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METAL SURFACE INFLUENCES ON THE STRUCTURE OF THE MICROBIAL COMMUNITY

A Thesis

Presented for the

Master of Science

Degree

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Raúl Orlando Díaz

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DEDICATION

I would like to dedicate this lengthy research to my mother, Carmen Iris Barreras, for her constant support, trust and love. For directing my first steps in the right direction surrounded with love and trust, I must recognize my grandmother, Rosita Rivera Meléndez de Barreras. To my aunt, Eliett Barreras, who has always provided me with encouragement and wisdom, I must say thank you. To my other aunts and uncles, specially Ana Rosa Barreras, who has always prayed for my sake, thank you. To the rest of my family members thanks for making all my Christmas Holidays so special. Finally, to my wife, Sandra K. Díaz, for providing her right shoulder when mine could not bare the load, allowing me to endure the long hours spent at University of Tennessee and to obtain this long dream.

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ABSTRACT

This research intended to study the role of the Sulfate Reducing Bacteria (SRB) in the corrosion of two different metal surfaces, 1010 mild steel (MS) and 304 stainless steel (SS) surfaces, and their influence on the microbial community that developed within the biofilm and corrosion products attached to the metal surfaces. A total of twelve metal surfaces of each type were exposed for a period of 56 days at three different aquatic sites, Douglas Dam Lake and two of its tributaries. The scrapped biofilm and corrosion products collected from the top side metal surface were evaluated by acridine orange direct counts, most probable number and 16s rRNA oligonucleotide probes, targeting the eubacteria and SRB community. The non-scrapped sides were suspended in a modified Bligh and Dyer solution from which the organic phase was collected and further processed. Of the organic phase, only the phospholipid fatty acid fraction was further separated and each component identified for a total of 54 samples. The metal sample corrosion rates were evaluated by the weight loss method and pit density. The inflow waters were evaluated in the laboratory by polarization resistance.

The biological results recovered by the above techniques indicated no significant differences for the total biomass, lipid biosynthesis and tran/cis ratio. However, the molecular and biochemical techniques indicated that SRB favored the colonization of the MS surfaces versus the SS surfaces under oxic conditions. The phospholipid ester-linked fatty acid fractions (PELFA) recovered from both surfaces exposed at three aquatic sites indicated that the microbial communities formed on the SS metal surfaces contained significantly higher concentration of polyunsaturated fatty acids (PUFA) than the MS metal surfaces. However, the monoenoic and iso and anteiso branched saturated fatty acids were recovered in significantly higher concentration on the MS surfaces than on the SS surfaces. Statistical analysis of the PELFA fraction separated the communities formed on the metals exposed at the three sites based on the influence exerted by the presence of biomarkers characteristics of eukaryotes and eubacteria.

The MS surfaces exposed to the sites experienced significantly higher corrosion rates under oxic conditions than the SS surfaces. Electrochemical evaluation of the MS surfaces did not result in significant corrosion rates between the collected water. However, the SS surfaces did not show significant differences

in pit density regardless of the exposed site. Overall, these *in situ* results indicate that microbial community structure is influenced by the type of metal substratum, while the MS corrosion rates are influenced by the presence of bacteria (i.e., SRB).

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PART I

INTRODUCTION

Microbially influenced corrosion (MIC) is currently one of the most difficult problems being faced by concrete structures (Tiller, 1990; Diercks et al., 1991; Mori et al., 1992) and gas, oil (Obuekwe et al., 1981; Phelps et al., 1991), pulp, paper (Hughes, 1968) and electric power industries (Marmo et al., 1990; Walsh et al, 1992). Some of the recent biofouling cases reported in the literature address the biofilm effect on systems such as network and buried pipes (Ahmad et al., 1988; Stranger-Johannessen and Norgaard, 1991), nuclear heat exchangers (Pope et al, 1982; Walsh et al., 1992) and cooling systems from geothermal (Mercado et al., 1987), atomic (Rajagopal et al. 1991a,b) and hydroelectric power plants (J.T. Johnson, personal communication).

Hydroelectric power industries use large quantities of untreated freshwater and marine sources to cool reactors and cooling systems (Pope et al., 1982; Mercado et al., 1987; Rajagopal et al. 1991a,b). The presence of aerobic and anaerobic bacteria in untreated waters can accelerate the corrosion rates by removing the metal ions from the anodic sites and utilizing the cathodic hydrogen from the cathodic sites, respectively, (Duquette and Ricker, 1985; Hamilton, 1987b) as energy sources, degrading applied protective coating (Mori et al., 1992) and utilizing inhibitors (Iverson, 1968, 1987; Nestorescu et al., 1977; Little et al., 1989; Franklin et al., 1991). The corrosion process induces serious monetary losses in some of the systems mentioned above resulting in shut down of processes (Hamilton, 1985; Weimer et al., 1988) and replacement of parts, equipments (Mercado et al., 1987) and sections (Islander et al., 1991).

Different monetary estimates have been calculated throughout the years in reference to the damages caused by MIC. Tiller (1982) in his review indicated that two different authors, Knox and Iverson have estimated the losses at $1 billion per year for the biodegradation of metals and at $500 million to billion per year for buried pipeline, respectively. Nevertheless, Walsh et al. (1992) indicated that the annual losses caused by MIC have been estimated in $30 to $40 billion per year.

Some of the expenses addressed above consisted of replacement of both corroded inexpensive industrial materials such as mild steel parts and expensive parts made of stainless steel and titanium as in the case of the geothermal Cerro Prieto I power plant (Mercado et al., 1987). This latter effect can result in higher corrosion complications such as structural failures through unknown corrosion mechanisms

(Cragnolino and Tuovinen, 1984).

The interactions of different bacteria within the mature biofilm, difference in dissolved O_2 concentration surrounding the exposed surfaces and presence of industrial wastes (e.g., paper industries) (Tatnall, 1981) can allow different corrosion reactions to take place enhancing the corrosion rates. Most of the recent studies performed in the area of MIC have concentrated in laboratory studies under control situations using mono or cocultures of isolated bacteria which have been previously recovered from corrosion tubercles (Obuekwe et al., 1987; Sand et al., 1987; Franklin et.al. 1988; Dowling et al., 1988 a,b), soil or water samples (Weimer et al., 1988), scrapped corrosion products (CP) and biofilm formed on the exposed metal surfaces. The majority of these studies have been performed in controlled situations using different electrolytes which have been sterilized by filtration and autoclaving. Either approach can remove some of the chemical elements (Walsh et al., 1992) or change the chemical composition of the electrolyte to which the surface is exposed to in nature.

Effort for the last 80 years has been directed toward the elucidation of the Sulfate Reducing Bacteria (SRB) corrosion mechanisms which are still far from being solved (Booth and Tiller, 1968; Iverson, 1979; Duquette and Ricker, 1985; Boopathy and Daniels, 1991; Dowling et al., 1991). Nevertheless, various researchers have been able to demonstrate the capacity of SRB to utilize as energy source the formed cathodic H_2 (Hardy, 1983; Pankhania et al. 1986). However, evaluation of bacterial interactions and reactions within the biofilm has been relegated to a second level. In this research, 1010 mild steel (MS) and 304 stainless steel (SS) surfaces, which are some of the most common materials used in plumbing applications such as handling potable waters (Reedy, 1973; Kain, 1990), electric power industries (Mercado et al., 1987) and underground structures (Iverson, 1987), were exposed to *in situ* conditions for a period of 56 days to study the effect of complex and different microbial communities on the exposed metal surfaces and to test these metallurgical materials in true environmental conditions. Currently, Tennessee Valley Authority (TVA) utilizes these metallurgical materials for construction of screens located at water intakes. Below are the main research objectives.

The tools used for the elucidation of the different microbial community structures present within

Principal Research Objectives of this *in Situ* Study.

- Compare the different type of microbial communities that develop on different and similar metal surfaces while exposed to similar and different environments.
- Compared the biomass measurements as indicated by the three different tools.
- Enumeration of the Sulfate Reducing Bacteria (SRB) present in the biofilm by three different tools. Are the SRB the major biotic factor in the corrosion of metal surfaces?
- Isolation and characterization of aerobic bacteria from each type of surface exposed to *in situ* conditions by off-line analytical tools such as gram stain, MIS and API techniques.
- Compare the rate of corrosion *in situ* of similar metal surfaces while exposed to two different environments.

the undisturbed biofilm consisted of the analysis of phospholipid ester-linked fatty acids (PELFA) and 16s rRNA oligonucleotide probe for eubacterial and SRB. The former tool has been validated and applied throughout the years in numerous microbial ecology environments (White et al., 1979; Schropp et al., 1988; Guckert and White, 1986; Parkes, 1987; Nichols et al., 1987; Ringelberg et al., 1989; Vestal and White, 1989; White et al., 1991; Rajendran et al., 1992), biofouling cases (Kerger et al., 1986,1987), soil samples (Rezanka et al., 1991; Zelles et al., 1992) and subsurface soil samples (White et al., 1991). However, molecular techniques, i.e. 16s rRNA oligonucleotide probe, has recently been applied to ecological studies with the initial work of Stahl et al. (1988).

Part II provides a complete overview of the five currently proposed corrosion mechanisms in the presence of SRB with further evaluation of possible chemical reactions that can occur within the biofilm in the presence of different bacterial species. Part III presents the PELFA and 16s rRNA results obtained from the exposure of different metal surfaces to three different ecosystems followed by Part IV which addresses the corrosion rates measured by the weight loss methods and electrochemical techniques.

PART II

LITERATURE REVIEW

1. BIOFILM AND CORROSION REACTIONS

The introduction of microorganisms either by human error or nature into industrial water systems as in the case of pharmaceutical, cosmetology (Butler, 1968) and oil industries (Hamilton, 1985, Cord-Ruwisch et al., 1987) has augmented the interest of the private sector for microbially influenced corrosion (MIC) research. The accumulation of extracellular polymeric substances (EPS) within the biofilm can induce the following effects within different industrial systems such as 1) reduction in the equipment performance demanding excess energy or producing less energy (e.g., industrial cooling water systems) (Porkiss, 1968; Nestorescu et al., 1977; Lappin-Scott and Costerton, 1989; Lakatos, 1990; Ludyansky, 1991; Rajagopal et al., 1991a,b), 2) release of contaminants into industrial products reducing product quality (Lappin-Scott and Costerton, 1989), 3) enhancement of biofouling situations (Characklis and Cooksey, 1983) and 4) release of produced H_2S gas by the Sulfate Reducing Bacteria (SRB) (Hamilton and Sanders, 1984; Hamilton, 1987; Edyvean, 1990; Islander et al., 1991) posing a health threat to employees (Iverson, 1968; Cord-Ruwisch et al., 1987; Diercks et al., 1991). These deleterious effects emphasize the need for better on-line non-destructive tools for early detection of bacteria within the producing system and understanding of different bacterial interactions within the biofilm.

The use of state of the art on-line non-destructive equipment such as scanning vibrating electrode technique (SVET) (Franklin et al., 1991) and electrochemical impedance spectroscopy (EIS) (Franklin et al.,1988; Dowling et al.,1988a) to measure corrosion rates and attenuated total reflection infrared spectroscopy (ATR/FT-IR) (White, 1986; Geesey and White, 1990; Bremer and Geesey, 1991; Nivens et al., 1992) and open circuit potential (OCP) (Mittelman et al., 1992) to determine the effects of biofilm formation have permitted these researchers to study different MIC aspects in aqueous environments. Recently, Fletcher et al. (1992) used two optical techniques, interference reflection and light section microscopies, to evaluate the integrity and interactions of polymer formed by bacteria when exposed to different electrolytes containing biocides. The use of the previously mentioned equipment has permitted the elucidation of biotic and abiotic corrosion mechanisms and their effects, testing of new and welded metallurgical materials in the presence of bacteria (Walsh, 1992) and recommendation of possible biocides

to reduce the corrosion rates observed in nature. The use of these techniques has brought to the MIC field a better understanding on the corrosion process mediated by microorganisms. Numerous symposium and review papers have been published in the area of MIC (Cragnolino and Tuovinen, 1984; Tiller, 1982; Dexter, 1985; Dexter and Videla, 1985; Hamilton, 1985; Howsam, 1990) without emphasizing the bacterial interactions within the biofilm.

The current understanding of abiotic corrosion mechanisms can be expressed in terms of the following equations. An exposed iron based metal surface in the presence or absence of O_2 surrounded with freshwater environment in a pH $\geq$ 7 can undergo corrosion by the following cathodic reactions (2 and 3)

Anodic Site: $4Fe \text{ ---> } 4Fe^{2+} + 8e^-$ (1)

Cathodic Site: $8H_2O + 8e^- \text{ ---> } 8OH^- + 4H_2$ O_2 absent (2)

$O_2 + 2H_2O + 4e^- \text{ ---> } 4OH^-$ O_2 present (3)

However, in situations where the pH < 7, two other different cathodic reactions (4 and 5) can occur at the cathodic site,

$8H^+ + 8e^- \text{ ---> } 4H_2$ O_2 absent (4)

$O_2 + 4H^+ + 4e^- \text{ ---> } 4H_2O$ O_2 present (5)

(Tiller, 1982; Hamilton, 1985; Buchanan and Stansbury, 1990).

The above reactions explain the abiotic mechanisms which exposed metal surfaces can experienced *in situ*. The presence of different bacteria, their activity and metabolism, may result in the formation of complex interactions within the biofilm exacerbating the biofouling situations (Hamilton, 1985; Tiller, 1990) and

cannot be fully addressed with the current knowledge. The combination of the above abiotic equations such as (2) and (3) or (4) and (5) in the presence of the biofilm can simultaneously occur on the exposed metal surface (Ford and Mitchell, 1990) depending mainly on the pH of the electrolyte surrounding the bare metal surface or the pH changes caused by the interactions of bacteria at the biofilm/metal surface interface.

The formation of a bacterial biofilm on the exposed surface (e.g., corrosion of concrete) can favor the developing of reactions (3) and (5) either simultaneously (Hamilton, 1985) or subsequently as in the case

of biofilm/concrete interface. As indicated by Diercks et al. (1991), *Thiobacillus neapolitans* and *T. intermedius* are capable of producing sulfuric acids which can diffuse through the biofilm lowering the pH at the biofilm/concrete interface, favoring the colonization of two other species, *T. thiooxidans* and *T. ferrooxidans*, increasing the corrosion rates by releasing acid compound (Cullimore, 1990b; Diercks et al., 1991; Mori et al., 1992).

