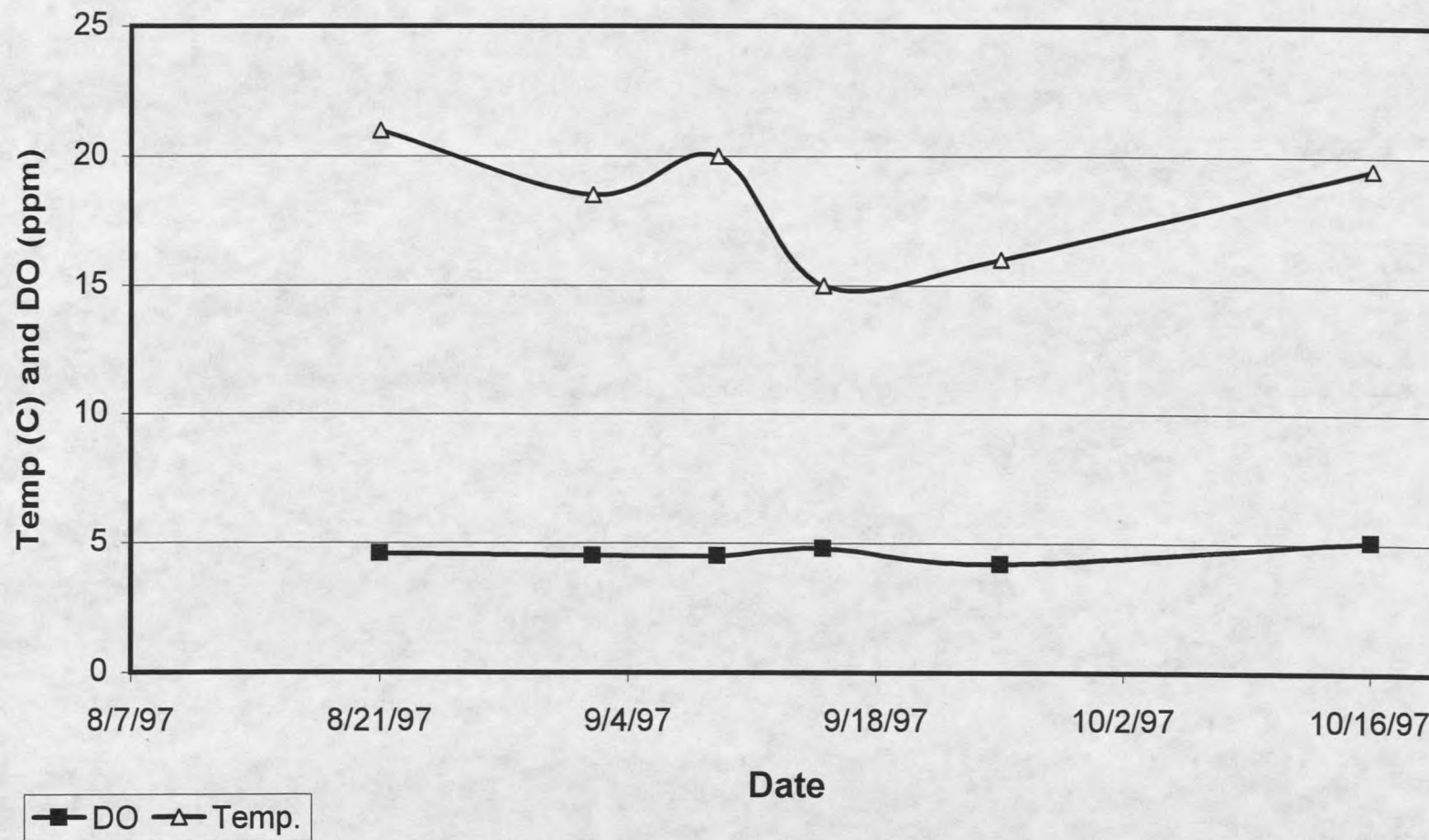
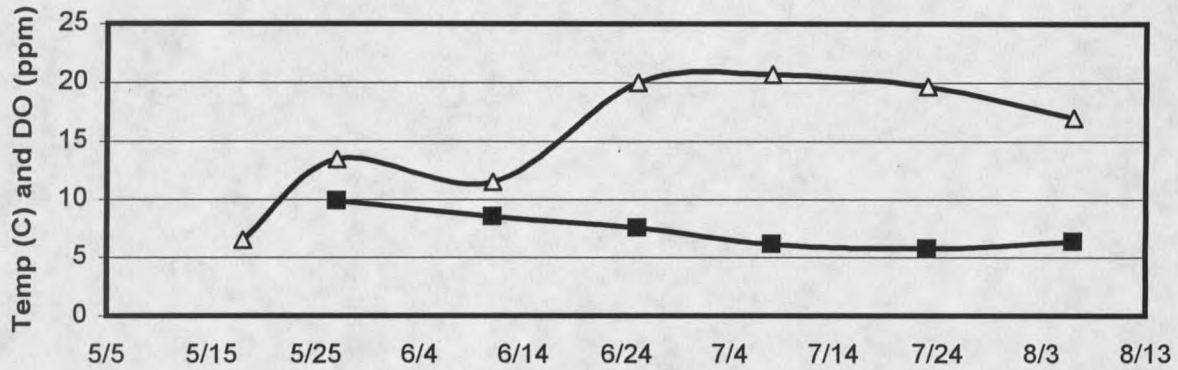


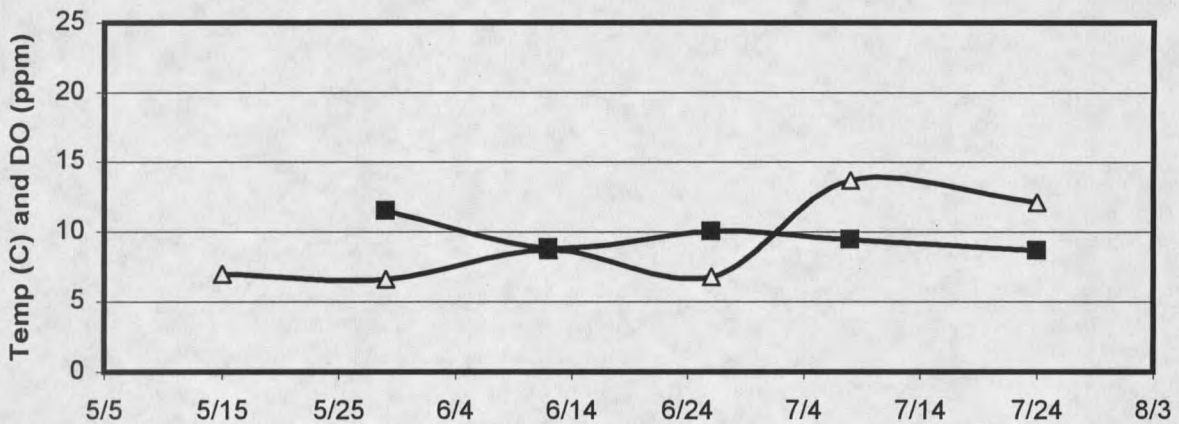
Figure 2.11

Dissolved Oxygen and Temperature Profiles for Streams - 1998

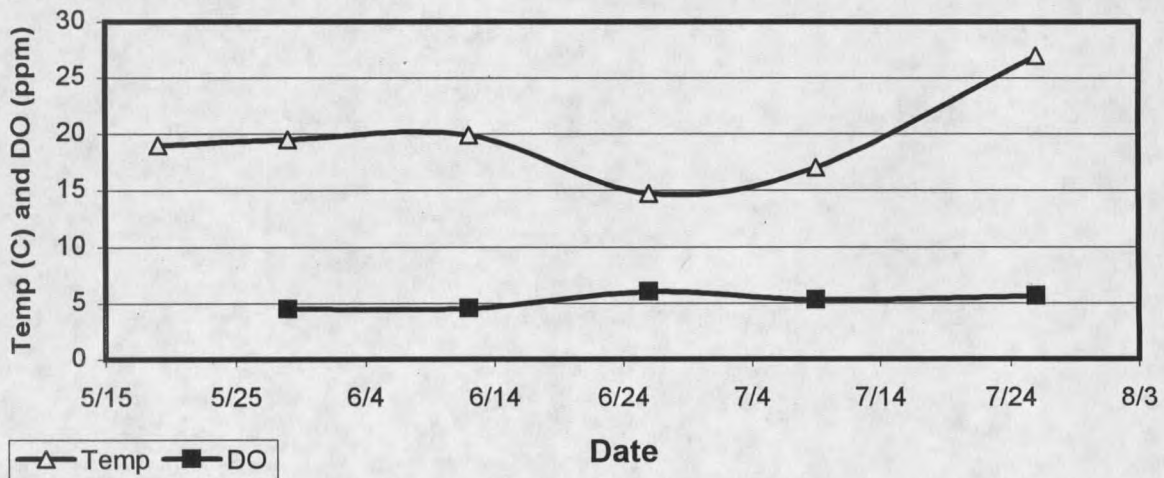
a. Roskie Creek 1998



b. Weasel Creek 1998



c. Metro Storm Drain Stream 1998



studies in July 1998. The coupons tested had been exposed *in situ* for 9 weeks. All randomly selected SS coupons removed from Roskie and Weasel Creeks tested positive for MnO_2 from the time we started spot testing throughout the remainder of the study. (Table 2.5) None of the SS coupons tested from MSD stream were positive for MnO_2 , nor did they enoble.