The formation of stratified and patchy biofilms on the exposed metal surface can allow the appearance of symbiotic interactions such as commensalism or mutualism within the biofilm accelerating the rate of corrosion (Pope et al., 1982; Walsh et al., 1992;). The formation of patchy biofilm resulting in O_2 concentration cells (Dowling et al., 1991) can cause a higher increase in corrosion rates by allowing surface area to be exposed to either reactions (3) or (5) becoming active cathodic sites and resulting in higher cathode/anode ratio (Lappin-Scott and Costerton, 1989) as demonstrated by equation A. The removal of the electrons by the reduction of O_2 at these sites mediated by the hydrolysis of the water or acidic conditions can allow further metal dissociation as shown in reaction (1) increasing the corrosion rate. Also, the presence of a thick biofilm decreases the O_2 concentration at the biofilm/surface interface (Cragnolino and Tuovinen, 1984; Hamilton, 1985, 1987a), allowing water hydrolysis or H_2 evolution to occur by the consumption of protons as shown in reactions (2) and (4), respectively. These situations allow the development of low redox and anoxic conditions (Hamilton, 1985) resulting in the formation of active cathodic site underneath the biofilm as in the case of stratified biofilm and higher corrosion rates. The latter can be enhanced by the continuous detachment and formation of biofilm (Iverson, 1979; Cragnolino and Tuovinen, 1984) and corrosion products (Booth and Tiller, 1968) increasing the rate of dissolution of the metal at the anodic site as shown in reaction 1.

It can be foreseen that the following cathodic reaction,

$$4H_2O + 8e^- \text{---> } 2O_2 + 4H_2 \quad \text{absence } O_2 \qquad (6)$$

can result in O_2 evolution. The presence of aerobic bacteria within the stratified and patchy biofilm, which include strict and facultative aerobes, can directly benefit of the end product of reaction (6) by actively removing the O_2 and developing differential aeration cells (e.g., industrial cooling water systems and heat

exchangers) (Pope et al., 1982; Hamilton, 1987a; Pankhania, 1988; Pedersen and Hermansson, 1991). The low concentration of O_2 within the biofilm, the presence of thick bacterial mat reducing the diffusion of O_2 through the biofilm (Lock et al., 1984) and production of different organic substratums allow the survival of anaerobic species such as SRB (Dowling et al., 1991) .

2. BACTERIA MECHANISMS IN MICROBIAL INFLUENCED CORROSION

SULFATE REDUCING BACTERIA

In an older report von Wolzogen Kuhr and van Der Vlugt (1934) proposed the theory of cathodic depolarization. This theory proposed that high corrosion rate can be caused by the removal of cathodic hydrogen from the cathodic site by hydrogenase positive SRB. These organisms can increase the corrosion rate by the mechanism shown in reaction (7), enhancing the removal of H^* from the cathodic site and facilitating the dissociation of metal at the anodic site as shown in (1).

$$H_2SO_4 + 8H^* \text{ ---> } H_2S + 4H_2O \qquad (7)$$

The previous theory in microbiological terms established that those organisms capable of utilizing the produced cathodic H_2 through the hydrogenase enzyme could increase the corrosion rate of exposed metal (Pope et al., 1982; Pankhania, 1988; Bryant et al., 1991). As in the case of SRB, the presence of hydrogenase enzyme enables the organism to utilize the electrons from the H_2 through the cytochrome C_3 (Cragnolino and Tuovinen, 1984).

Several researchers have reported the presence of hydrogenase enzyme in some of the studied SRB species such as *Desulfovibrio, Desufotomaculum, Desulfobulbus, Desulfomonas and Thermodesulfobacterium species* (Pankhania, 1988) and other bacterial species such as *Clostridium spp., Shewanella spp.* (Bryant et al., 1991), *Methanogenic spp.* (e.g., *Methanosarcina barkeri, M. thermolithotrophicus, M. thermoautotrophicum, M. bryantii,* and *M. hungatei* (Daniels et al., 1987)), *Chromatium spp.* and *Chlorobium spp.* (Margulis et al., 1986). Mara and Williams (1971, 1972) performed polarization studies in the presence of hydrogenase positive organisms such as, *E. coli., Proteus mirabilis, Serratia marcescens* and *Pseudomonas aeuriginosa* concluding that their presence was the main reason for the observed corrosion rates. However, these authors in two different papers failed to address the metabolic status of the studied bacteria (Mara and Williams, 1971,1972). Recently, Pedersen and Hermansson (1989, 1991) have shown that two marine isolates, *Serratia marcescens* and *Pseudomonas sp. S9,* provide protection to exposed metals. Also, Booth and Tiller (1968) demonstrated that the rate of corrosion was independent of hydrogenase enzyme while Donlan (1992) did not detect hydrogenase enzymes on four different surfaces

from which SRB have been recovered. These results suggest that the presence of hydrogenase enzyme alone cannot be used as a sole corrosion indicator as recently done by Bryant et al. (1991). Also, it emphasizes that bacterial interactions within the biofilm are more important than one single parameter or organism.

Costello (1974) proposed a modification of reaction (7) to explain the cathodic depolarization theory,

$$4H_2 + SO_4^{2-} \text{---> } H_2S + 2H_2O + 2OH^- \quad (8)$$

$$2H_2S + 2e^- \text{---> } 2HS^- + H_2 \quad (9)$$

$$2HS^- + Fe^{2+} \text{---> } FeS + H_2 \quad (10)$$

As outlined in the above reactions the presence of SO_4^{2-} and absence of O_2 allows the SRB to utilize the cathodic H_2 as an energy source resulting in H_2S formation (8) which can be further oxidized into HS^- (9) and FeS (10).

Hamilton (1985) proposed similar chemical reactions as in equation (8) but with different end products. As seen in equation (11), the main products of Hamilton's reactions resulted in no OH^- formation suggesting that reaction (13) proposed by von Wolzogen Kuhr and van Der Vlugt (1934) must depend on the hydrolysis of water as depicted on equation (2) or (3). However, FeS has not been detected in nature (McNeil and Little, 1990) suggesting it as intermediate step in the process of the corrosion products (CP).

$$4H_2 + SO_4^{2-} \text{---> } 4H_2O + S^{2-} \quad (11)$$

$$S^{2-} + Fe^{2+} \text{---> } FeS \quad (12)$$

$$3Fe^{2+} + 6OH^- \text{---> } 3Fe(OH)_2 \quad (13)$$

The previous proposals favor the oxidation of atomic and cathodic hydrogen as the possible corrosion process for SRB (Hardy, 1983; Daniels et al., 1987; Pankhania, 1988) resulting in the production of FeS as shown in reactions (10 and 12), which has been considered as a second mechanism for SRB corrosion.

FeS is one of the corrosion products detected in numerous laboratory corrosion studies where SRB presence has been detected (Booth and Tiller, 1968; Weimer et al., 1988; Tiller, 1990; McNeil and Little, 1990). FeS has been shown to behave as a cathodic active agent toward iron (Booth and Tiller, 1968;

Cragnolino and Tuovinen, 1984) by increasing the cathode/anode ratio resulting in a higher cathodic area. By increasing the cathodic area, FeS directly increases the corrosion exchange current (I_o) as shown in equation (A) (Little et al., 1989b),

$$I_o = i_o A_c \text{ where} \quad (A)$$

i_o = exchange current density

A_c = cathode area.

H_2S, which is produced as seen in reaction (8), has also been considered as a cathodic (Obeukwe et al., 1981; Tiller, 1982; Hardy, 1983; Duquette and Ricker, 1985; Videla, 1985; Hamilton, 1987a; Cullimore, 1990a; Boopathy and Daniels, 1991) and anodic active agent (Booth and Tiller, 1968; Cragnolino and Tuovinen, 1984) but its corrosion capabilities depend on its accumulation within the biofilm, causing pitting and stress-corrosion cracking in copper pipes (Little et al., 1989a), intergranular stress corrosion and embrittlement in stainless steel (Tiller, 1990; Walsh et al., 1992) and cracking in concrete (Mori et al., 1992). However, two other theories have been proposed to explain the effect of SRB in MIC (Hamilton, 1987).

Iverson (1979) postulated the formation of a highly corrosive reduced iron product, Fe_2P, behaving as a cathodic active agent as seen in reaction (14). Formation of this product must be assisted by the action of the iron oxidizing bacteria (IOB) which facilitate the production of Fe^{3+} and basic conditions which can be facilitated by the hydrolysis of water as seen in reaction (3). Also, the Fe^{2+} release at the anodic site can result in the formation of Fe_2P through an abiotic reaction as seen in reaction (15). This reaction favors the possible process which Iverson observed within laboratory setting. Weimer et al. (1988) found different corrosion products under different phosphate concentration emphasizing that phosphate products such as vivianite ($[(Fe_3(PO_4)_2)\text{-}8H_2O)]$) only occur in the presence of high phosphate concentration, which is likely to occur in natural water ecosystems receiving run off from agricultural land.

$$Fe(OH)_3 + OH^- + PO_4^{2-} + Fe^{3+} \text{ ---> } Fe_2P + 2H_2O + 3O_2 \quad (14)$$

$$4OH^- + 2PO_4^{2-} + 4Fe^{2+} \text{ ---> } 2Fe_2P + 2H_2O + 5O_2 \quad (15)$$

Nevertheless, Schaschl (1980) proposed an additional theory where the H_2S is abiotically oxidized into S^0

through intermediate reactions. Reaction 16 presents the abiotic oxidation step in which the cathodic formed H_2S is oxidized onto HS^-. Then, the formed bisulfide ion react with the released Fe^{2+} at the anodic site, resulting in the formation FeS (10) and differential aeration cell mainly of sulphur.

$$4H_2S + O_2 + 4e^- \text{---> } 4HS^- + 2H_2O \quad (16)$$

METHANOGENIC BACTERIA

Recently, Daniels et al. (1987) demonstrated that methanogenic bacteria (MB), which can use H_2 as electron donor and CO_2 as sole carbon source (Boopathy and Daniels, 1991), produced CH_4 in the presence of Fe^0 as e^- donor using H^+ as energy source. They postulated that methanogenic bacteria must be capable of using the atomic or cathodic hydrogen as shown in reaction (17). Further studies with *Methanococcus deltae, M. thermolithotropohicus* and *M. barkeri* conducted by Boopathy and Daniels (1991) confirmed the possible role that they can play in MIC.

$$8H^* \text{ or } 4H_2 + 4Fe^0 + CO_2 \text{---> } CH_4 + 4Fe^{2+} + 2H_2O \quad (17)$$

Daniels, et al.'s (1987) results indicated the inhibitory capabilities of Fe^{2+} in the growth of methanogenic bacteria. However, the production of $2HS^-$ and S^- by the SRB as indicated by reactions (10 & 12), could reduce the inhibitory capability of Fe^{2+} resulting in the formation of FeS and mutualism interactions in natural ecosystems between the SRB and MB. The symbiotic interactions of these two species in complex environmental cases can have detrimental effects in a MIC situation resulting in enhancement of the corrosion rates. As Kristjanson, et al. (1982) indicated, the production of CH_4 and H_2S by the MB and SRB, respectively, are not mutually exclusive processes in the presence of excess H_2, as seen in the iron based metal surfaces.

The survival of *Desulfovibrio vulgaris* and H_2-utilizing methanogenic bacteria in coculture studies in the absence of sulphate (McInerney and Bryant, 1981; Tomei et al., 1985) suggests that the action of SRB as shown in reactions (10) or (12) and the action of MB as seen in reaction (17) could result in the removal of Fe^{2+} by the biotically produced S^- or by the oxidation of IOB (18) resulting in low level of H_2S and/or Fe^{2+}. The above reactions suggest that microbial corrosion can be a complex event in microbial environments (Pankhania et al., 1986) where the aerobic bacteria and eukaryotes (e.g., fungi), within the

biofilm can maintain low levels of O_2 resulting in the survival of anaerobic species and intensification of the corrosion process by the H_2 utilizers (e.g., SRB and MB) allowing the survival of obligate proton reducers (OPR) (e.g., *Syntrophomonas wolfei, S. wolinii*). OPR have been found to survive in syntrophic interactions with SRB and MB in the presence of butyrate (Tomei et al., 1985).

IRON OXIDIZING BACTERIA

The presence of iron oxidizing bacteria (IOB) within the biofilm at neutral pH such as *Gallionella, Sphaerotilus, Leptothrix* and *Creuothrix* favors the development of tubercles in potable water distribution pipelines and industrial cooling systems enhancing the dissolution of iron based metal (Iverson, 1968, 1985, 1979; Tiller, 1982, 1990; Videla, 1985; Hamilton, 1987a; Dowling et al., 1991). The consumption of the O_2 at the biofilm/water interface and the build up of EPS allow the survival of the anaerobic bacteria such as SRB at the biofilm/surface interface in well-oxygenated water such as natural rivers and estuaries (Little et al., 1989a; Gomez de Saravia et al., 1990).

The following chemical reactions (Brock and Madigan, 1988) explain how these organisms can increase the corrosion rates:

$$2Fe^{2+} + 1/2O_2 + 2H^+ \text{---> } 2Fe^{3+} + H_2O \qquad (18)$$

The arrangement of equation (13) as seen in reaction (19) allow further interaction of the biotically produced Fe^{3+} from reaction (18).

$$Fe^{3+} + 3OH^- \text{---> } Fe(OH)_3 \qquad (19)$$

The corrosion products, $Fe(OH)_3$, produced can further enhance the corrosion rates by interacting with the PO_4^{2-} available in solution and the cathodic hydrogen resulting in reaction (14). The formation of tubercles by IOB and the presence of biofilm can favor the adsorption of chemical anions such as chlorides and SO_4^{2-} (Tiller, 1990; Dowling et al., 1991) increasing the corrosion rates in metal surfaces such as SS (Iverson, 1987) through crevice corrosion mechanism.

FERMENTATIVE BACTERIA AND EUKARYOTES

The presence of acid producers such as fermentative bacteria, i.e., *Lactobacillus delbrueckii* and fungi, i.e., *Cladosporium resinae* (Videla, 1985; Iverson, 1987; Ford and Mitchell, 1990) within the biofilm/metal surface interface can increase the corrosion rates by the secretion of corrosive metabolites such as organic and inorganic acids (Iverson, 1968; Pope et al., 1982; Gomez de Saravia et al., 1990; Pedersen and Hermansson, 1991) increasing the concentration of H^+ (Videla, 1985) and enhancing the cathodic reaction as shown in reaction (4). Their metabolites can serve as the carbon sources for the SRB, developing symbiotic interactions within the biofilm (Dowling et al., 1988a). The presence of acidic corrosive metabolites can reduce the protective characteristics of the oxide layer present in SS metal surfaces (Gomez de Saravia et al., 1990) and detrimental damages to paint coated surfaces (Iverson, 1968; Stranger-Johannessen and Norgaard, 1991). Tiller (1982) indicated that the corrosion mechanisms which the metal surface can experience under acidic conditions depends mainly on the capability of the metal surface to produce (21) or not produce H_2 (23).

$$2[H^+ acid\ org^-] + 2e^- \text{---> } 2H_2 + 2\ org^- \quad (20)$$

$$Fe^{2+} + 2\ org^- \text{---> } Fe(org)_2\ (salt) \quad (21)$$

$$2[H^+ acid\ org^-] + 2OH^- \text{---> } 2H_2O + 2\ org^- \quad (22)$$

$$Fe^{2+} + 2\ org^- \text{---> } Fe(org)_2\ (salt) \quad (23)$$

3. PHOSPHOLIPID ESTHER-LINKED FATTY ACIDS

As indicated above, the interactions of different organisms within the biofilm can increase the corrosion rates by 1) the formation of different corrosion products increasing the surface area, 2) removal of the atomic or cathodic hydrogen from the cathodic site(s) 3) and removal of the Fe produced at the anodic site(s). The isolation of different bacteria groups such as phototrophic, chemoorganotrophic, lithoautotrophic, heterotrophic and aerobic chemolithotrophic from concrete, sandstone, brick surfaces, tubercle deposits and in the water column of aquatic environments (Tuovinen et al., 1980; Mallory and Saylers, 1983; Daniels et al., 1987; Diercks et al., 1991) emphasize the possible complex interactions that can develop within the biofilm (Hamilton, 1985; Ford and Mitchell, 1990).

The task of isolating and enumerating those organisms forming complex interaction in complex communities such as the one found in different surfaces and soils by classical methods such as viable plate, direct counts and other chemical applications such as ATP extractions and enzymatic activities can be tedious (Geesey and White, 1990), expensive (White et al., 1979) and time consuming (Hughes, 1968; Sand et al., 1987). The previous methods can provide results which underestimate the microbial population counts (Francisco et al., 1973; Costerton and Geesey, 1979; Mallory and Sayler, 1983; White, 1986; Parkes, 1987; Holben and Tiedje, 1988) and give few details of the microbial community structure (Lipski et al., 1992; Zelles et al., 1992).

As indicated by Diercks et al. (1991) the presence of *Thiobacillus spp.* in corrosion products collected from exposed surfaces (e.g., concrete) could suggest the actions of MIC. Phospholipid ester-linked fatty acid (PELFA) biomarkers for *Thiobacillus spp.* have been previously identified by White and co-workers from monocultures (Kerger et al., 1986, 1987), cocultures (Sand et al., 1987) and environmental samples (Sand et al., 1984). Similarly, PELFA biomarkers for other bacteria species such as SRB have been studied in monocultures (Edlund et al., 1985), tricultures (Dowling et al., 1988b) and in complex environmental communities (Phelps et al., 1991). PELFA biomarkers have also been used to define microbial communities *in situ* which have been documented by White and co-workers since 1977 (King et al., 1977) and to study quantitatively and qualitatively different microbial communities.

This destructive technique has permitted the detection of viable organisms capable of degrading toxic compounds (Nichols et al., 1987; Ringelberg et al., 1989), differentiation of different microbial communities structures formed on natural and man-made surfaces (Bobbie et al., 1978; Nickels et al., 1981), determination of the effect of stressors such as pollutants in the microbial community (Schropp et al., 1988, Vestal and White, 1989; Guckert et al., 1991) and elucidation of community structures (Gillan and Hogg, 1984; Smith et al., 1986; Guckert et al., 1991; Phelps et al., 1991).

The detection of prokaryote and eukaryote PELFA within the biofilm suggests their presence and active role confirming the formation of complex microbial interactions on the exposed surfaces. The presence of complex communities living within the biofilm emphasize that microbial corrosion must be treated as a complex process (Videla, 1985) where all the previously discussed reactions can take place at the same time or in sequence (Iverson, 1968, 1987). Numerous theories have been proposed to explain the MIC process. However, the complex bacterial interactions that can develop in MIC cases in nature emphasize the use of off-line destructive tools such as PELFA to understand the possible type of microbial interactions (Dowling et al., 1987), obtain accurate accounts (Costerton and Geesey, 1979) and propose possible MIC mechanisms. Further work in which the lipid of other bacterial isolates such as IOB are validated will provide better understanding as the major components of *in situ* microbial communities.

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PART III

FORMATION OF COMPLEX MICROBIAL COMMUNITIES ON EXPOSED METAL SURFACES

1. INTRODUCTION

Microbially influenced corrosion (MIC) is currently being experienced by the gas, oil (Phelps et al., 1991), pulp, paper (Hughes, 1968), nuclear and hydroelectric power industries (Walsh et al., 1992) and water treatment plants (Jones, 1992). The electric power industries (e.g., nuclear, hydroelectric and geothermal power) use large quantities of untreated water from freshwater and marine sources (Pope et al., 1982; Mercado et al., 1987) as mean of cooling systems and reactors. The presence of debris in these untreated fresh waters in combination with living micro and macroorganisms (Characklis and Cooksey, 1983) can accelerate the corrosion rates by establishing biological coatings on the exposed inorganic surfaces interfering with the formation of oxide film, and enhancing its removal (Iverson, 1968).

Biochemical analysis of phospholipid ester-linked fatty acids (PELFA), which are essential parts of all living cellular membranes (Parkes, 1987), has been the desired method for the study of microbial communities in nature because of their ubiquity, rapid extracellular turnover in viable and dead cells and constant relative concentration in microbial membrane (Vestal and White, 1989). This tool has been applied in numerous microbial communities studies (White et al., 1979a; Schropp et al., 1988; Guckert and White, 1986; Parkes, 1987; Vestal and White, 1989; White et al., 1991; Rajendran et al., 1992), validation of bacterial biomarkers (Nichols et al., 1987; Ringelberg et al., 1989), biofouling cases (Kerger et al., 1986, 1987) and in lipid modification studies in response to impose abiotic (Nordström and Laakso, 1992; Suutari and Laakos, 1992) and biotic changes (Bringer et al., 1985; Guckert and White, 1986).

The development of stratified and patchy biofilm on the exposed metal surfaces can result in the formation of complex microbial communities (Hamilton, 1985; Tiller, 1990) enhancing the appearance of symbiotic interactions which can be confirmed by the identification of specific biomarkers for organisms such as SRB, algae, etc, (Parkes, 1987). The presence of complex microbial communities and symbiotic interactions can increase the corrosion rates through the combination of different chemical reactions as shown previously. These reactions can occur simultaneously or subsequently within the

biofilm resulting in acceleration of the electrochemical corrosive process (Pope et al., 1982; Walsh et al., 1992).

The use of molecular techniques such as species specific probe (i.e., 16s rRNA oligonucleotide probe) and biochemical analysis (e.g., PELFA extraction) permits the characterization and quantification of the total viable eubacterial biomass present in natural communities (White, 1983; Ogram and Sayler, 1988; Stahl et al., 1988; Britschi and Giovannoni, 1991) to include nonculturable and unknown organisms (Holben and Tiedje, 1988; Britschi and Giovannoni, 1991; Wilson, 1992). Also, the application of these tools permits the phylogenetic group-specific and taxonomic identifications of bacterial isolates (Stackebrandt et al., 1985; Kerger et al., 1988; Delong et al., 1989; Urdaci et al., 1990; Witzel, 1990; Distel et al., 1991; Tsai et al., 1991; Amman et al., 1992; Lovley et al., In press) enhancing the identification process of clinical (Veys et al., 1989; Wilson, 1992) and environmental specimens (Lovley et al., In press). The use of molecular techniques in an ecology setting has increased within the last fourteen years. They have been widely employed in diversified studies such as detection of bacterial symbiosis (Distel et al., 1991), detection of specific problematic organism within the food processing industries (Wang et al., 1991), etc. The 16s rRNA oligonucleotide probe has been the method of choice because the technique has low detectable limits (Amy and Hiatt, 1989) and the gene, consisting of highly conserved sequence (Tsien et al., 1990; Britschgi and Giovannoni, 1991), is present (Wilson, 1992) and abundant (Amman et al., 1992) in all living cells.

In this part, I describe the phospholipid ester-linked fatty acids (PELFA), 16s rRNA oligonucleotide probes, most probable number (MPN) and acridine orange direct count (AODC) results in term of the microbial community structures, biomass, and metabolic status of the communities. Separation of the PELFAs in different groups such as polyunsaturated, which are characteristics of eukaryotic organisms (White, 1983; Lechevalier and Lechevalier, 1988) and saturated, monoenoic and branched (i.e., iso and anteiso) fatty acids, which are associated with eubacteria (White, 1983; Lechevalier and Lechevalier, 1988), was performed to facilitate the analysis of the communities and to determine the effect of different man-made surfaces on the microbial community structure. The results

of the tran-cis ratio of specific PELFAs recovered from isolated bacterium or communities have been applied to evaluate the profile changes caused by nutrient deprivation as in the case of *Vibrio cholerae* (Guckert et al., 1986). Recently, Rajendran et al. (1992) utilized the measured ratio to evaluate the impact of possible stressor on the recovered PELFAs from sediments collected at eutrophic bays. I also used the measured ratios to evaluate the effect that different surfaces and contaminants present in the water column could have caused on the community structures.

2. MATERIALS AND METHODS

SITE DESCRIPTION

Douglas Reservoir impounds the French Broad River at mile marker 32.3. Tennessee Valley Authority (TVA) manages this reservoir located within central eastern Tennessee at Sevier County. The corrosion study was performed at three subsites, within the reservoir, mile 32.4, and three subsites within two of the previous studied inflows (Díaz, unpublished work) (Figure 2.1). The Nolichucky and Pigeon Rivers were sampled at mile 10.3 and 5.5, respectively (Figure 2.1 and 2.2). It is important to define the term subsites and sites. Throughout this work the term subsite means one location within the study aquatic environment site while the term site consists of three evaluated subsites.

The reservoir contains approximately 30,400 surface hectares and drains a watershed of approximately 1,427,383 hectares. The main uses of the restrained water in this reservoir are generation of electrical power, flood control and recreation (TVA, 1985).

The French Broad River is joined by two other inflows, the Nolichucky River and the Pigeon River. The Nolichucky River originates in North Carolina and flows northeasterly toward the Tennessee state boundary. At the state line this river encounters a mica, feldspar and kaolin clay mining area (Blevins and Pancorbo, 1986; Schacher, 1988). It also receives industrial discharges and landfill leachates (Blevins and Pancorbo, 1986). The Pigeon River, which is located in the eastern part of Tennessee, receives coffee-colored discharges from Champion International Corporation (CIC) paper mill which has been operating since 1908 (Schacher, 1988). CIC is operating a chlorine kraft pulp bleaching paper mill located in Canton, North Carolina, 6.5 miles from the Tennessee state line (Blevins, 1991). This river is also currently employed in the generation of electric power at the Tennessee/North Carolina state boundaries by the Carolina Power and Light Company (CPLC) (Díaz, personal observation).

The reservoir subsites were chosen in this part of the experiment because recent microbiological influenced corrosion (MIC) related problems at the reservoir intakes and cooling systems have been observed (J. Johnson, TVA, personal communication). TVA is currently releasing liquid O_2 during the

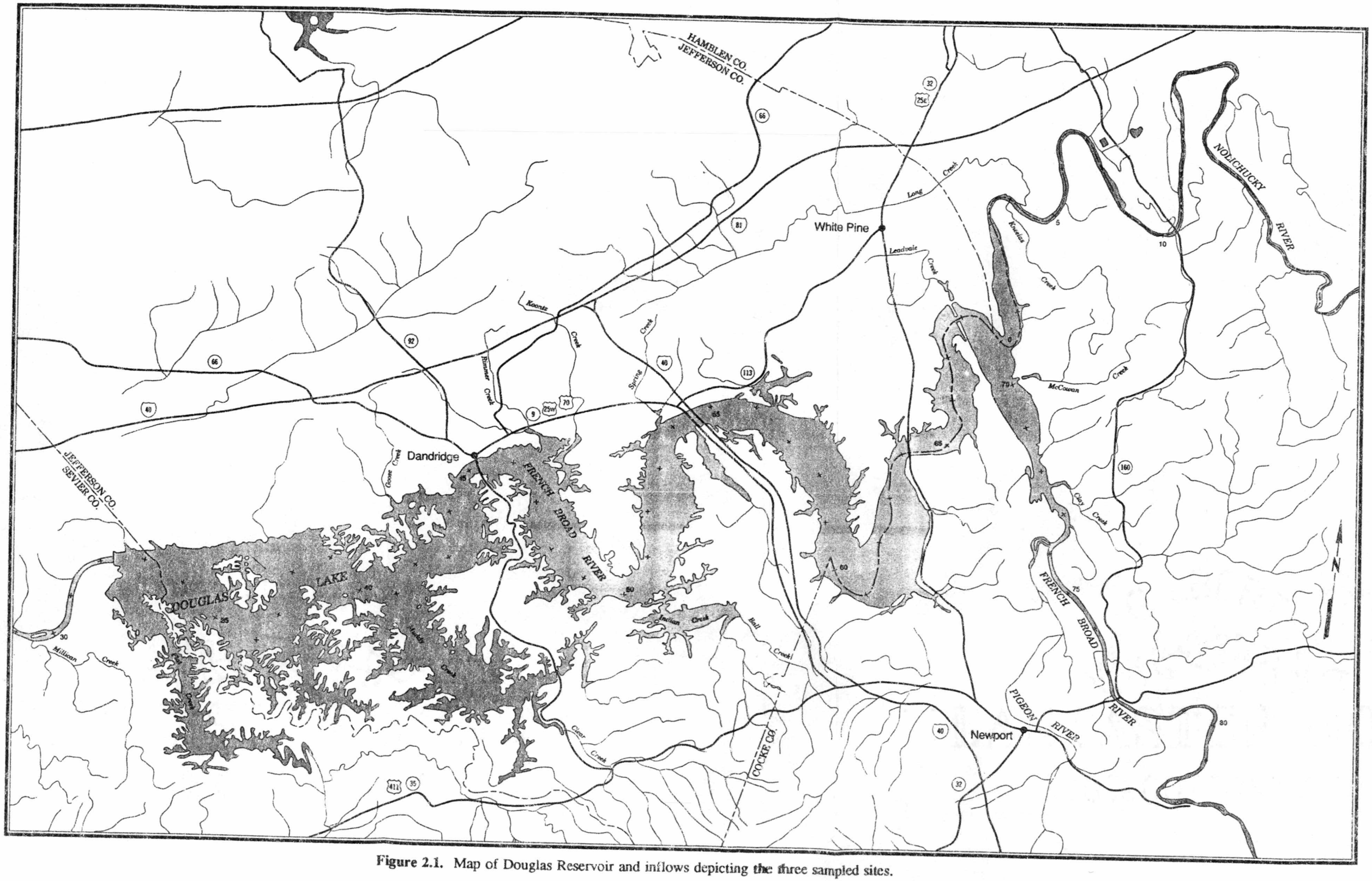

Figure 2.1. Map of Douglas Reservoir and inflows depicting the three sampled sites.

Figures 2.2.1 A. Douglas Dam Reservoir sampled subsites. **B.** Nolichucky River sampled subsites.