SS coupons from Hebgen Lake were tested for MnO_2 also. (Table 2.6) Coupons removed on 7/17/98 did not test positive for MnO_2 and none had ennobled by this date. On 7/31/98, three coupons were removed from each frame. The coupons at 3m and 7m tested positive for MnO_2 , and they were ennobled to 281 and 118 mV respectively. The coupon tested from the 15m frame was negative for MnO_2 and E_{corr} was -93 mV. On 8/14/98, the coupon from the 3m frame was positive for MnO_2 , and coupons from 7m and 15m were negative. Corrosion potentials from these frames were +196, -5, and -14 mV respectively. On 8/28/98, coupons from the 3m and 7m frames were positive, and the coupon from 15m was negative. Corrosion potentials from these frames were +153, +201, and -104 mV respectively.

Total Organic Carbon. TOC at Cliff Lake from 7/14/97 through 8/20/97 ranged between 1.8-2.8 ppm for all three depths, with the exception of one spike on 7/25/97 at the 3-m depth, which was 5.5 ppm. (Figure 2.12a) Water at Hebgen Dam was unstratified, and as expected TOC levels were similar at 1, 3 and 5 meters and ranged from 2.9-4.2 ppm with the exception of one spike on 8/1/98 at the 3-meter level of 5.9 ppm (Figure 2.12b). The TOC range for

TABLE 2.5 Fiegel's MnO₂ Spot Test Results - Streams 1998

Roskie Creek			Weasel Creek			MSD		
Date	pos/neg	E _{corr} (mV)	Date	pos/neg	E _{corr} (mV)	Date	pos/neg	E _{corr} (mV)
7/23/98	+	286	7/24/98	+	326	7/26/98	-	-14
8/6/98	+	333	8/7/98	+	343	8/12/98	-	-10
8/20/98	+	327	8/21/98	+	334	8/21/98	-	-41

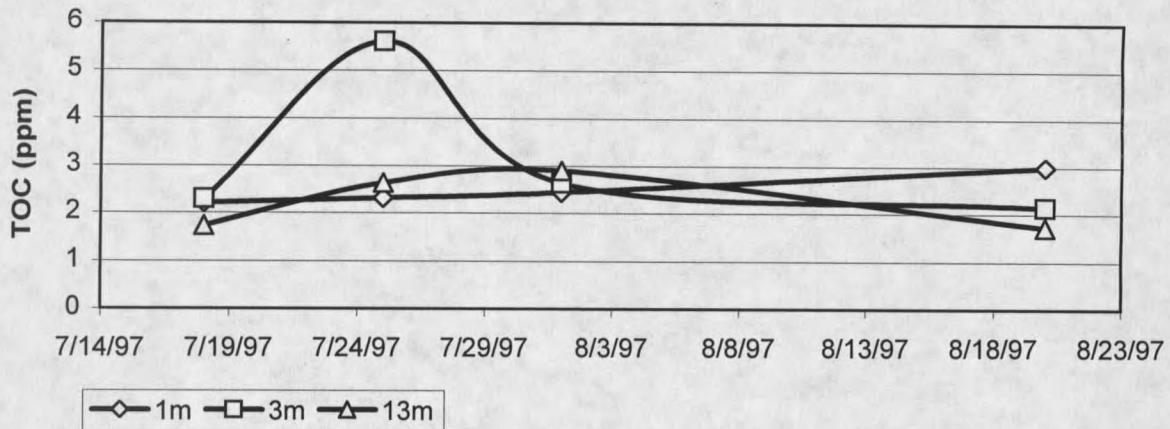
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TABLE 2.6 Fiegel's MnO₂ Spot Test Results - Hebgen Lake 1998

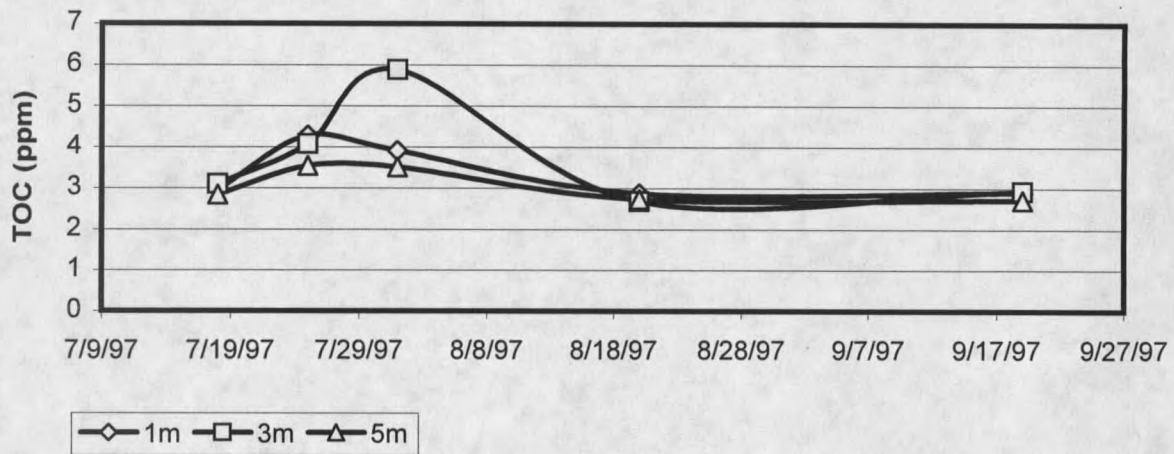
Hebgen 3m			Hebgen 7m			Hebgen 15m		
Date	pos/neg	E _{corr} (mV)	Date	pos/neg	E _{corr} (mV)	Date	pos/neg	E _{corr} (mV)
7/17/98	-	-137	7/17/98	-	-42	7/17/98	-	-170
8/14/98	+	196	8/14/98	+	-5	8/14/98	-	-14
8/28/98	+	153	8/28/98	+	201	8/28/98	-	-104

Figure 2.12 Total Organic Carbon 1997

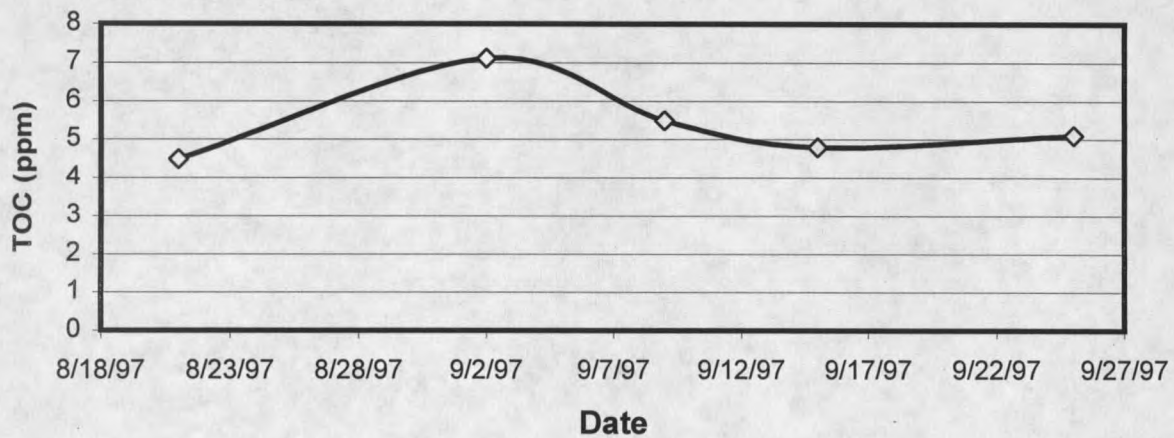
2.12.a Cliff Lake



2.12.b Hebgen Dam



2.12.c MSD Pond



Hebgen Dam was 2.9-4.2 ppm, with the exception of the one data point mentioned above. The TOC at MSD pond between 8/20/97 and 9/22/97 ranged between 4.3-7ppm. (Figure 2.12c)

Figure 2.13 shows TOC levels in Hebgen Lake at 3,7 and 15 meters. In general, TOC levels were elevated in the epilimnion (3m), between 3-5 ppm. TOC levels at 7 and 15 m seemed to follow a trend where levels increased from 2 ppm in early June to peak at 4 ppm in late July, and then declined again to approximately 2.5 ppm in late August.

Typically, TOC levels fluctuated in the streams more than in the lakes. TOC levels at Roskie Creek ranged anywhere between 3-10 ppm from 5/15/98 to 8/21/98 (Figure 2.14). The peak TOC levels were during early June and late July. Weasel Creek TOC levels were typically the lowest and ranged from 1.5-6 ppm for the same period. MSD stream TOC levels were very consistent, ranging from 4.5 to 5.5.

ICP analysis for manganese recovered from exposed SS coupons. In general, the longer the exposure the higher the concentration of manganese recovered from the surface of the SS. Coupons were sampled from one week mark during the 1997 season, but in 1998 coupons were exposed for about 6 weeks before coupons were removed and analyzed for manganese deposition.

Figure 2.15 shows results from all sites except Hebgen Lake. Roskie Creek had the highest amounts of manganese recovered from the SS, ranging from 14 $\mu\text{g}/\text{cm}^2$ at twenty days exposure to 42 $\mu\text{g}/\text{cm}^2$ at ninety-four days in 1998.

Figure 2.13 Total Organic Carbon - Hebgen Lake 1998

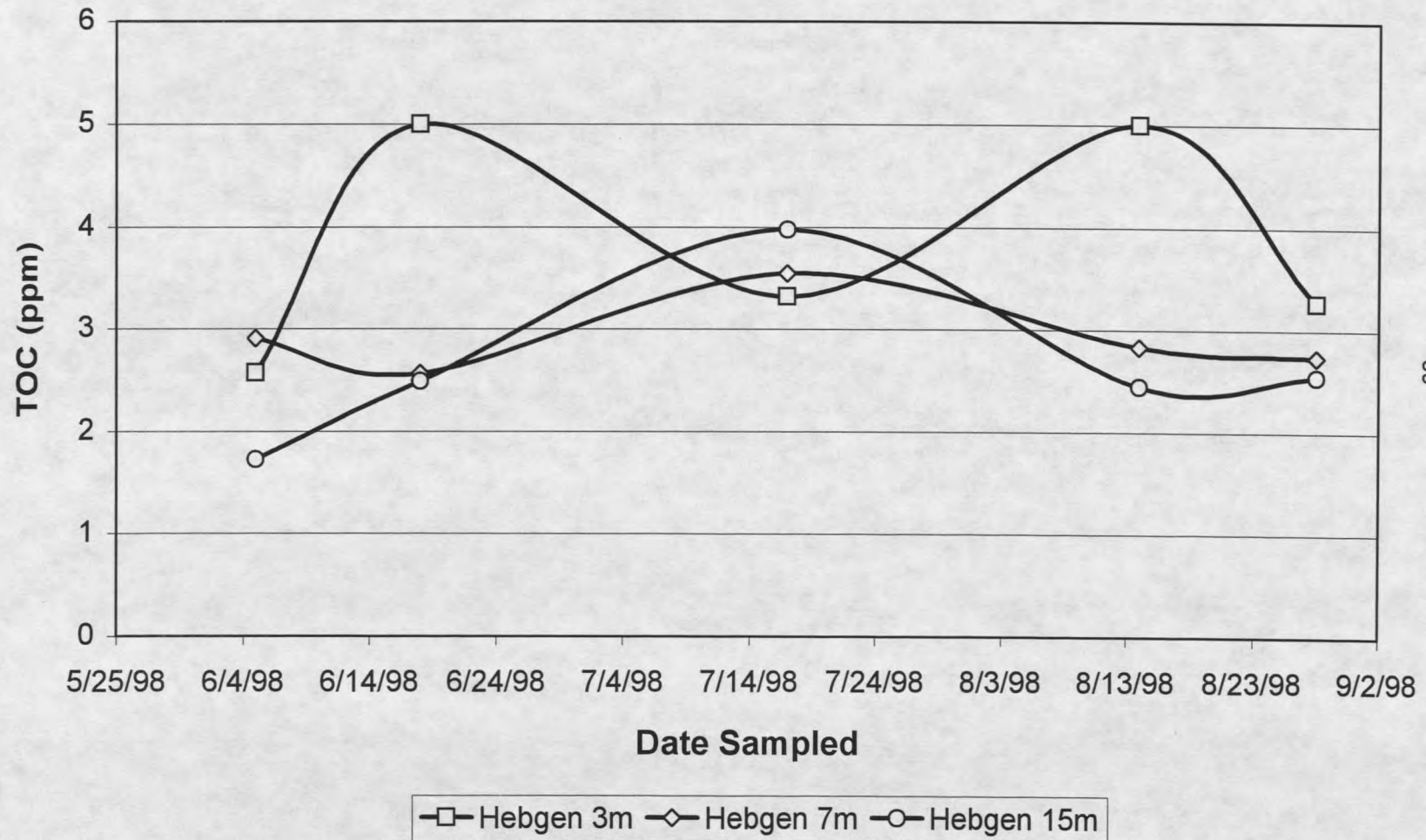


Figure 2.14 Total Organic Carbon- Streams 1998

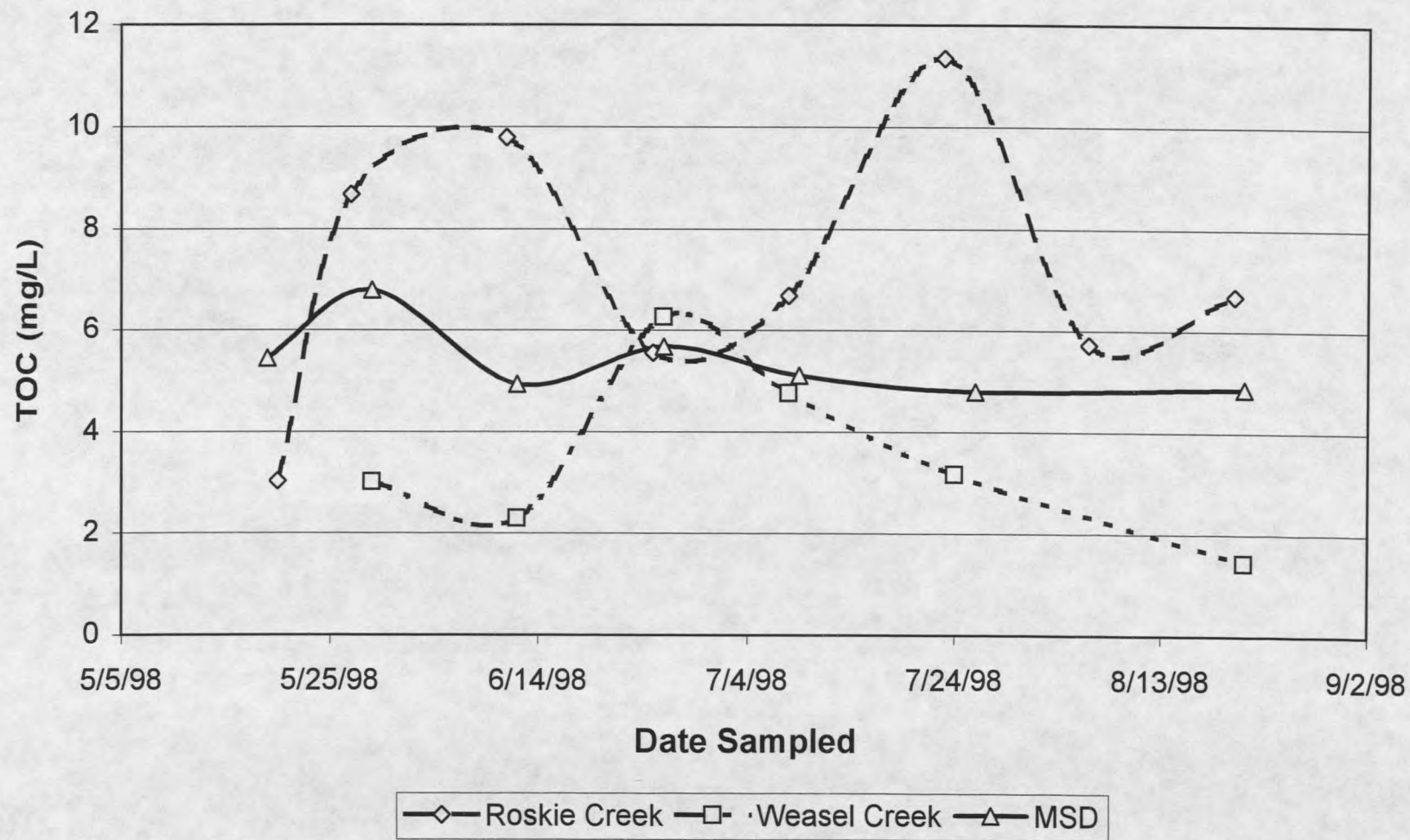
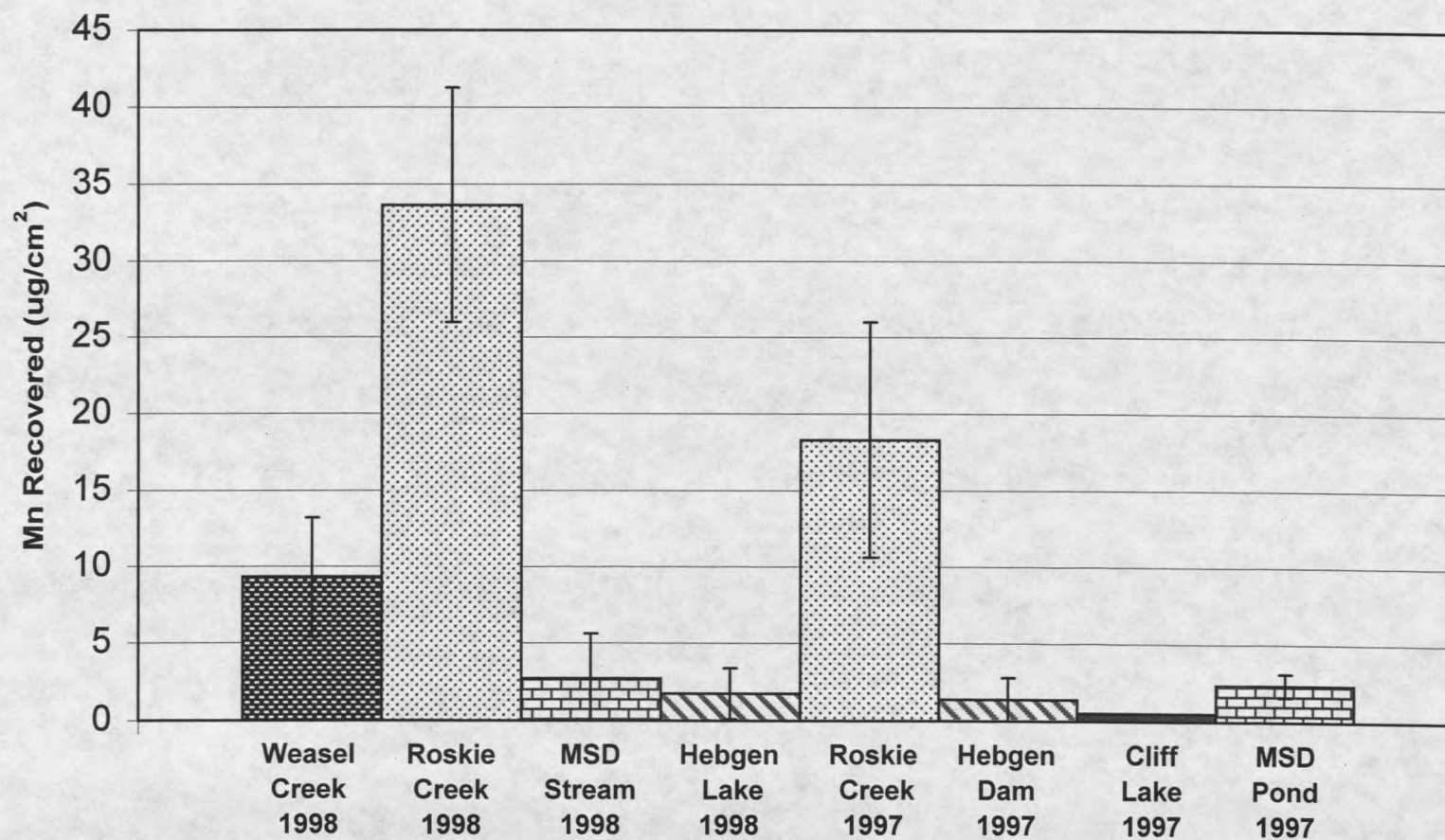


Figure 2.15 Manganese from SS Coupons

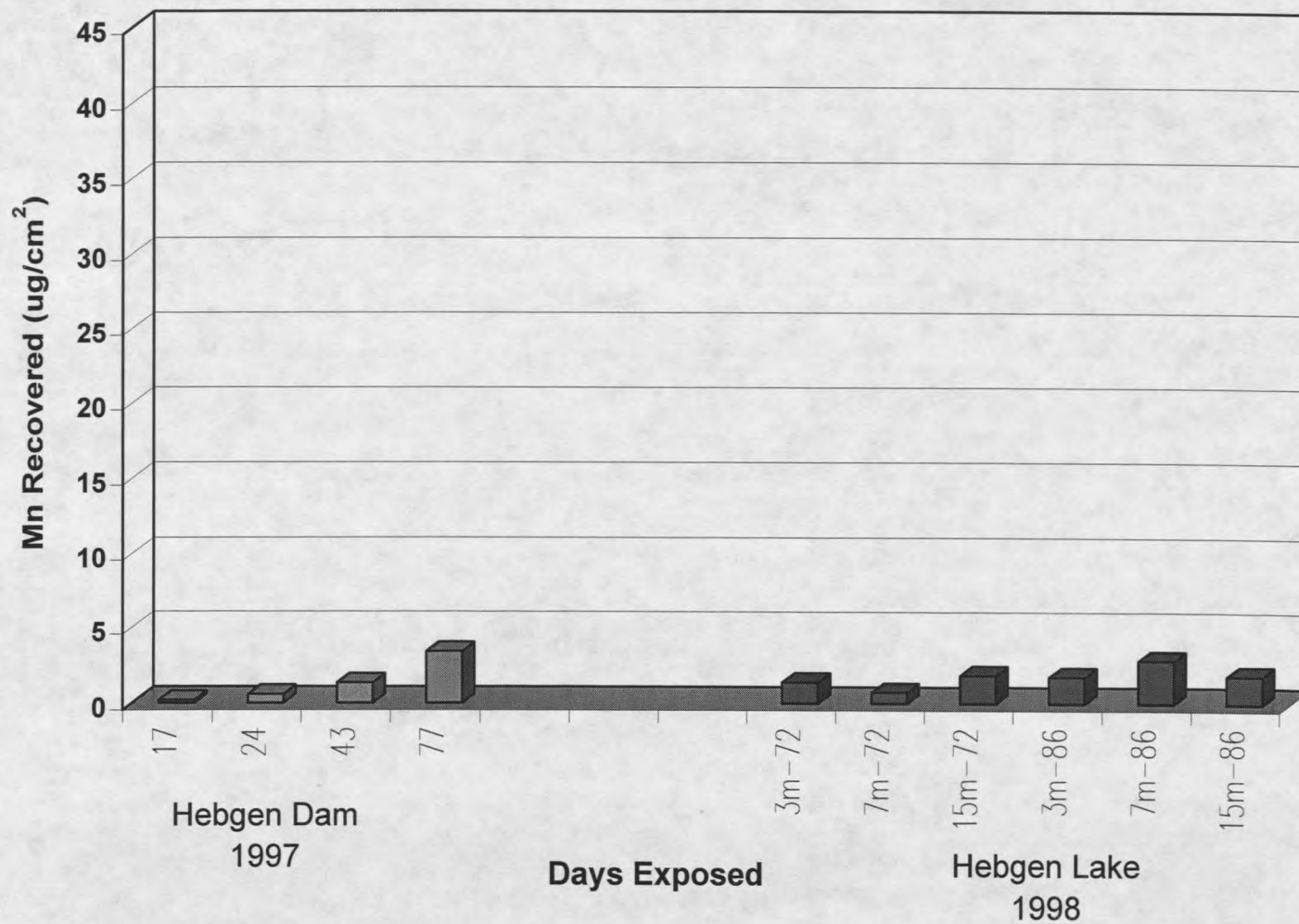


Lowest manganese recovery was from SS coupons exposed in Cliff Lake. 0.50 $\mu\text{g}/\text{cm}^2$ was recovered from one coupon at 105 days in 1997. At MSD values did not increase with longer exposure. Manganese recovered from SS coupons in the pond in 1997 ranged from 1.4 to 2.4 $\mu\text{g}/\text{cm}^2$, with peak amounts recovered at day 33. Manganese recovered from coupons suspended in the stream ranged from 1.6 to 6.5 $\mu\text{g}/\text{cm}^2$ with peak amounts recovered at day ninety-four. Figure 2.16 compares Hebgen Dam and Hebgen Lake. In Hebgen Dam coupons gradually accumulated more manganese from day seventeen, 0.2 $\mu\text{g}/\text{cm}^2$ to day seventy-seven, 3.4 $\mu\text{g}/\text{cm}^2$. At Hebgen Lake, recovered manganese ranged from 0.7 to 2.8 $\mu\text{g}/\text{cm}^2$ at seventy-two and eighty-six days, respectively.