Figures 2.2.2 C. Pigeon River sampled subsites.

summer months to increase the dissolved O_2 (DO) concentrations levels in the tailwaters (Eng. M. Mobley, TVA, personal communication) to 5 and 6 mg L^{-1} as required by the Environmental Protection Agency (EPA) and TVA, respectively (Schacher, 1988). The inflows evaluated during the *in situ* study permitted a comparison between the water chemistry and bacterial communities present in their waters and possible effects at the reservoir. Exposure of two different metal surfaces, 1010 mild steel (MS) and 304 stainless steel (SS), permit the evaluation of the different microbial communities developed on the exposed surfaces and their corrosion capabilities while facing different O_2 concentrations. The differences previously observed in dissolved oxygen *in situ* at the three studied sites (Díaz, unpublished work) and measured during this study (tables 2.1-2.3) corroborate the point in case.

ENVIRONMENTAL PARAMETERS

The temperature, pH, redox potential, conductivity and dissolved oxygen of the water column were measured at the same subsites where the metal samples were suspended with a Hydrolab Surveyer II provided by TVA. The abiotic parameter measurements were taken at the time of suspending and retrieving the metal coupons (tables 2.1-2.3). The pH values were within the 6.5 to 9.0 safe range established by the Environmental Protection Agency (EPA) for aquatic life (U.S. EPA, 1986). Calibration of the instrument took place before and after conducting the sampling as described in the Hydrolab Surveyor II operating manual (Hydrolab Corporation, 1985) and no significant differences were detected. The previous reference established that the *in situ* redox potential readings can be normalized to the standard hydrogen electrode (SHE) values by adding +285 mV to each individual reading.

SAMPLE SIZE

A total of twelve MS samples and twelve SS samples were suspended at three randomly selected subsites within each of the aquatic communities. These materials were selected because of their availability and their constant use in fresh water applications such as cooling and potable water systems (Reedy, 1973; McCaul and Geld, 1978; Mercado et al., 1987; Ahmad et al., 1988). The 24 samples were divided equally within the three subsites and assembled in PVC frames (Industrial Plastics Work,

Table 2.1. Douglas Dam Reservoir water column physical parameters measured at the time of suspending (1) and retrieving (2) the metal samples.

Physical Parameters	Average Value (1)	Standard Deviation	Average Value (2)	Standard Deviation
Temperature (°C)	20.50	1.61	23.57	0.47
pH (unit)	6.70	0	6.57	0.06
DO (mg/L)	0.13	0.06	0.37	0.21
Cond. (umhos/cm)	148	1.73	153.3	0.58
E_h (mV vs SHE)	589	101.66	580.67	5.69
Depth (meters)	33.63	0.29	27.17	2.92

DO=Dissolved O_2, Cond.=Conductivity. The redox potential was measured in reference to Standard Hydrogen Electrode (SHE). The average value and its standard deviation corresponds to one measurement made at each subsite for an n=3.

Table 2.2. Nolichucky River water column physical parameters measured at the time of suspending (1) and retrieving (2) the metal samples.

Physical Parameters	Average Value (1)	Standard Deviation	Average Value (2)	Standard Deviation
Temperature (°C)	25.7	0.26	17.4	0.36
pH (unit)	7.53	0.06	8.30	0.10
DO (mg/L)	7.43	0.15	9.37	0.23
Cond. (umhos/cm)	194	13.08	309	26
E_h (mV vs SHE)	576.33	20.03	608.67	5.13
Depth (meters)	0.97	0.15	0.4	0.26

Legend same as Table 2.1.

Table 2.3. Pigeon River water column physical parameters measured at the time of suspending (1) and retrieving (2) the metal samples.

Physical Parameters	Average Value (1)	Standard Deviation	Average Value (2)	Standard Deviation
Temperature (°C)	22.8	0.1	18.3	0
pH (unit)	7.33	0.06	7.83	0.06
DO (mg/L)	8.6	0.1	9.53	0.15
Cond. (umhos/cm)	183.33	0.58	249.67	0.58
E_h (mV vs SHE)	554.33	9.07	616	3.46
Depth (meters)	0.7	0.1	0.8	0.1

Legend same as Table 2.1.

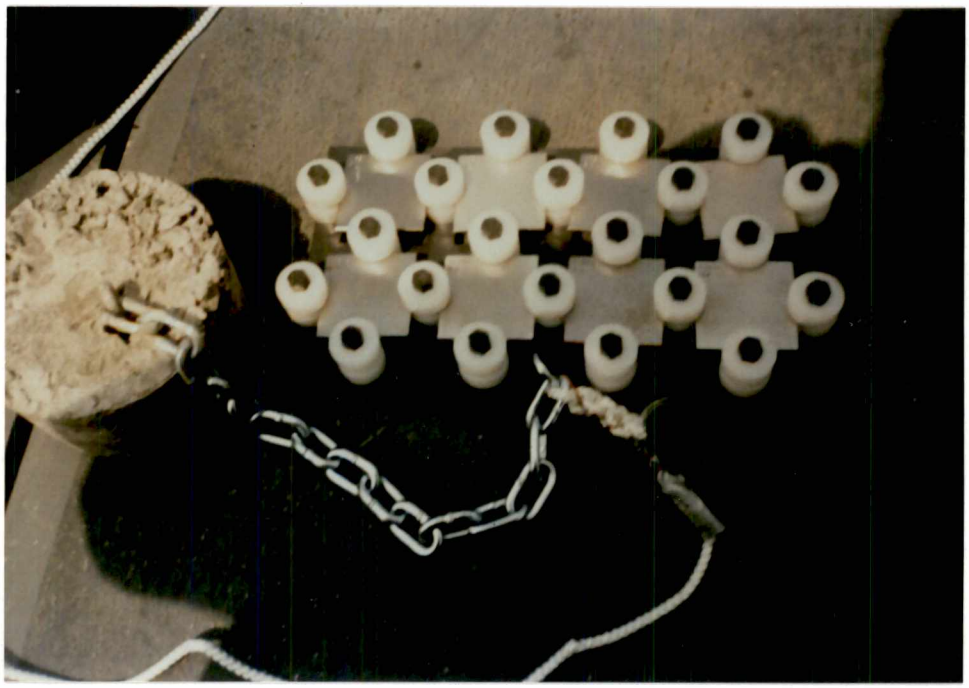

Figure 2.3. PVC frame and concrete slab used for the suspension of the metal coupons *in situ*.

Knoxville, TN) (figure 2.3).

The PVC frames were suspended 1 foot from the bottom at each subsite and attached to a 2 pound concrete slab by a chain link. The concrete slab was prepared by adding tap water to Sure Mix concrete powder as the manufacturer (W.R. Bonsal Co., Charlotte, NC) indicated. A two gallon plastic pot

provided the correct size for the desired slab weight. The mixture was allowed to dry overnight.

The samples were exposed at the reservoir from 31 July to 25 September 1991 and at the inflows from 1 August to 27 September 1991 for a total period of 56 days at each subsite. Existing polystyrene floats at the reservoir allowed identification of the subsites and easy retrieval of the samples. However, the suspended samples at the inflows were identified by attaching nylon lines (Omniflex, Columbia, SC) to the PVC frames and attaching them to existent bridge bases (figure 2.2 B-C).

METAL COMPOSITION

MS and SS coupons with the following measurements (2" X 2" X 1/8") (Metal Samples Inc. Munford, AL) were used as substrata for the development of microbial biofilms and as a tool to evaluate the water's corrosiveness. Table 2.4 contained the metal samples chemical composition with their unified numbering system designations.

Table 2.4. Chemical composition of the metal samples tested in the different environments.

Elements	MS (G10100)	SS (S30400)	Elements	MS (G10100)	SS (S30400)
Carbon	0.08	0.06	Nickel	0	8.03
Chromium	0	18.11	Phosphorous	0.007	0.034
Iron	99.446	71.472	Sulfur	0.017	0.004
Manganese	0.45	1.83	Silicone	0	0.46

The values are expressed in percentage.

METAL PREPARATION

The cleaning procedure consisted of preparing the MS and SS samples as described in ASTM G-1-

88 (ASTM, 1989). The MS surfaces were polished with an uniform 600 grit silicon carbide paper and cleaning the surface with three rinses of 18 mohm-cm water followed by degreasing the coupon surfaces with acetone. The SS samples were prepared with the same cleaning procedure described in the next section and degreased prior to storage. Upon cleaning the metal sample surfaces, the samples were wrapped in Kimwipes and stored in a desiccator until further use. Twenty four hours prior of suspending the metal samples, they were assembled in PVC racks and sterilized by ethylene oxide/CO_2 gas 80/20 for four hours at 50 °C at University of Tennessee Medical Center. This sterilization method reduced the possibility of causing early chemical corrosion of the MS surfaces as in the case of wet steam autoclaving (Franklin et al., 1988).

CORROSION PRODUCT CLEANING PROCEDURES

The procedure to clean the corrosion products from the metal samples has been described in ASTM G-1-88 Annex A.1 (ASTM, 1989). It consisted in exposing for 25 minutes at room temperature the MS samples in a solution prepared by mixing 20 g/L of Sb_2O_3 and 50 g/L of $SnCl_2$ (Mallinckrodt Specialty Chemicals Co, NY) in 1 liter of HCL (37%). The SS samples were cleaned in a mixture of 100 ml of HNO_3 and 900 ml of 18 mohm-cm for 20 minutes at 60 °C. All the chemicals employed throughout this research were reagent grade. Thereafter, the cleaning procedure was the same for both type of metals. The samples were ultrasonically washed in a mixture of 18 mohm-cm water and organic soap (25% v/v) for 25 minutes and rinsed with 18 mohm-cm water. The samples were degreased with acetone and dried in a desiccator for 24 hours.

Three control coupons for each type of material were not exposed to the *in situ* conditions but were cleaned as previously described to detect metal weight loss due to the chemical cleaning and lipid extraction. Metal weight loss for the MS and SS averaged 0.04359 and 0.0016 mil per year (mpy), respectively.

SAMPLE COLLECTION

Upon retrieving the metal samples, a total of six exposed metal surfaces, which consisted of three MS and three SS surfaces per subsite, were selected for biological evaluation. Collection of the

corrosion products (CP) and biofilm consisted in scrapping with an sterile teflon spatula the top side of the exposed metal surface and transferring them into a 15 ml centrifuge tube containing 1 ml of sterilized mineral water salt (MWS) solution. In between scrapping the spatula was rinsed with 90 percent ethanol and air dried. After removing the corrosion products and biofilms, the tubes were placed in ice and transported to the laboratory for further evaluation. The samples were processed within 72 hours at the laboratory.

BACTERIAL COUNTS AND BIOMASS

The collected corrosion products and biofilm were serially diluted into anaerobic crimp top tubes containing sterilized mineral water solution and 10 S/P solid glass beads. The MWS solution was prepared by mixing the following substances expressed as mg L^{-1} of distilled water: NH_4CL, 53.49; $MgSO_4$*7H2O, 500; KH_2PO_4, 272.2; $CaCl_2$*2H2O, 50; and 10 ml Hutner's Mineral Base (Gerhardt et al., 1981). The tubes were pressurized with 20% (v/v) CO_2 in N_2, sealed with a rubber stopper and aluminum cap and sterilized in the autoclave at 121 °C for 15 minutes at 20 psi. Serial dilutions were performed for each sample. Three different set of analytical procedures; 1) phospholipid ester-linked fatty acids (PELFA), 2) 16s rRNA oligonucleotide eubacterial probe and 3) acridine orange direct counts (AODC), were used to determine total microbial biomass. Also, enumeration of SRB consisted of three similar applications as explained below.

Sulfate Reducing Bacteria by the Most Probable Number Method.

The SRB counts from the scrapped corrosion products and biofilm were measured by the standard MPN techniques (Collins and Lyne, 1976). Three mls of the first three dilution tubes were used to inoculate triplicate anaerobic crimp top tubes containing Sulfate API BROTH media (Difco Laboratories, Detroit, MI). The tubes were incubated for 22 days at 28 °C. Those tubes that turned black, which is indicative of the reduction of the ferrous ammonium sulfate present in the solution to FeS, were scored positive and evaluated based in the MPN table. One positive tube was kept for further isolation and identification of the SRB.

Sulfate Reducing Bacteria and Eubacteria by 16s rRNA Probe Technique.

Two ml of the collected sample were transferred into two 1.5 ml Eppendorf microcentrifuge tubes, one ml per each tube. The sample was centrifuged at 10,000 x g for 10 minutes and the supernatant decanted. The pellet was suspended in a buffer containing sodium dodecyl sulfate and RNAse stabilization reagent included with the Gen-Probe kit (Gen-Probe Company, San Diego, CA). Two different probes were used to determine the total biomass of Eubacteria and SRB present in the environmental sample. The SRB probe measures the presence of two types of SRB, *Desulfotomaculum spp.* and *Desulfovibrio desulfuricans* (Gen-Probe Co. SRB I and SRB II kits, respectively). The 16s rRNA oligonucleotide SRB probe (OSRBP) procedure was performed as described by Mittelman (1991). The incubations were done for 1 hour at 60 °C, followed by 10 minute hydrolysis at 60 °C to obtain higher sensitivity. The relative light units (RLU) emitted by the bound acridinium ester-linked oligonucleotide DNA probe and the bound RNA were measured with a LEADER I Luminometer (Gen-Probe Co., San Diego, Ca).

The relative light units measured by each of the collected samples were correlated to a serial dilution of *Desulfovibrio desulfuricans* DSM 1924 obtained from Deutsche Sammlung von Mikroorganismen Gmbh (DSM) Braunschweig, Germany. One ml of *D. desulfuricans* culture grown in API medium was serially diluted (10^{-7}) using the MWS solution. Three ml from each dilution were divided into 1 ml for direct counting using the AODC, as explained below. The other 2 mls were split in half and transferred into 1.5 ml microcentrifuge tubes for further evaluation of the total SRB by the 16s rRNA OSRBP. All measurements were done in duplicate. This approach correlated the measured RLU from environmental samples using Eubacteria and SRB probe to AODC values obtained with pure culture of SRB.

The measured data points for each parameter were fitted to a first order linear equation using a linear regression method (SAS, 1985). The correlation coefficient for the first model, AODC vs Eubacteria probe (figure 2.4), was 0.9993 with $p< 0.01$ and for the second model, AODC vs SRB probe (figure 2.4), was 0.9977 with $p< 0.01$. Based on the linear regression equation the established detection limit was 1.9×10^3 cells for the Eubacteria probe and 3.2×10^3 cells for the SRB probe which agree

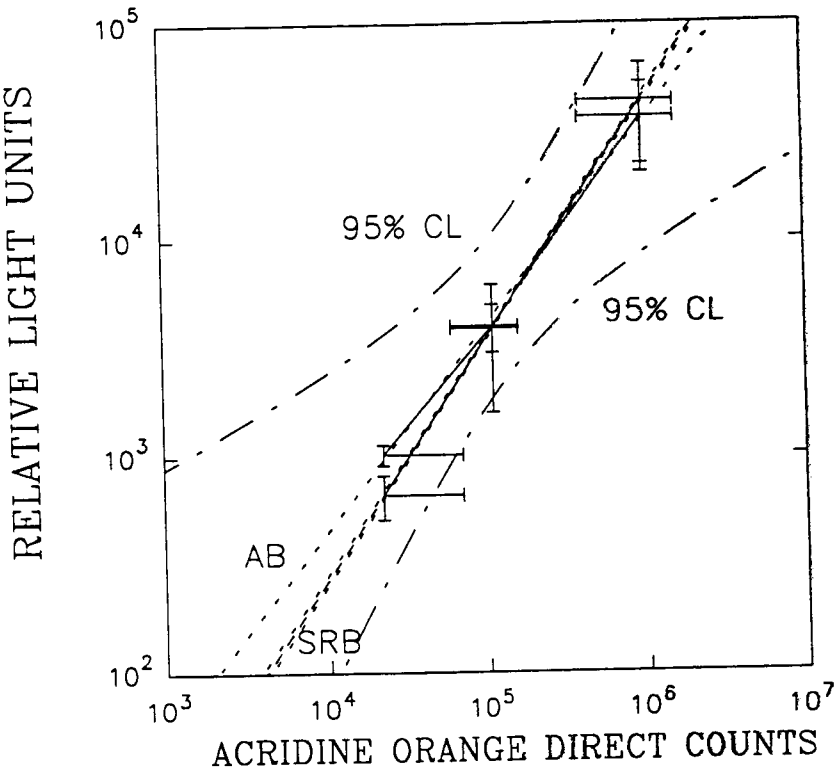

Figure 2.4. Regression results of a linear equation for two different models. AB and SRB stands for eubacterial and sulphate reducing bacteria probes, respectively. AB and SRB graphics are indicated by the single and double dotted lines, respectively. CL stands for confidence limits. The horizontal bars at each respective points represent the standard deviation while the vertical lines indicate the range for each value.

with the manufacturer detection limit of 5 X 10^3 for both probes.

Biomass Measured by Acridine Orange Direct Count.

In compliance with the procedure described by Hobbie et al. (1977), 1 ml from the last dilution tube, containing 2% (v/v) glutaraldehyde, was filtered by suction onto a 0.2 µm polycarbonate filters (PF) (Nucleopore Corporation, Pleasanton, CA) placed on top of a cellulose filter (Nucleopore Corporation, Pleasanton, CA.) The PF was stained for 5 minutes with 2 ml of acridine orange (AO) solution at a final concentration of 0.01 percent and enumerated. The filters were air dried and fixed onto glass slides with immersion oil. Prior to examining the stained filters under epifluorescence illumination, a drop of immersion oil was placed on the stained filter and sealed with a coverslip. A total of 15 fields per sample were randomly counted recording at least 100 bacteria per total count.

Biomass Measured by Phospholipid Fatty Acid Extraction.

The bottom side of the exposed metal surface was lipid extracted by a modified Bligh and Dyer extraction (Bligh and Dyer, 1959; White et al., 1979b). The metal sample was deposited in a screw-capped teflon covered jar (QARPAK bottles, Baxter), previously washed with ethanol followed by chloroform and air dried for 24 hours at 60 °C. The possible loss or contamination because storage or freezing was reduced by commencing extraction on site. The one-phase chloroform:methanol:PO_4 buffer mixture (35:70:28 ml) was allowed to extract for a period of 24 hours under slow continuous agitation provided by a rotary shaker. The mixture was decanted into a 250 ml separatory funnel and the single phase was separated into organic and aqueous phases by the addition of 35 ml chloroform and 35 ml PO_4 buffer. This step was allowed to separate for 24 hours at room temperature. Then, the organic phase was filtered through a fluted Whatman 2V filter paper into a round bottom flask to dry in vacuo at 37 °C. The filtered organic phase was subsequently dried and further treated to obtain the ester-linked phospholipid fatty acids from the samples as previously described by Ringelberg et al. (1989).

PHOSPHOLIPID ESTER LINKED FATTY ACID NOMENCLATURE

Designation of PELFA consists of total number of carbon chain length as indicated by the number before the colon. The number following the colon stands for the number of double bonds

within the carbon chain length. The position of the double bond as indicated by the symbol "w" correlates with the number of carbon from the CH_3 end of the molecule. The cis (c) or trans (t) arrangement indicates the geometry of the double bond configuration. For example, 20:1w8t is a PELFA with a total of 20 carbons with one double bond at the 8 carbon from the methyl end group in the trans configuration. The iso (i) or anteiso (a) designates the branched fatty acids. The cyclopropyl (Cy) fatty acids are designated by the total number of carbons.

BACTERIAL LIPID BIOSYNTHESIS

Bacterial lipid biosynthesis was measured in triplicate in two different artificial surfaces exposed at each subsite. The coupons were randomly selected and the bacterial lipid biosynthesis measured by adding 0.5 ml of 2 μCi ml^{-1} (56 mCi $mmol^{-1}$) ^{14}C-1-acetate (New England Nuclear, Boston, MA). The 0.5 ml of ^{14}C-1-acetate was added onto three areas within the surface of the coupon by a clamped o-ring extractor (Nivens, personal communication). The incubation was carried out at ambient temperature for a period of 30 minutes. Thereafter, a mixture of one-phase chloroform:methanol mix (0.63:1.26 ml) was added to stop further incorporation. The previous mixture was added in increments of 0.63 ml to ensure the complete removal of the total lipids and not to exceed a total of 1.89 ml. The total lipids were transferred into test tubes previously heated for 24 hours at 360 °C. The total lipids single phase were separated by the addition of 0.63 of chloroform and 0.63 ml of water. The organic phase was transferred to a 5 ml scintillation vial and dried under nitrogen stream. Each scintillation vial received 4 mls of scintillation cocktail (Ecolume, Irvine, CA) and was counted in the LKB model 1212 (Turku, Finland) using a single label protocol and external standard.

BACTERIAL ISOLATION

Isolation of the bacteria consisted of plating 0.1 ml of the highest MWS dilution (10^{-9}) onto Heterotrophic Aerotolerant Colony Forming Unit (HACFU) media. The HACFU media consisted of 900 ml of DW and 100 ml of mineral salts, as previously described, with the following additions by g L^{-1}: Bacto Agar (Difco, Detroit), 15; HEPES 99 percent, 1.31; Sodium Succinate, 0.2; TSB, 0.51; Yeast Extract, 0.16. The media was sterilized by autoclaving at 121 °C for 15 minutes at 20 psi. The plates

were incubated for a period of 96 hours at 28 °C.

Isolation of the aerobic colonies was based on the colony's morphology and pigmentation (Gerhardt et al., 1981). Purification of the isolates consisted in repeatedly picking and streaking of colonies until one colony morphology was obtained. Identification of the individual bacterial isolates consisted of using gram stain as indicated by Gerhardt et al. (1981), oxidase tests (Marion Scientific, Kansas City, Missouri) and identification by the Microbial Identification Systems (MIS) (Hewlett-Packart, 1985) with an alternate confirmation by the API 20E or RAPID NFT (Analytab Products, Plainview, NY).

STATISTICAL ANALYSIS

Analysis of the collected data was performed using SAS, a System for Elementary Statistical Analysis. All the measured parameters were evaluated by the two sample t-test procedure, when applicable. All the discussed results are only addressed when $p<0.05$, unless otherwise specified. The T-test procedure allows the evaluation of the collected data based on the assumption that the mean values for the two evaluated groups are equal (**NULL HYPOTHESIS**) and permit the rejection of the previous hypothesis if they are not equal (**ALTERNATIVE HYPOTHESIS**). Comparison between the dissimilar surfaces was performed for all three environments.

ANOVA procedure permitted the statistical analyses of the different PELFA detected on the different surfaces. This procedure allows the evaluation of the variation of the data controlling for the experimental error. Also, all the reported results are only for those results where $p<0.05$. Anova for each lipid was applied to compare the two metal surfaces while holding constant the sites. Analysis of all the PELFAs recovered from the microbial communities through principal component analysis (PCA) detected the major principal PELFA(s) which experienced the highest variations (positive or negative) within the studied aquatic sites and metal surfaces. Each detected PELFA values formed the data matrix. From the matrix of correlations, SAS PCA derived the eigenvalues, eigenvectors and principal components.

3. RESULTS

BIOMASS

Comparison Between Different Metal Surfaces.

Phospholipid Ester-linked Fatty Acids

The biomass collected from 1010 mild steel (MS) (Appendix A.1 and A.3) and 304 stainless steel (SS) surfaces (Appendix A.2 and A.4) exposed at the Pigeon River (PR) and the Nolichucky River (NR) indicated no significant differences for the former ($p<0.64$, Appendix B.1) and the latter inflows ($p<0.21$, Appendix B.1). However, the biomass retrieved from the MS surfaces (Appendix A.5) and SS (Appendix A.6) surfaces exposed at Douglas Reservoir (DR) resulted in significantly lower biomass on the SS surfaces than on the MS ($p<0.0005$, Appendix B.1).

16s rRNA Oligonucleotide Eubacteria Probe

The predicted biomass collected from the two different metal surfaces exposed at the PR resulted in significantly higher biomass on the SS surfaces than in the MS surfaces ($p<0.009$, Appendix B.2). However, no significant differences were noted for the 2 different surfaces exposed to the NR ($p<0.38$, Appendix B.2) and DR ($p<0.47$, Appendix B.2).

Acridine Orange Direct Counts

The biomass collected from the two different surfaces exposed to the PR indicated significantly higher biomass on the SS than on the MS surfaces ($p<0.02$, Appendix B.3). However, the second inflow and DR showed no significant differences on biomass resulting in the following probabilities for the former ($p<0.19$, Appendix B.3) and for the latter ($p<0.10$, Appendix B.3).

SULFATE REDUCING BACTERIA

Comparison Between Different Metal Surfaces.

Sulfate Reducing Bacteria Phospholipid Ester-linked Fatty Acids Biomarkers

Sulfate Reducing Bacteria (SRB) phospholipid ester-linked fatty acids (PELFA) biomarkers, *i17:1, 10Me16:0, a17:1w7c* and *Cy17:0,* recovered from two different surfaces exposed to the PR's waters resulted in significant higher SRB PELFA concentration on the MS than on the SS ($p<0.0002$,

Figure 3.1). Similar results were found for the surfaces exposed to the NR ($p<0.005$, Figure 3.1). However, those surfaces exposed to the reservoir did not show significant differences ($p<0.55$, Figure 3.1).

Most Probable Number

The results obtained by the most probable number (MPN) indicated no significant differences on both types of surfaces exposed to the PR ($p<0.08$), the NR ($p<0.84$) and DR ($p<0.20$) (Appendix C.1).

16s rRNA Oligonucleotide Probe

The 16s rRNA oligonucleotide probe results indicated that the SRB presence on SS were below the detection limit for all three sites (Figure 3.2). The detection limit was estimated to be $3.2X10^3$ while all the MS had values above the estimated detection limit.

POLY UNSATURATED

Comparison Between Different Metal Surfaces.

Analysis of the poly unsaturated fatty acids (PUFA) recovered from the different surfaces exposed to the PR, NR and DR indicated a significantly higher concentration of PUFA on the SS than on MS surfaces ($p<0.006$), ($p<0.004$) and ($p<0.002$), respectively (Figure 3.3). The PUFA percentages from the total phospholipid ester-linked fatty acids recovered from the MS surfaces was 18, 11 and 1 for NR, PR and DR, respectively while for the SS surfaces the percentages were 24, 22 and 19 in the same order.

ISO AND ANTEISO SATURATE AND MONOENOIC

Comparison Between Different Metal Surfaces.

Analysis of the total percentage of iso and anteiso (i,a) saturate and monoenoic PELFA recovered from MS and SS surfaces exposed to the PR, NR and DR resulted in significantly higher presence on the MS than on the SS surfaces for all three sites with the following probabilities in the same order ($p<0.002$), ($p<0.004$) and ($p<0.0001$) (Figure 3.4).

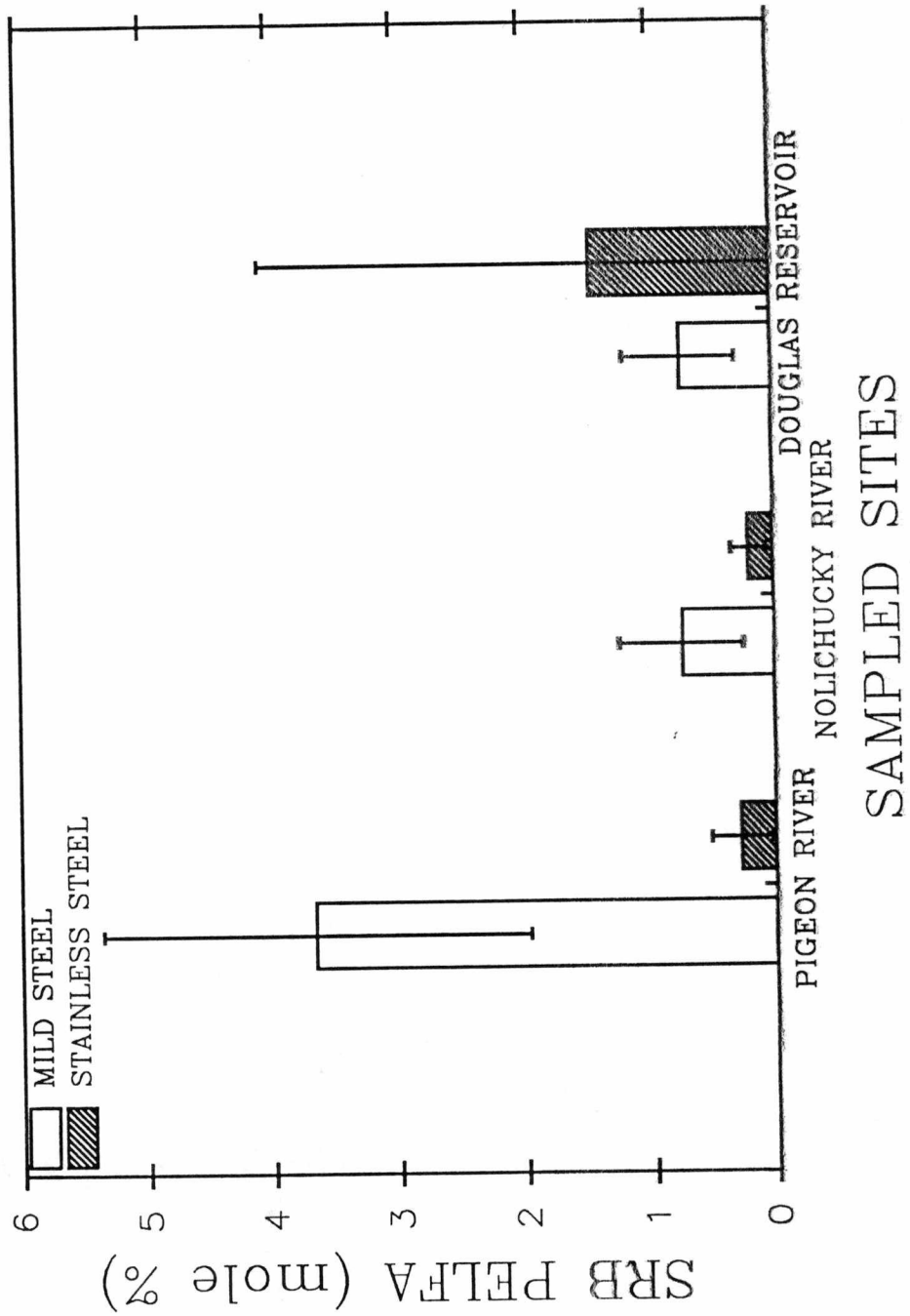

Figure 3.1. Percentage of designated SRB PELFA biomarkers, *i17:1*, *10Me16:0*, *a17:1w7c* and *Cy17:0*, recovered from two different surfaces exposed at three aquatic sites. N=9 except for the SS surfaces values from the Pigeon River (PR) and Douglas Reservoir (DR). The N value for the former is 8 and for the latter is 6 because the loss of one coupon and 3 values below the detection limit, respectively. The vertical line indicates the sample standard deviation.