Light. Visible light, measured by the secchi disc, fluctuated within the epilimnion in both the stratified lakes. At Cliff Lake, secchi measurements were obtained while the lake was stratified only. Visible light was detected within 1 meter of the thermocline, increasing from 5.6 m at the beginning of the study to 7.0 m on 8/20/97. Secchi disc measurements at this location were obtained between 11:00 a.m and 2:00 p.m.

At Hebgen Lake, visibility was 4 m at the beginning of the study on 5/30/98, when the lake was well mixed. Maximum visibility measurements of 8m were obtained during late June and July. When the lake was clearly stratified by 7/31/98, visibility just reached the thermocline at 5 m. Secchi disc measurements at Hebgen Lake were obtained between 12:00 and 3:00 p.m.

Figure 2.16 Manganese from Hebgen SS Coupons



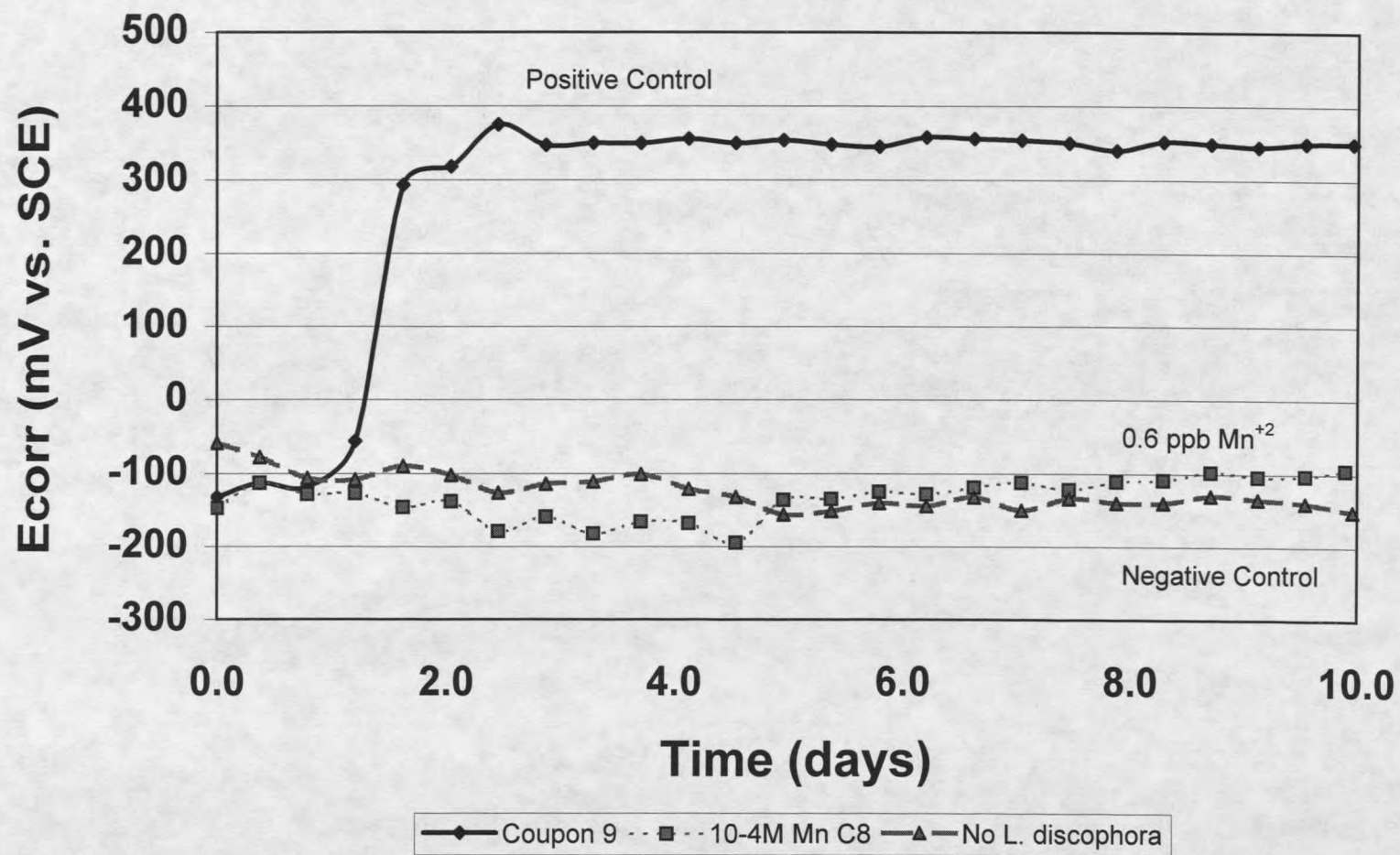
At Hebgen Dam, where the water was well mixed, visible light fluctuated between 2.5 and 3.5 m. Secchi disc measurements at this location were obtained between 1:00 and 3:00 p.m.

Roskie and Weasel Creek experienced some turbidity during spring snowmelts and otherwise remained clear during study periods. At MSD stream, turbidity fluctuated drastically, coinciding with fluxes in local precipitation. The frame was staked into the stream bed so the coupons were within 8 inches of the water surface yet there were days when the frame with coupons was not visible. For the majority of the study, water was cloudy but coupons were visible. In contrast, turbidity at MSD pond was not impacted by fluxes in run-off like the stream. It was clear in early summer but turbidity increased, so that by the end of the study, in mid October, the frame with coupons were only slightly visible.

Laboratory Results

Two flow-cell bioreactors ran in parallel for each experiment with a connecting salt bridge. Corrosion potentials were read for each individual coupon in the reactor vs. a SCE reference electrode inserted in the salt bridge. Potentials were recorded every 30 minutes via the data acquisition system. By manipulating pH, dissolved manganese and pyruvate concentrations in the flow-cell bioreactor it was possible to study the relationship between these factors and the biological oxidation of manganese and ennoblement of SS coupons.

Figure 2.17 Ennoblement Curves for Standard Controls

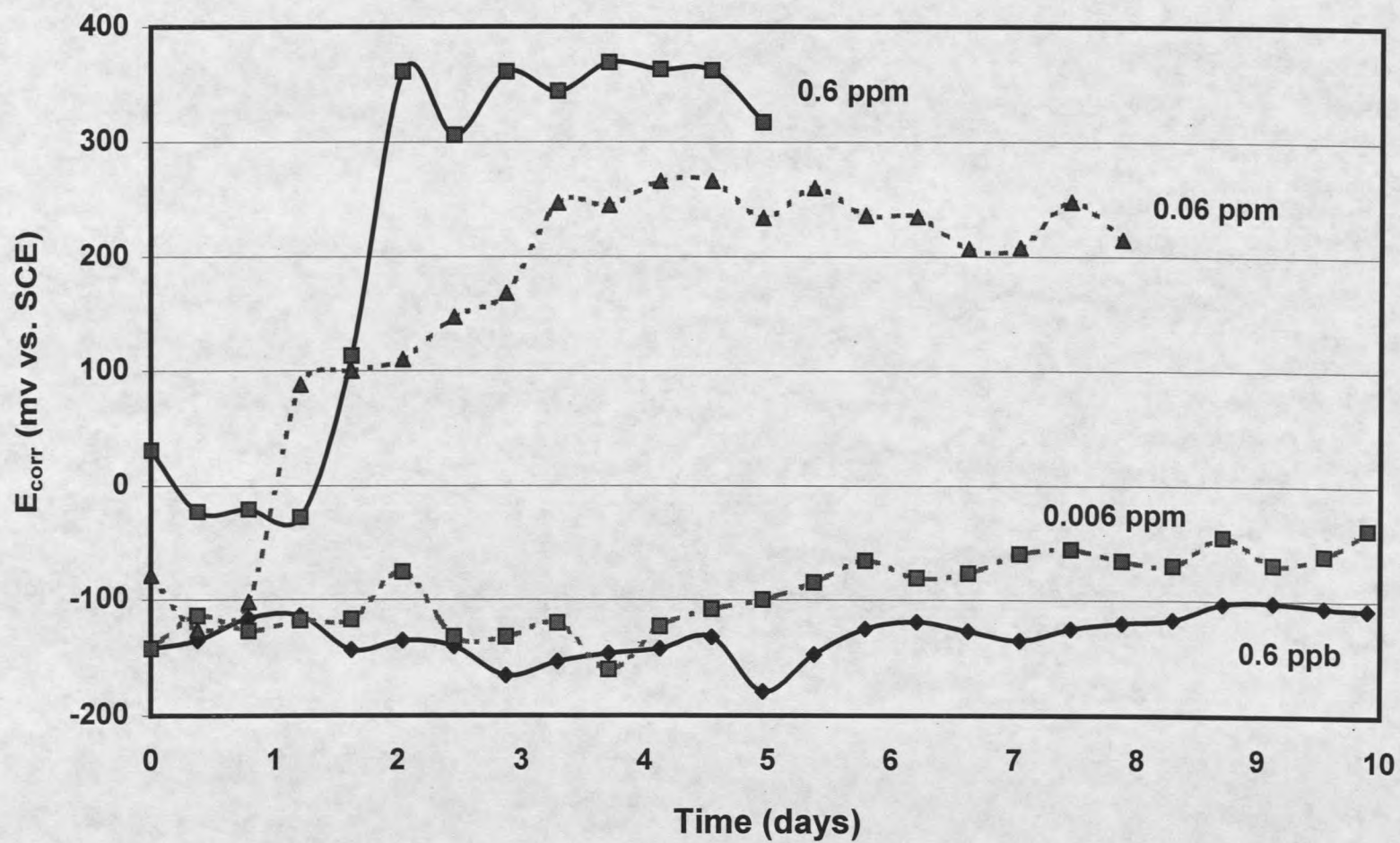


The standard control for the laboratory study was *L. discophora* grown in MSPV media with vitamins, supplemented with 200 ppm sodium pyruvate, 10^{-4}M (approximately 6 ppm) MnSO_4 , at pH 7.2 and 25°C (Figure 2.17). DO in the reactor ranged from 4.6-5 ppm. The standard control reactor contained an appreciable black precipitate (MnO_2) after 10 days of culture, and SS coupons fully ennobled within 48 hours. Data generated from the standard control were used as a reference level of ennoblement for future experiments. A negative control experiment was conducted using the same growth conditions, in the absence of *L. discophora*. In the negative control there was no MnO_2 deposition detected at the surface of the SS coupons and no ennoblement.

Dissolved Manganese. Manganese concentrations were systematically reduced 10 fold in each experiment to observe the effects on ennoblement. All other parameters of the growth media were held constant. As manganese was diluted from 10^{-5}M to 10^{-6}M , it took longer for the SS to enoble. (Figure 2.18) Finally, at concentrations of 10^{-7}M , the coupons did not fully enoble, and they stabilized at zero mV vs. SCE. At 10^{-8}M , the coupons did not enoble or tend toward an increased potential. There was an appreciable biofilm on the SS but it retained the unchanged opaque color of the bacteria with no MnO_2 deposition.

pH. For these experiments, pH in the feed media was reduced to 6.0 and 6.9, respectively. All other conditions remained constant, as in the standard control. There was visible cell growth at 24 hours in both reactors fed with 6.0 and 6.9 pH media.

Figure 2.18 Ennoblement Curve for $[\text{Mn}^{+2}]$



Oxidation of manganese became apparent in the reactor fed with 6.9 pH media at about 24 hours (Figure 2.19). Reactor pH, monitored daily from the effluent, remained stable between 6.9-7.0 over the 8 day experiment period. Ultimately, SS coupons in this reactor ennobled and remained stable after 3 days, similar to the positive control. All coupons tested positive for presence of MnO_2 at day 8.

There was no evidence of manganese oxidation in the reactor fed with a 6.0-pH media after 24 hours. After 3 days however, pH in this reactor slowly began to increase. At days 3, 5, 7, 9, and 10 the pH was 6.3, 6.3, 6.3, 6.6, and 6.5, respectively. Coinciding with this increase in pH, the corrosion potentials of the coupons began to increase as well. By day 8, the coupons had fully ennobled. All coupons tested positive for presence of MnO_2 at day 8.

Sodium Pyruvate. Sodium pyruvate was the carbon and energy source supplied to the bacteria in the growth media. 200 ppm pyruvate added to the MSVP medium was the recommended amount for supporting maximal growth of *L. discophora*.⁶ Pyruvate concentrations in the growth medium were reduced to 100 and 25 ppm. E_{corr} was monitored for 15 days with the following results (Figure 2.20). Reduction of pyruvate to 100 ppm resulted in delayed ennoblement. Full ennoblement and stabilized potentials resulted after 7 days, as opposed to 2 days in experiments where 200 ppm pyruvate was added. No ennoblement was observed after 15 days when pyruvate concentrations were reduced to 25 ppm.