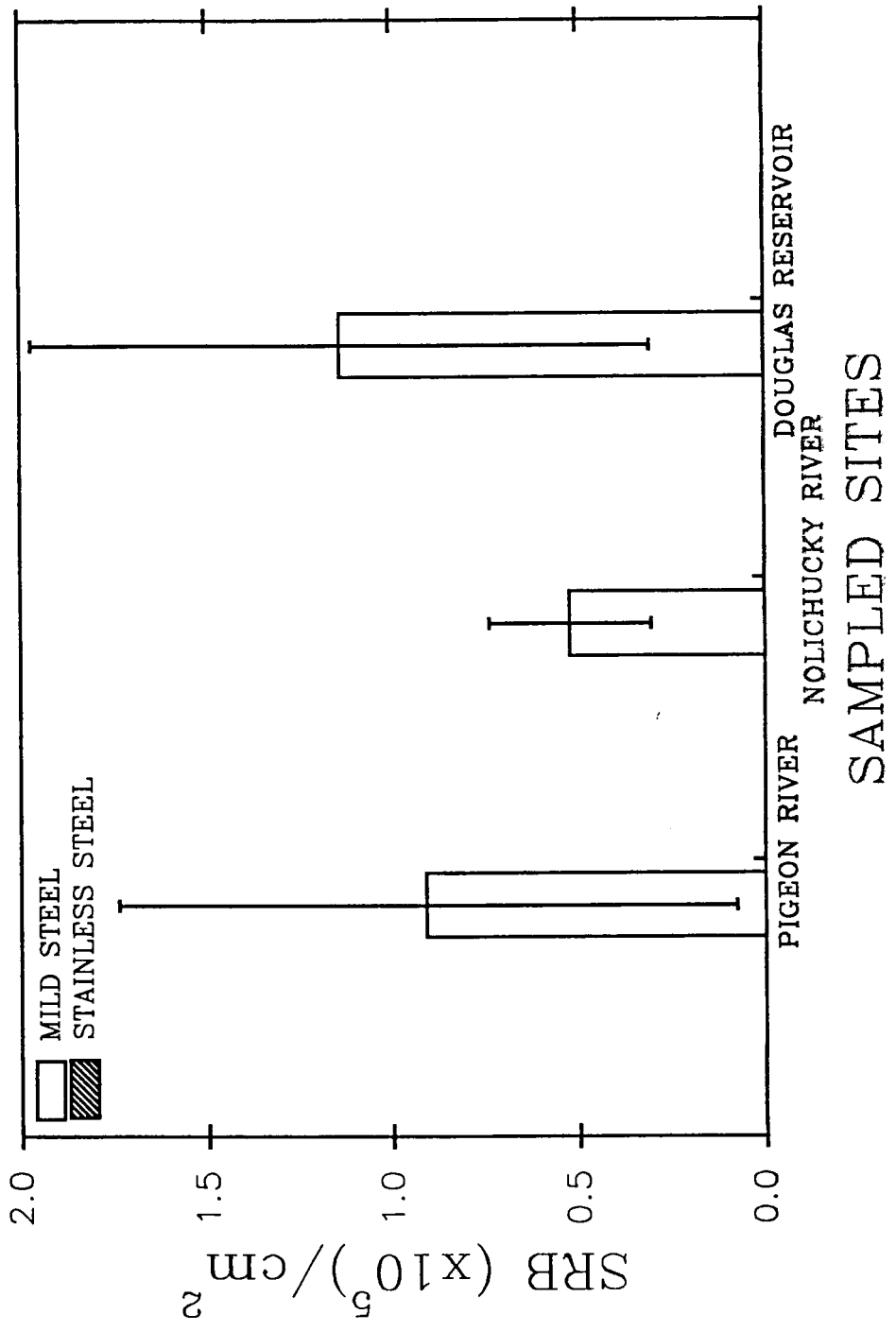

Figure 3.2. Estimated counts of SRB collected from two different surfaces exposed at three different aquatic sites and estimated by the 16s rRNA oligonucleotide SRB probe. N=9, 8 and 7 for the MS surfaces exposed to the NR, PR and DR, respectively. No SRB were detected on the SS surfaces exposed to the three sites as well as on 1 and 2 of the MS surfaces exposed to the PR and DR, respectively. The vertical line indicates the sample standard deviation.

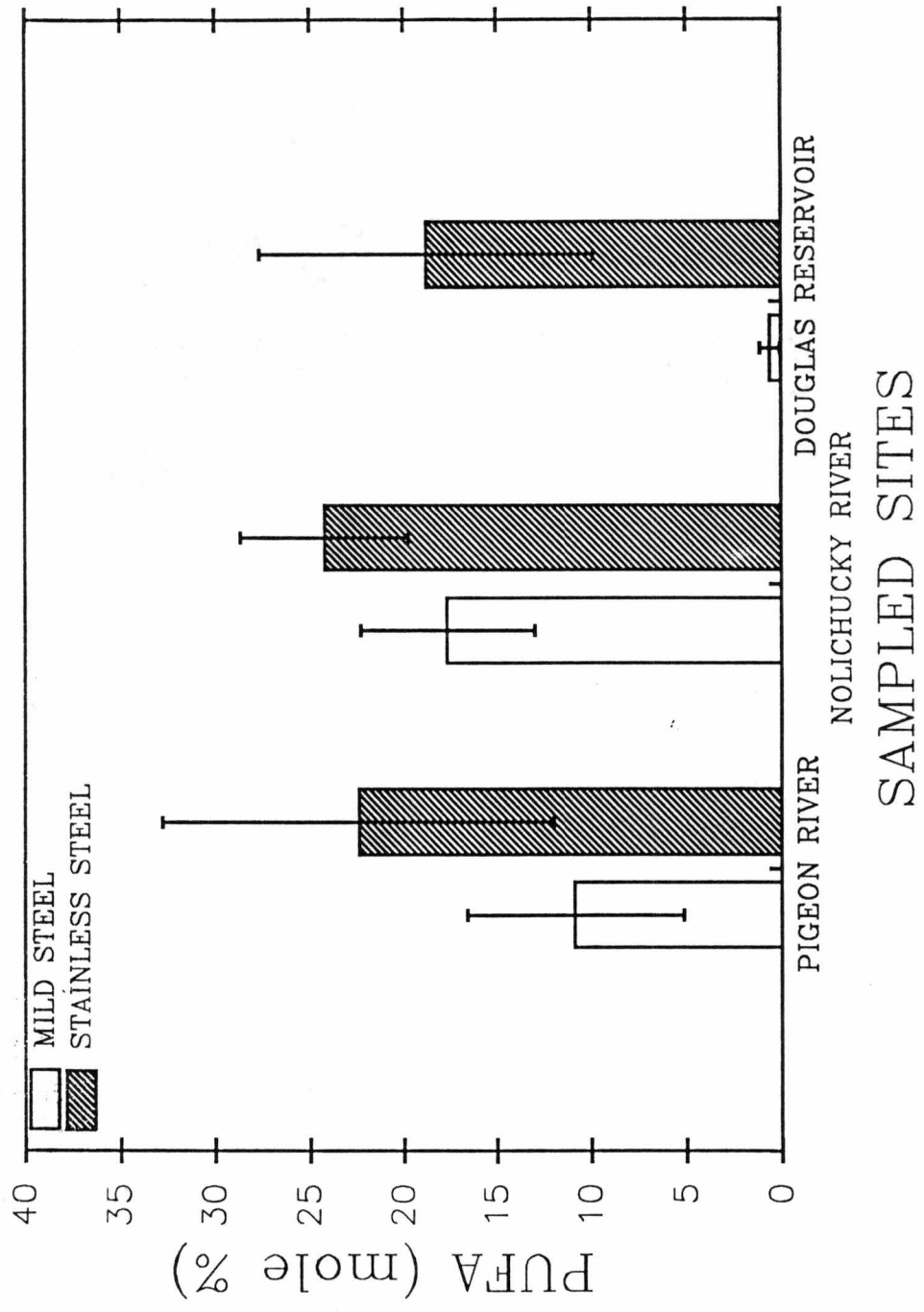

Figure 3.3. Total PUFA detected on two different surfaces exposed at three aquatic sites. N=9 except for the SS surfaces values from the Pigeon River (PR) and Douglas Reservoir (DR). The N value for the former is 8 and for the latter is 6 because the loss of one coupon and 3 values below the detection limit, respectively. The vertical line indicates the sample standard deviation.

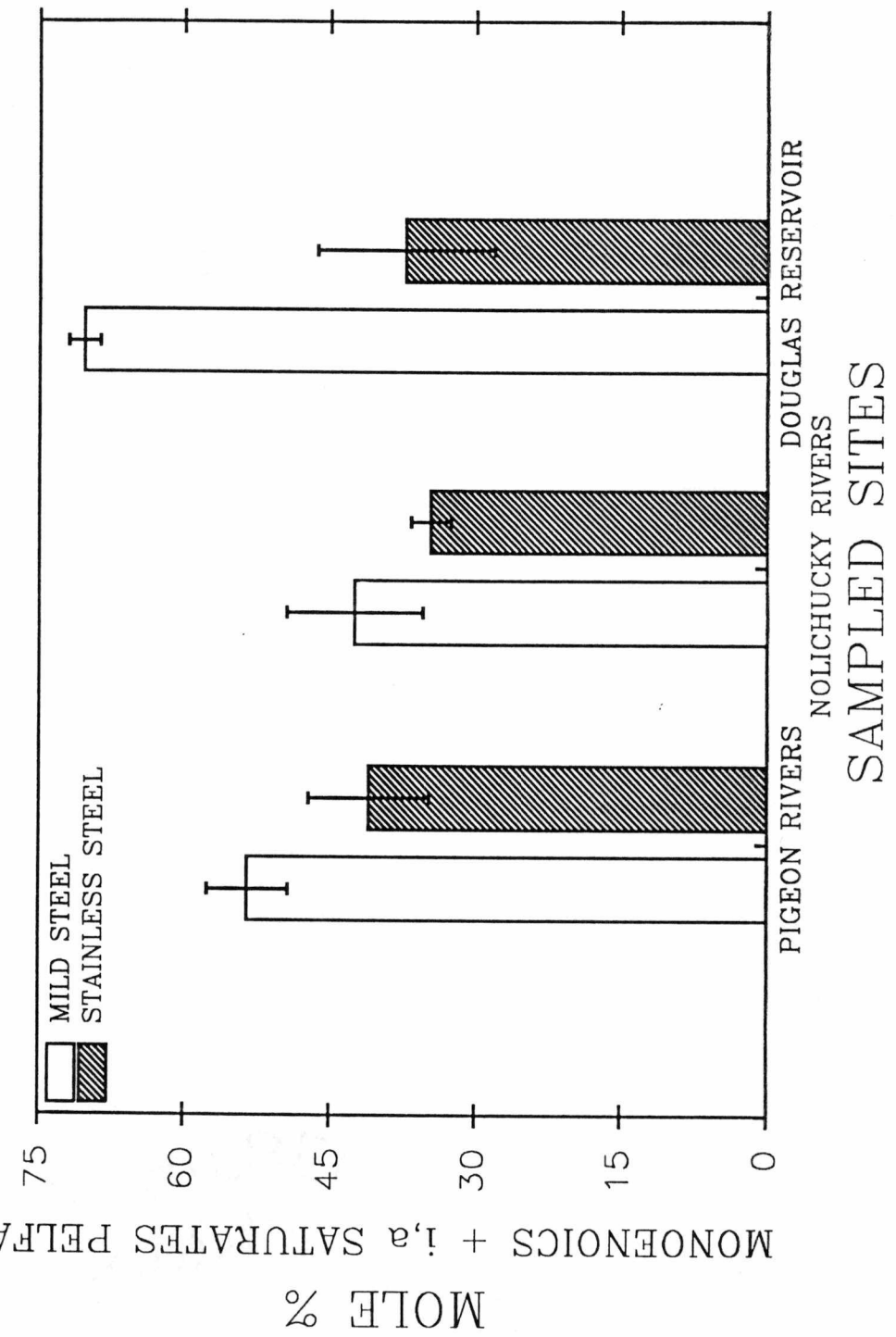

Figure 3.4. Total i,a saturate and monoenoic PELFA detected on two different surfaces exposed at three aquatic sites. N=9 except for the SS surfaces values from the Pigeon River (PR) and Douglas Reservoir (DR). The N value for the former is 8 and for the latter is 6 because the loss of one coupon and 3 values below the detection limit, respectively. The vertical line indicates the sample standard deviation.

BACTERIAL LIPID BIOSYNTHESIS AND TRAN/CIS RATIO

Comparison Between Different Metal Surfaces.

The bacterial lipid biosynthesis measured on two different metal surfaces resulted in no significant differences while exposed to PR, NR and DR resulting in the following probabilities ($p<0.24$), ($p<0.38$) and ($p<0.07$), respectively (Appendix D).

The tran/cis (T/C) ratio recovered from the 2 surfaces exposed to the PR water resulted in significantly higher T/C ratio on the MS than in the SS surfaces ($p<0.004$, Appendix E). However, the NR and DR did not show significant differences within the two exposed metal surfaces ($p<0.14$) and ($p<0.47$), respectively (Appendix E).

PHOSPHOLIPID FATTY ACIDS DETECTED ON TWO DIFFERENT SURFACES EXPOSED AT THE SAME SITE.