Figure 2.19 pH Related Ennoblement

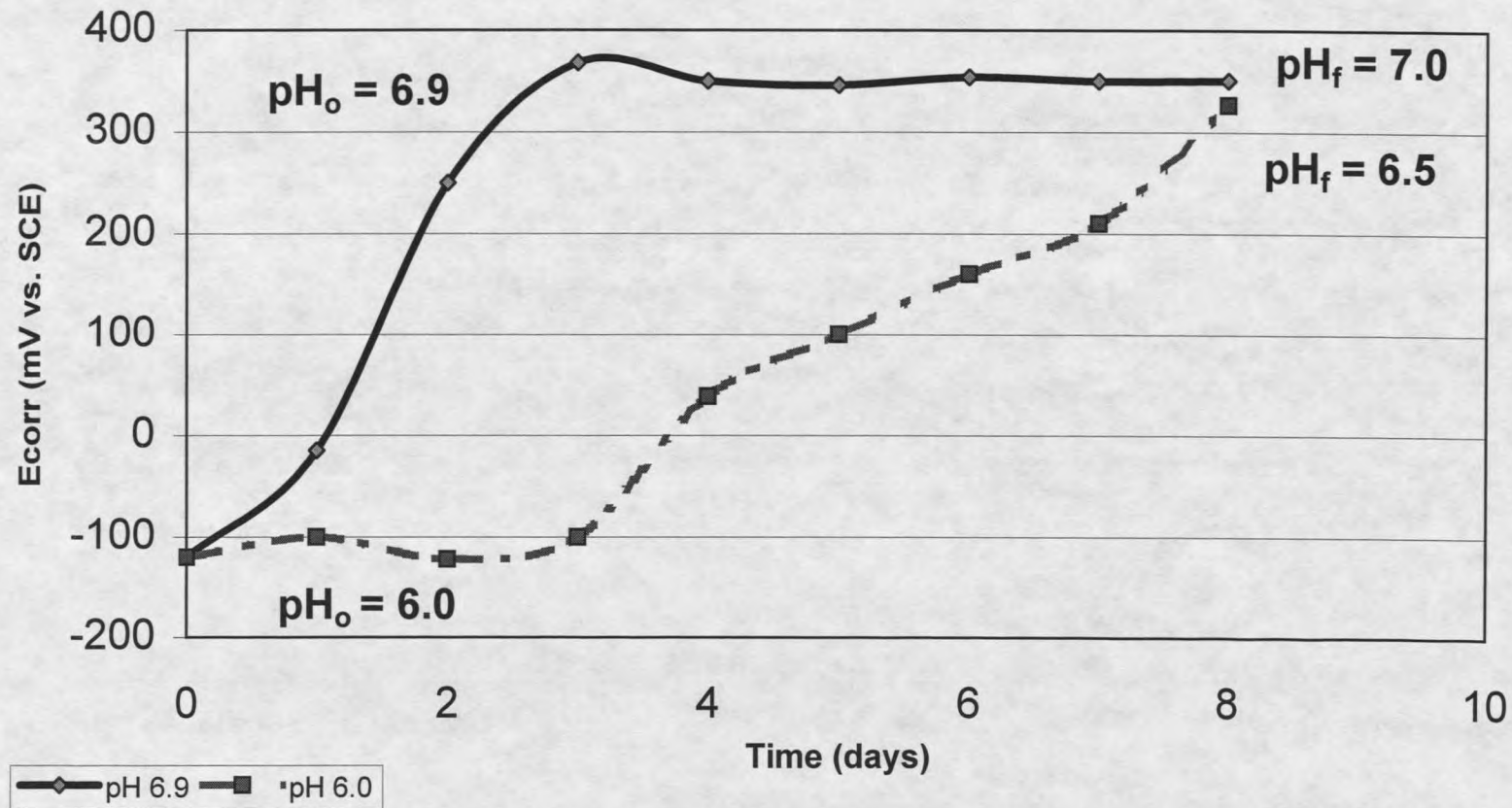
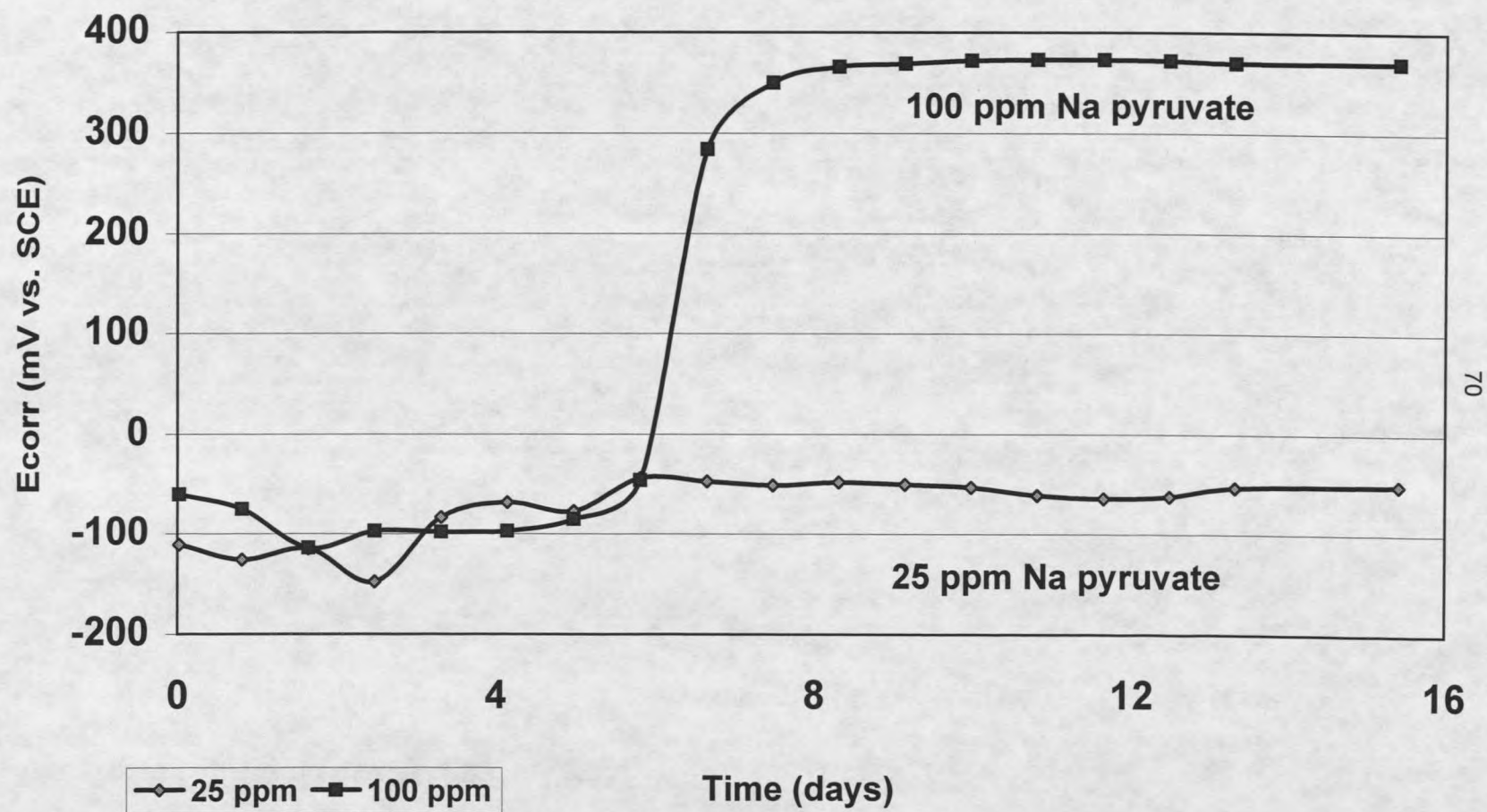


Figure 2.20 Na Pyruvate Related Ennoblement



Results from the field studies and laboratory experiments support the original hypothesis. Ennoblement did occur at different rates, or not at all, depending on the environmental characteristics in different natural water systems. Through careful selection and monitoring of independent environmental parameters in the field, it was possible to isolate some critical factors which put 316L SS at risk for ennoblement.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Rates and/or occurrence of ennoblement varied widely among the natural fresh water environments studied. Results from this study demonstrate that corrosion potentials are influenced by the characteristics of the water at the metal surface and the presence or absence of manganese oxidizing microorganisms in the environment.

Of the environmental parameters studied for their influence on ennoblement of SS, dissolved manganese concentration and pH stand out. Organic carbon is a necessary element in the growth and maintenance of a biofilm; however in the environments studied here, TOC concentrations did not appear to be limiting. Dissolved oxygen is necessary for biological oxidation of manganese, yet DO was not limiting in the fresh water environments studied. A possible exception is the 13 m depth at Cliff Lake, where no ennoblement was observed at the entire site throughout the study. Ennoblement of 316L SS was observed at temperatures between 6-22°C, in the field, and 25°C in the lab, therefore the range of water temperatures were not prohibitive of ennoblement, but rather influenced the rate at which coupons ennobled.

It is a logical assumption that the most opportunistic microorganisms colonize the newly introduced surfaces first. If these organisms do indeed oxidize manganese, and all the conditions favor oxidation, rates of ennoblement are high

soon after exposure (Roskie, Weasel Creek). In the case where manganese oxidizing microorganisms are perhaps few or colonize the stainless steel at a slower rate because of other environmental limitations, shift in E_{corr} may be very slow or go undetected for the entire study period. Maybe they are there but grow slowly or oxidize the manganese slowly. On the other hand, if microorganisms lacking the ability to oxidize manganese initially colonize the stainless steel, this biofilm may prevent contact of any later forming manganese oxide deposits and inhibit any electrochemical effects at all.

Manganese oxide deposition on the SS coupons varied from site to site. Ennoblement rates at Roskie and Weasel Creeks were comparable, yet amounts of manganese recovered from coupons at these sites differed significantly. This again confirms that ennoblement is not dependent on large concentrations of dissolved manganese in the bulk fluid. Roughly four time more manganese was recovered from coupons exposed at Roskie Creek than at Weasel. They both fully ennobled in the same amount of time and remained stable for the duration of the study. These results are comparable to those of Dickinson (6,7) and Lindhardt, (20) who showed that SS coupons ennobled to some stable potential and remain stable irrespective of the amounts of manganese dioxides accumulated past a few $\mu\text{g}/\text{cm}^2$.

Dissolved manganese concentrations at Roskie were roughly 2 to 4 times greater than Weasel. Yet, coupons at both sites ennobled in the same amount of time and remained stable for the duration of the study. Results from the

laboratory studies of dissolved manganese correlated well with field results. Coupons exposed in the bioreactor bulk fluid with lower Mn^{+2} concentrations of 0.6 (10^{-5}M) and 0.06 ppm (10^{-6}M), still ennobled but at a decreased rate. At 0.006 ppm (10^{-7}M) none of the coupons "fully" ennobled, but there was a slight trend toward increasing potentials. At 0.6 ppb (10^{-8}M) coupons no longer ennobled and there were no visible MnO_2 deposits on the coupons. Dissolved manganese in the range between .01 and .006 ppm are sufficient to induce ennoblement if Mn-oxidizing bacteria are present, based on this study.

Dissolved manganese concentrations from 0.04-0.09 ppm at Roskie Creek seemed to enhance biological manganese oxidation without indications of toxicity. Increased mixing resulting from run-off is more likely to enhance ennoblement in fresh water systems. Comparing data collected from both Hebgen Dam and Hebgen Lake, which basically have the same water chemistry, it is interesting that increasing corrosion potentials were observed on several coupons within 50 days in the lake, and only 1 coupon showed an increase in E_{corr} after 80 days exposure at the dam. Experiments were set up on 7/8/97 at the dam, and in early spring at the lake, 5/20/98. As shown in figures 4 and 6, ennoblement curves generated from data collected in the spring of 1998 occurred at a faster rate than the 1997 study, which started in the summer. There is no explanation for this. However in the spring there is considerable run-off from rainfall and snowmelt in these areas, which creates a lot of mixing of water and sediment. This probably increases the amount of dissolved

manganese and TOC in the water. Increased manganese means more available for oxidation, in the presence of Mn-oxidizing microorganisms

Although coupons did not "fully" enoble in Hebgen Lake to 350+ mV, four of the coupons at 3 m did enoble to 230-305 mV by day 50. There was clearly a trend toward ennoblement in water that had 0.01 ppm or less dissolved manganese. Coupons at 7 and 15 m lagged behind coupons at 3 m in achieving elevated potentials. 15 m coupons reached 0 mV by the end of the experiment, but coupons exposed at 7 m attained the same level of ennoblement at 3 m by day sixty-four.