Table 3.1 contains the statistical results performed for each lipid collected from different surfaces exposed to three different environments. The presence of specific PELFA detected on specific surface exposed to specific site such as 10Me18:0, a19:0, 19:1w12c, 20:1w7c, etc. and within sites such as *10Me16:0, 20:2w3, 22:5w3, etc.* indicates the development of specific microbial communities. The communities developed at DR seems to be represented by those species capable of surviving under anoxic conditions only. All the lipids recovered from the surfaces exposed to the reservoir were also found on the surfaces exposed to the inflows with the exception of PELFA, *15:1w6c*, indicating that the inflow communities allowed the development of stratified biofilm resulting in the survival of anaerobic microbial species under oxic conditions (Hamilton, 1985). The absence of some lipids from the surfaces exposed to anoxic conditions such as *16:1w13t*, which is found in green algae (Vestal and White, 1988), *16:3w4, 20:3w3/i20:0, 22:4w6* (PUFA), *21:0 and 24:0* (saturates) detected on the surfaces exposed to oxic conditions suggest their validation as aerobic indicators. However, the recovery of 0.47 percent of the total PELFA for *Cy17:0* and *Cy19:0(w7,8)* from those surfaces exposed to anoxic conditions versus 0.72 percent under oxic conditions confirm that cyclopropyl fatty acids are indicative of anaerobic (Guckert et al., 1985) and aerobic communities as well.

Specific PELFAs such as *16:1w7c* and *16:1w7t* were found in significant higher concentration on the MS surfaces than on the SS for all three environments. However, PUFA *20:5w3* showed opposite pattern, suggesting some microbial metal surface selection (Valeur et al., 1988).

Table 3.1. PELFA detected on two different surfaces while exposed to the same aquatic environment.

PELFA	THREE DIFFERENT AQUATIC ENVIRONMENTS		
	PIGEON RIVER	NOLICHUCKY RIVER	DOUGLAS RESERVOIR
10Me16:0	NSD	-	-
10Me18:0	FOUND ONLY MS	-	-
14:0	NSD	NSD	MS > SS
15:0	NSD	MS > SS	FOUND ONLY MS
15:1w6c	-	-	FOUND ONLY MS
16:0	NSD	NSD	NSD
16:1w5c	NSD	NSD	NSD
16:1w7c	MS > SS	MS > SS	MS > SS
16:1w7t	MS > SS	MS > SS	MS > SS
16:1w9c	NSD	MS > SS	NSD
16:1w13t	NSD	NSD	-
16:3w4	NSD	NSD	-
17:0	NSD	NSD	NSD
17:1w6c	NSD	NSD	FOUND ONLY MS
18:0	NSD	NSD	SS > MS
18:1	-	NSD	NSD
18:1w5c	NSD	NSD	FOUND ONLY MS
18:1w7c	NSD	NSD	SS > MS
18:1w7t	NSD	NSD	FOUND ONLY MS
18:1w9c	NSD	-	SS > MS
18:2w6	NSD	NSD	NSD
18:3w3	SS > MS	NSD	NSD
18:3w6	SS > MS	NSD	NSD
18:4w3	NSD	NSD	FOUND ONLY MS
19:1w12c	-	FOUND ONLY SS	-
20:0	NSD	NSD	NSD
20:1w7c	-	FOUND ONLY SS	-
20:2w3	-	NSD	-
20:3w3/i20:0	NSD	NSD	-
20:4w3/20:3w6	FOUND ONLY SS	NSD	-
20:4w6	SS > MS	NSD	SS > MS
20:5w3	SS > MS	SS > MS	SS > MS
21:0	NSD	NSD	-
22:0	-	FOUND ONLY SS	-
22:2w6	-	FOUND ONLY SS	-

PELFA	THREE DIFFERENT AQUATIC ENVIRONMENTS		
	PIGEON RIVER	NOLICHUCKY RIVER	DOUGLAS RESERVOIR
22:4w6	NSD	NSD	-
22:5w3	-	NSD	-
22:6w3	FOUND ONLY SS	NSD	-
24:0	FOUND ONLY SS	FOUND ONLY SS	-
Cy17:0	MS > SS	MS > SS	NSD
a15:0	NSD	NSD	FOUND ONLY MS
a17:0	NSD	NSD	FOUND ONLY MS
a17:1w7c	NSD	-	FOUND ONLY MS
a19:0	-	FOUND ONLY SS	-
br19:1?	NSD	MS > SS	FOUND ONLY MS
Cy19:0(w7,8)	NSD	NSD	FOUND ONLY MS
i14:0	NSD	NSD	FOUND ONLY MS
i15:0	NSD	MS > SS	MS > SS
i16:0	NSD	NSD	FOUND ONLY MS
i17:0	NSD	NSD	FOUND ONLY MS
i17:1	NSD	-	FOUND ONLY MS

The abbreviations used in table 3.1 stand for; MS=mild steel, SS=stainless steel, NSD=No significant differences at a $p<0.05$, >=significantly higher at a $p<0.05$ and -=not detected.

PRINCIPAL COMPONENT ANALYSIS

Evaluation of the detected PELFAs by principal component (PC) analysis indicated that the first two components explained 71 percent of the variance while the third component explained 15 percent more, resulting in a total of 85 percent of the variation. The other two components explained the other 15 percent. Table 3.2 contains the eigenvectors with the highest positive and negative values which exerted the differentiation between surfaces and communities.

As seen in figure 3.5, the separation of the three different environments was influenced by the significant concentration of the PELFAs *16:1w13t*, *21:0* and *18:2w6* which were found in significantly higher concentrations on those surfaces exposed to the NR than on those exposed to the PR, and the former was not detected on the surfaces exposed at the reservoir. However, PELFA, *22:5w3*, was detected only on those surfaces exposed at the NR, while the iso and anteiso *17:1* showed opposite pattern and the PUFAs *20:3w3* and *20:4w3* were not detected on the surfaces exposed at the reservoir.

The two different metal surfaces were separated based on the following factors regardless of the site. PELFA *16:1w7c* was found to be in significantly higher concentration on the MS surfaces than

Table 3.2. Highest positive and negative eigenvector values for the components of the first two PCs.

PELFA	PRINCIPAL COMPONENT 1	PELFA	PRINCIPAL COMPONENT 2
16:1w13t	20.4%	i15:0	25.7%
21:0	20.2%	15:0	25.3%
18:2w6	19.8%	a15:0	25%
18:1w5c	19.6%	16:1w7c	22.5%
22:5w3	18.5%	18:1w7t	22.6%
20:3w3/i20:0	18.4%	16:0	21.9%
20:4w3/20:3w6	18.7%	17:1w6c	19.5%
18:1w7c	-17.3%	18:0	-25.3%
16:1w7t	-14.0%	20:4w6	-23.5%
i17:1	-13.9%	20:0	-22%
16:1w5c	-13.6%	20:5w3	-20.3%
a17:1w7c	-12.6%	18:1w9c	-19.8%
15:1w6c	-11.9%	18:1w7c	-14.2%

on the SS surfaces for all three sites while PUFA *20:5w3* had opposite pattern. Besides these two factors, PUFA *20:4w6* showed similar pattern as *20:5w3*, but not for the surfaces exposed at NR. *i15:0* and *15:0* had similar results as *16:1w7c* but only for the surfaces exposed at NR and DR except *15:0*, which was only detected on the MS surfaces exposed at the reservoir. At the reservoir, PELFAs *a15:0*, *18:1w7t* and *17:1w6c* were only found on the MS surfaces.

BACTERIAL ISOLATES

Table 3.3 contains two isolates recovered from different water sources. Isolate R22B was isolated from the French Broad River waters at 0.1 meter from the sediment (Díaz, unpublished work) while isolate 48 was isolated from MS surface exposed to the Pigeon River. Both isolates were identified by the API 20E system as *Enterobacter cloacae* while the Microbial Identification Systems (MIS) identified the former as *Salmonella spp.* and the latter as *Kluyvera spp.*

Table 3.3. Bacterial isolates recovered from two different water sources.

BIOCHEMICAL TEST	ISOLATE #	
	R22B	48
ONPG	+	+
ADH	+	+
LDC	-	-
ODC	+	+
CIT	+	+
H_2S	-	-
URE	-	-
TDA	-	-

BIOCHEMICAL TEST	ISOLATE #	
	R22B	48
IND	-	-
VP	+	+
GEL	-	-
GLU	+	+
MAN	+	+
INO	-	-
SOR	+	+
RHA	+	+
SAC	+	+
MEL	+	+
AMY	+	+
ARA	+	+
OXI	-	-
KOH	-	-
GRAM	-	-
MORPHOLOGY	ROD	ROD
EMB COLONY/COLOR	SMALL COLONY RED	LARGE COLONY BLUE CENTER RED BORDER

Table 3.3 results were obtained with the API 20E System (Analytab Products, Plainview, NY). The abbreviations used in the above table stand for: ONPG=orthonitrophenol and indicates the presence of beta-galactosidase, ADH=arginine dihydrolase and transform the arginine into ornithine, LDC=lysine decarboxylase causes the transformation of lysine into an amine, ODC=indicate the presence of ornithine decarboxylase releasing amine from ornithine, CIT=citrate only carbon source, H_2S=hydrogen sulfide produced from thiosulfate, URE=indicate the presence of urease by the release of ammonia, TDA=tryptophane deaminase resulting in the formation indolepyruvic acid, IND=indole formation from the metabolism of tryptophane, VP=acetoin formed from sodium pyruvate, GEL=gelatin and confirms the presence of proteolytic enzymes. GLU=glucose, MAN=mannose, INO=inositol, SOR=sorbitol, RHA=rhamnose, SAC=sucrose, MEL=melibiose, AMY=amygdalin and ARA=(L+)arabinose which indicates the utilization of different carbon sources, OXI= oxidase, the presence of NO_2 and N_2 gases indicate the reduction of nitrate and GRAM=gram positive or negative based on the symbol used. The symbols used, + or -, throughout the table mean positive and negative, respectively.

Table 3.4 contains the isolates identified by API rapid NFT and MIS systems. Isolates L31A, L21A1, L21G, L51A and L21A2 were isolated from the surface waters of Douglas Reservoir while isolate L54C was isolated at 0.1 meters from the sediments (Díaz, unpublished work). They were identified by the API rapid NFT system as follows; L54C and L21G as *Pseudomonas spp.*, L31A, L21A1, and L21A2 as *Flavobacterium spp.* and L51A as *Pasteurella haemolytica*. However, the MIS system provided different results. L51A as *Pseudomonas vesicularis.*, L21A1 and L21A2 as *Bacillus spp.*, and the other isolates, L31A, L54C, and L21G, as *Kurthia spp.*, *Arthrobacter spp.* and *Aureobacterium spp.*, respectively.

Table 3.4. Bacterial isolates recovered from different sites within the reservoir.

BIOCHEMICAL TEST	ISOLATE #					
	L31A	L21A1	L54C	L21G	L51A	L21A2
NO_3	-	-	+	-	+	-
TRP	-	-	-	-	-	-
GLU	-	-	-	-	-	-

BIOCHEMICAL TEST	ISOLATE #					
	L31A	L21A1	L54C	L21G	L51A	L21A2
ADH	-	-	-	-	-	-
URE	-	-	-	-	-	-
ESC	+	+	-	+	+	+
GEL	+	+	-	-	-	+
PNPG	+	+	+	+	+	+
GLU	+	+	+	+	-	-
ARA	-	-	-	-	-	-
MNE	+	+	+	-	-	+
MAN	+	+	+	-	-	+
NAG	-	-	+	-	-	-
MAL	+	+	+	-	-	+
GNT	-	-	+	-	-	-
CAP	-	-	-	-	-	-
ADI	-	-	+	-	-	-
MLT	+	+	+	-	-	+
CIT	+	+	+	-	-	+
PAC	-	-	+	-	-	-
OXI	-	-	-	-	-	-
KOH	-	-	-	-	+	-
GRAM	-	-	-	-	-	-
MORPHOLOGY	ROD	COCCI	COCCI	ROD	ROD	ROD

Table 3.4 results were obtained with the API Rapid NFT System (Analytab Products, Plainview, NY). The above table represents two different type of tests. The biochemical tests consists of the following parts and its abbreviations stand for: NO_3 reduction which is confirmed by the production of NO_2 gas, TRP=tryptophanase which form indole by the metabolism of tryptophane, GLU=glucose fermentation, ADH=arginine dihydrolase and transform the arginine into omithine, ammonia and CO_2, URE=indicate the presence of urease by the release of ammonia, ESC=esculin and it confirms the presence of beta-glucosidase, GEL=gelatin and confirms the presence of proteolytic enzymes and PNPG=p-nitro-phenyl-beta-galactopyranoside which is hydrolysed by beta-galactosidase.

The assimilation tests which are: GLU=D-glucose, ARA=(L+)arabinose, MNE=D-mannose, MAN=D-mannitol, NAG=N-acetyl-D-glucosamine, MAL=maltose, GNT=D-gluconate, CAP=caprate, ADI=adipate, MLT=L-malate, CIT=citrate and PAC=phenyl-acetate which indicates the assimilation of different carbon sources resulting in growth. Also two other tests were performed, OXI= oxidase and GRAM=gram positive or negative based on the symbol used. The symbols used, + or -, throughout the table mean positive and negative results, respectively.

Table 3.5 provides the biochemical results obtained with the isolates recovered from the metal surfaces exposed to three different environments. Isolates 54B and 4 were the only isolates identified by the API Rapid NFT System. They were identified as *Ps. maltophilia*. However, the MIS system identified them as follows: 54B and 4 as *Ps. maltophilia*, 54A, 29C and 29A as *Brevibacterium spp.*, 21B as *Bacillus spp.* Recent work of Lipski, et al. (1992) showed that physiological test system alone are of limited values for identification of isolates from biofilters. Brander et al. (1992) found similar results while working with clinical isolates resembling *Mycobacterium spp.* The use of total lipid for identification of bacteria under controlled environment such as temperature and media has been the foundation of the MIS system.