Manganese was recovered from SS coupons at MSD, where neither MnO_2 nor ennoblement was ever detected. A thick biofilm formed on these coupons. They had the characteristic ochre color of iron oxide deposits and elevated amounts of iron were recovered from the SS coupons. Therefore, deposition of manganese may be attributed to autocatalytic binding to iron oxides. Testing for iron oxides was not specifically addressed in this study.

Overall, field results show E_{corr} is dependent on pH. Neutral or near neutral pH was favorable for manganese oxidation. pH greater than 8.5 favors precipitation of manganese compounds, i.e., MnCO_3 . (1) pH less than 6.4-6.5 favors dissolved iron, manganese and manganese reduction. Results in the lab at elevated pH were inconclusive, since massive precipitation occurred in the filters and reactor. No manganese oxidation was observed and no ennoblement occurred.

In the laboratory experiments where pH in the feed medium was decreased to 6.9 and 6.0 respectively, the results correlated nicely with field data. The ennoblement curve for pH 6.9 resembles the positive control. Coupons ennobled in 2 days and tested positive for MnO_2 . However the ennoblement curve for pH at 6.0 did not ennoble until day eight. pH in the reactor remained near 6.0 until day three, when gradually it began to increase. The bacterial cells gradually changed the pH in the bulk fluid, and as pH increased to between 6.3 and 6.5, the coupons began to ennoble. These coupons fully ennobled by day 8 and tested positive for MnO_2 . Based on results of these studies, formation of manganese dioxide and ennoblement require pH in the bulk fluid greater than 6.3-6.4.

Laboratory pH experiments nicely supported field results at MSD where the average pH was 6.4. Mn-oxidation was not detected by Fiegel's spot test (13) and the coupons did not ennoble, even in the presence of extremely high concentrations of dissolved manganese (6.5-8.0 ppm). This water was most probably toxic to many microorganisms. Greene and Madgewick (15) observed that a rate limiting factor in microbial manganese oxide synthesis is the inhibition of the microbes by high concentrations of manganous ion in batch culture. At MSD, there are several elevated heavy metal concentrations, for example, Zn, Fe, Mn, Cu, and Al. High concentrations of these metals may be toxic to organisms that oxidize manganese. Mine waters containing ferrous iron are particularly objectionable because ferrous compounds are strong reducing agents and tend to denude the stream of its dissolved oxygen. They tend to

encourage the growth of filamentous iron bacteria, which flourish in acid waters containing iron and some organic matter. These bacteria can oxidize soluble ferrous salts to ferric compounds resulting in the accumulation of slimy reddish-brown deposits containing the bacteria and ferric hydroxide. (18) Turbidity, caused by the oxidation of ferrous iron can prevent animal life from establishing itself at the bottom of the stream by cutting off light. (34) Other objectionable effects of mine waters on a stream are reduction in pH value, precipitation of iron compounds, and increases in total hardness, iron and sulfate. MSD water, bed and banks exhibit the telltale 'ochre' color associated with mine waters. DO of 5 ppm at MSD was not low enough to be the limiting factor for ennoblement, because we did see ennoblement at the thermocline in Hebgen Lake where the DO was also around 5 ppm. Neither manganese oxidation nor ennoblement were detected.

Manganese oxidizing microorganisms generally have the ability to oxidize iron as well. (10) Iron oxidation is the most predominant metal oxidation among these organisms (2) and is a faster reaction than manganese oxidation. (1,2) At MSD it is possible that the high iron concentrations are conducive to biological oxidation of iron instead of manganese. (Data from iron removed from coupons) This is another possible explanation for the lack of ennoblement at MSD.

Ennoblement occurred at faster rates in flowing streams. This can be attributed to several reasons; 1) flow increases the probability that an organism which oxidizes manganese would contact the SS surface, then colonize it, 2) flow

increases mixing of sediments in the water, thus more manganese remains suspended and available for oxidation, 3) flow increases aeration of the water, thus increasing the dissolved oxygen content of the water available for microbial growth and oxidation and 4) increases flux of organic carbon to the surface where it can be used by the bacteria.

Ennoblement was observed at temperatures ranging from 6°C in Roskie and Weasel Creek to 22°C at Roskie, Weasel, and Hebgen Lake. All SS coupons exposed at other sites were within this temperature range and did not ennoble. Therefore, temperatures within the range studied were not a critical factor for ennoblement.

However, temperature did influence ennoblement rates initially, as shown in previous studies. (29) Field studies here confirmed that once a coupon is ennobled in natural fresh water, it remains ennobled, independent of the temperature. Temperature affects several parameters in fresh water environments. As water temperature decreases, certain microorganisms are lower in number; they may die off, slow their metabolism, or go into a dormant phase until optimal growth conditions return. Nutrients become scarce. Snow and ice cover limit light availability during winter months and affect photosynthetic microorganisms. All of these may influence the activity of the Mn-oxidizing bacteria on the SS surface.

It is not practical to make comparisons between the TOC data collected from the field and the results from the laboratory experiments. Sodium pyruvate is an

easily assimilable carbon source for cells. Total organic carbon in nature is more complex and not as easy to assimilate as pure pyruvate. Therefore, these results are discussed independently.

In nature where 2-12 ppm TOC was observed, only a percentage of that amount is utilized by microorganisms. These concentrations are considerable lower than the pyruvate concentrations in the lab. Virtually all pyruvate is utilizable by microorganisms. Possible explanations for this is that degradation of pyruvate in the culture media is probable, since pyruvate is sterily added to 25°C medium at start up of the reactor and it takes 3 days to utilize 20L. Another unknown is the actual pyruvate concentration in the flow cell reactor. Cells colonize the entire system, tubing, mixing chamber and reactor. A considerable amount of pyruvate may be consumed before it even reaches the flow cell reactor and becomes available to the cells which colonize the SS coupons.

Reduction of pyruvate concentrations in the feed media correlated with a reduction in corrosion potentials. Ennoblement of SS did occur with half reduction of the standard control influent pyruvate concentration to 100 ppm, but it took 6-7 days for the coupons to enoble as opposed to 2.

From organic carbon analysis it was determined that TOC was not a limiting factor at any of the *in situ* sites. TOC overall ranged from 2-12 ppm and ennoblement was observed at both extremes.

Conductivity did not vary significantly among exposure sites, ranging between .04-.05 megmhos, and for purposes of this study, no apparent trends

about the influence of conductivity on the ennoblement phenomenon were observed.

In the hypolimnion of Hebgen Lake, where ennoblement was delayed compared to the thermocline and epilimnion, combinations of environmental factors are limiting. Concentrations of dissolved oxygen were low (2-5 ppm), temperatures were low (6-10°C), and visible light was limited. A trend toward ennoblement was observed at week 15. The combination of low temperature, low DO and low light, or any one of these individual parameters are likely to influence ennoblement kinetics.

These results lead to a better understanding of ennoblement. Electrochemical data collection gives insight as to the corrosion status of stainless steel in its environment. Corrosion potentials of SS are determined by the alloy composition and the surface condition (type, density and size of defects). In addition, corrosion potentials of stainless steels vary depending on the characteristics of the bulk fluid in which they are exposed. Important constituents are chloride and manganese concentrations, and pH. Trace amounts (0.01-0.006 ppm) of manganese dissolved in the bulk fluid are sufficient for biological oxidation of Mn^{2+} and thus SS ennoblement. *In situ* and laboratory studies confirm that pH ranging from 6.3-8.3 are optimal for biological oxidation of Mn^{2+} and ennoblement.

This study identified specific risk factors for ennoblement of stainless steel and potentially, risk factors for SS corrosion. These results allow for more

informed decisions on water quality requirements for industrial processes utilizing SS components.

Recommendations for Future Work

Pyruvate studies should be repeated with the addition of more pyruvate concentrations. Influent and effluent pyruvate concentrations should be measured at regular intervals to determine losses due to degradation.

I also recommend expansion of the pH experiments. Maintaining constant pH in the reactors may better define the pH limits of manganic oxide related ennoblement. In addition, attempts to overcome precipitation problems, by changing the chemical composition of the medium would make it possible to better define the upper limits of pH and the effects on ennoblement.

Exposure sites should be expanded to include industrial water systems and other passive metals. Future studies might include a pilot study, controlling pH and manganese fluxes upstream.

Finally, biofilms from the SS coupons exposed during the 1998 studies were crypreserved. They are available for more extensive study through cryosectioning and staining techniques utilizing 16SrRNA probes.

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