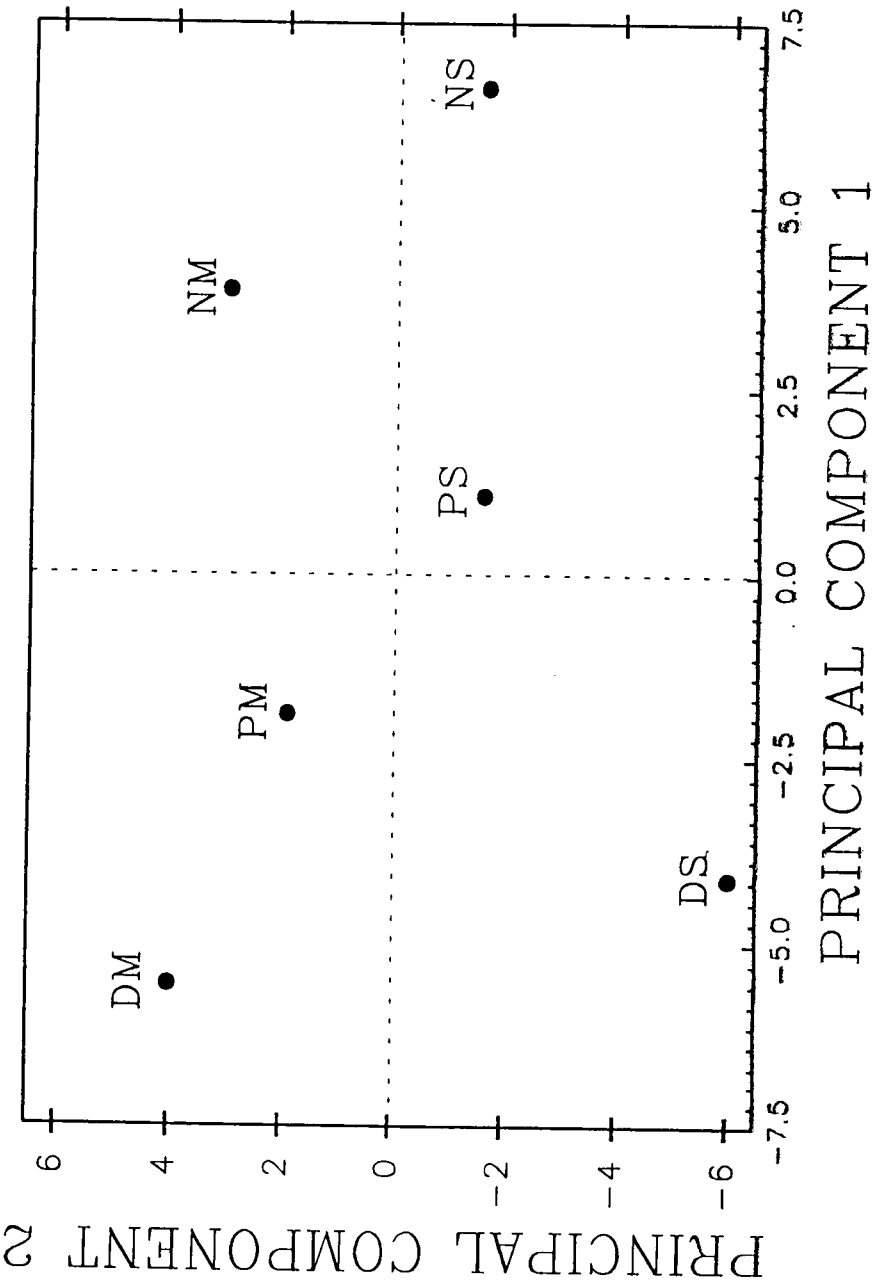

Figure 3.5. Principal component one versus principal component two. The abbreviations used in this figure mean as follow; the first letter stands for the site while the second letter stands for the type of metal surface. D=Douglas Reservoir, P=Pigeon River, N=Nolichucky River, M=Mild Steel and S=Stainless Steel.

Table 3.5. Bacterial isolates recovered from different metal surfaces and sites.

BIOCHEMICAL TEST	ISOLATE #					
	4	29C	29A	54B	54A	21B
Sampled Site	DR	NR	NR	PR	PR	NR
Metal Surface	SS	SS	SS	SS	SS	MS
NO_3	-	-	-	+	-	-
TRP	-	-	-	-	-	-
GLU	-	-	-	-	-	-
ADH	-	-	-	-	-	-
URE	-	-	-	-	-	+
ESC	+	+	+	+	-	-
GEL	+	+	+	+	+	+
PNPG	+	+	+	+	+	-
GLU	+	+/-	+	-	+/-	-
ARA	-	+/-	+	-	-	-
MNE	+	+/-	+	+	+/-	-
MAN	-	+	+	-	+/-	-
NAG	+	+	+	+	-	+
MAL	+	+	+	+	-	-
GNT	-	+	+	-	-	+
CAP	-	-	-	-	-	-
ADI	-	+/-	-	-	-	-
MLT	+	+	-	+	-	+
CIT	+	+	-	+	-	-
PAC	-	-	-	-	-	-
OXI	-	-	-	-	-	+
KOH	+	-	-	+	-	-
GRAM	-	-	-	-	-	-
MORPHOLOGY	ROD	COCCI	COCCI	ROD	COCCI	ROD

Legend similar to table 3.4. The sampled site abbreviations stand for PR=Pigeon River, DR=Douglas Reservoir and NR=Nolichucky River. The metal surface abbreviations stand for MS=mild steel and SS=stainless steel.

The MIS and biochemical identification test allowed the identification of the isolates recovered from the different sites. However, only 14 percent of the MIS results agreed with the identification

provided by the biochemical results. Eighty three percent of the isolates from table 3.5 gave positive results for beta-galactosidase, did not ferment glucose and were gram-negative bacteria. Nevertheless, the test, which is designed for non-fermentative enteric gram-negative bacteria, did not identify 67 percent of the isolates. These results indicate that the gram stain provided wrong results and should have been verified by an alternate test such as KOH before the API tests were performed. Based on the KOH results 11 of the 14 isolates showed gram positive results, indicating that identification of the 11 isolates should have been performed with the gram positive API test. However, the MIS identification for 9 of the 11 isolates (Tables 3.4 and 3.5), which were identified as gram positive, did agree.

Table 3.3 results did not provided enough information to differentiate the isolates. Based on the different colony morphologies and colors, the EMB media confirmed that the two isolates are different and reject the API identification. Based on the results listed on table 3.3, I can conclude that the isolates belong to the *Enterobacteriaceae* family. Further testings will be required for the differentiation to the species level. Of the 14 isolates, only 3 were identified as gram negative by gram stain and KOH and 2 of them were correctly identified by both methods. These results emphasize that MIS provides reliable information and serves to confirm the actual biochemical identification results. This work agree with a recent statement made by Stahl et al. (1988), indicating that the current classification scheme does not fit the characteristics of environmental isolates, resulting in unreliable and misleading identification.

4. DISCUSSION AND CONCLUSION

BIOMASS

The phospholipid ester-linked fatty acids (PELFA) results suggested that no significant differences in periphyton biomass were found between the two distinct bottom metal surfaces exposed to well-oxygenated waters. Evaluation of the samples collected from the two different top metal surface sides by 16s rRNA oligonucleotide eubacterial probe (OEP) and the acridine orange direct counts (AODC) resulted in similar microbial biomass results for all three studied environments. The use of 16s rRNA OEP directed toward the eubacterial kingdom relied on conserved regions present throughout the kingdom (Stahl et al., 1988).

The following conversion factors, 10^{12} cells of the size of *Pseudomonas spp.* gram^{-1} of dry weight and the presence of 50 µmol lipid phosphate gram^{-1} per dry weight (White, 1979b), allowed the conversion of the biomass results obtained by the PELFA technique, resulting in values within a range of 10^7 to 10^8 bacteria cm^{-2} of the size of *Pseudomonas spp.* for the exposed surfaces regardless of the sites. However, these numbers can deviate from the actual values because the above conversion factors assumed that all the recovered bacterial cells had the same size and disregard the eukaryote contribution to the total biomass results. However, the AODC results indicated that the bottom side surface regardless of the metal type had 1.5 fold lower biomass than the upper side surface. Lock et al. (1984) reported similar findings while studying exposed surfaces in northern boreal streams.

The 16s rRNA OEP and AODC results did not agree with the PELFA results. The total eubacterial biomass estimated by the 16s rRNA OEP method was six order of magnitude lower than the AODC values depending on the type of metal surface. This difference could have resulted because the presence of quiescent organisms. Lock et al. (1984) results indicated that bacteria colonize opposite side surfaces in similar proportion. If similar proportion of eubacteria were colonizing the bottom surface, then the estimated 10^8 bacteria cm^{-2} of the size of *Pseudomonas spp.* could have consisted of at least 10^2 eukaryotes such as diatoms, fungi and protozoa. The detection of eukaryotes biomarkers such as *16:1w13t, 16:3w4, 18:1w9, 18:2w6* (green algae) *14:0, 18:3w6, 20:5w3* and *22:4w6* (diatoms and microeukaryotes) (Nickels

et al., 1981; Gillan et al., 1984; Vestal et al., 1989) on the bottom side surfaces support the above statements. Also, diatoms have been previously recovered from sites lacking direct sunlight such as heat exchanger tube (Characklis and Cooksey, 1983).

While the three biomass indicators resulted in different biomass concentration the differences in their results can be explained in terms of the different metabolic status of the microbial population which each tool evaluates. The biomass values estimated by the PELFA and 16s rRNA OEP methods, which measure viable biomass (White, 1983; Stahl et al., 1988), suggest that the AODC values included eubacterial and eukaryotic cells and non active cells. The obtained AODC results were in close agreement with the biomass results obtained by Edwards (1987) and Guckert et al. (1991), who found 10^{10} bacteria cm^{-2} in two southeastern blackwater rivers and a third order stream, respectively. However, the AODC results are in disagreement with the biomass results obtained by Geesey et al. (1978), Lock et al. (1984), Stock and Ward (1989) and Haack and McFeters (1982), who measured within a 10^6 to 10^9 cells cm^{-2} in unpolluted mountain streams, boreal rivers, a small stream and the outlet stream of an alpine lake, respectively.

The above results suggest that; 1) bacterial numbers were equally distributed regardless of the sampled sides and sites and 2) total biomass was not influenced by the type of metal surfaces and sites.

SULFATE REDUCING BACTERIA

The use of different tools to evaluate the presence of different sulfate reducing bacteria (SRB) on two different metal surfaces exposed to three different aquatic environments resulted in distinct patterns. The detection of biomarkers for *Desulfobacter spp.* (Dowling et al., 1986; Parker, 1987) and *Desulfovibrio spp.* (Smith et al., 1986b; Parker, 1987; Dowling et al., 1988), which are bacteria capable of oxidizing lactate (Postgate, 1984), and the results obtained by the 16s rRNA oligonucleotide Sulfate Reducing Bacteria probe (OSRBP), which measured the presence of lactate oxidizers in the presence of sulfate, *Desulfotomaculum spp.* and *Desulfovibrio desulfuricans*, (Postgate, 1984) indicate that the MS surfaces exposed to the inflows had significantly higher concentration of SRB than the SS surfaces.

Evaluation of the collected samples, consisting of corrosion products and biofilm, from the top side metal surfaces by the most probable number (MPN) method showed no significant differences for SRB

lactate utilizers between the different surfaces exposed to the three different aquatic systems, indicating similar amounts of lactate utilizers. The 16s rRNA OSRBP results, regardless of the site, indicated that the collected samples from the top side of SS surfaces contained SRB below the established detection limit, 3.2 X 10^3. However, the samples collected from the top side of the MS surfaces contained between 10^4 to 10^5 SRB. The 16s rRNA OSRBP detected significantly higher SRB on the top side of the MS surfaces exposed to the reservoir than on the SS surfaces, emphasizing that SRB tend to colonize those surface experiencing uniform corrosion. The concentration of SRB based on the MPN and 16s rRNA OSRBP results were similar for the MS surfaces exposed to the inflows, but the former resulted in higher SRB values, 10^4 to 10^5, for the SS surfaces exposed to the inflows than the 16s rRNA OSRBP results. Also, the MPN values indicated approximately 1.6 fold lower SRB values for the surfaces exposed to the reservoir than the values determined by the 16s rRNA OSRBP method. The lower amount of SRB on the surfaces exposed to the anoxic conditions in contrast with the ones exposed to the oxygenated conditions emphasizes that SRB can flourish in oxygenated conditions as previously found by Little et al. (1989). These results indicate that the lactate utilizer population of SRB can vary within distinct surfaces exposed to the same aquatic environment. The differences measured in SRB numbers by the above techniques suggest the presence of different SRB communities growing on different surfaces, requiring different carbon sources. The MPN values obtained in this study were 1 to 2 order higher than the results obtained by Hamilton and Sanders (1984) in marine environments. The MPN method has the following disadvantages: 1) low precision (Battersby, 1988), 2) requires longer incubation time and 3) contains only one carbon source. The latter disadvantage favors selection against those SRB which require other carbon sources as in the case of *Desulfotomaculum acetoxidans* (Postgate, 1984) and *Desulfobacter spp.* (Dowling et al. 1986; Cord-Ruwisch et al., 1987; Devereux et al., 1989) which require acetate. *Desulfovibrio spp.*, which can also use formate and acetate as carbon sources, (Devereux et al., 1989, 1990) were probably measured by the MPN and 16s rRNA OSRBP methods, indicating that both methods could have measured the same SRB population. While the MPN method failed to identify those SRB requiring other carbon sources as explained above, the 16s rRNA OSRBP failed to identify other SRB species such as *Desulfobacter spp.*, providing reasonable explanations

for the observed differences in values between both methods.

Recent results of Devereux et al. (1990) indicated that 16s rRNA sequences of *D. desulfuricans* ATCC 27779 and *Desulfomonas pigra* had 95% sequence similarities while *Desulfovibrio vulgaris* had 91% sequence similarity to the two previous organisms. In previous work, Devereux et al. (1989) also indicated that *Desulfovibrio gigas* belonged to the same line within the 16s rRNA phylogenetic tree in which the above three bacteria are contained. Their results suggest that the target site of the 16s rRNA OSRBP used in this study could have detected other *Desulfovibrio spp.* besides the manufacturer identified species.

The use of these three techniques indicates that different metabolic SRB organisms are present in nature, and future studies must take into consideration such factor. These results also suggest: 1) SRB tend to colonize different metal surfaces but preferred those surfaces experiencing uniform corrosion where the release CP accumulate on the exposed surface, developing an stratified biofilm with lower redox and higher anoxic conditions (Hamilton, 1985) at the biofilm/metal interface, 2) studies of SRB alone might not provide sufficient information to elucidate the mechanism of microbial corrosion and to explain the corrosion rates seen in the field and 3) molecular and chemical techniques must be employed in future studies to determine the microbial structure community and diversity.

POLYUNSATURATED FATTY ACIDS

The presence of significantly higher polyunsaturated fatty acids (PUFA) (i.e., *18:2w6, 18:3w3,* etc.) on the SS than on the MS surfaces regardless of the studied sites suggests that eukaryotic microflora and microfauna (White, 1983; Guckert et al., 1985) tend to colonize the exposed SS surfaces. Similar behavior was observed for PUFA *20:5w3* regardless of the site. These results also suggest that surface colonization could have occurred on both metal surfaces in equal rates but predation by nematodes and bacterial grazers (i.e., protozoa) could have increased the rate of lipid synthesis (Bobbie et al., 1978) on those surfaces where the predators were present in higher concentration. However, the evaluation of the lipid synthesis by the incorporation of ^{14}C-1-acetate into the total lipid did not show significant differences within the different surfaces, regardless of the site.

The presence of arachidonic acid *(20:4w6)*, which is associated with bacterial grazers and

nematodes (Nickels et al., 1981) and the following PUFAs, *(20:5w3)* and *(22:6w3)*, associated with eukaryotic algae, (Lechevallier and Lechevalier, 1988), were found to be in significantly higher concentration on the SS than in the MS surfaces for all the studied sites with the exception of the NR where both type of surfaces exhibited no significant differences for *20:4w6* and *22:6w3*. Also, *22:6w3*, was not detected on the surfaces exposed to the reservoir and on the MS surfaces exposed to the PR.

The significantly higher concentration of copper measured in the water column of the PR (see Part IV, Result section) than in the other two aquatic studied sites suggest that bacterial grazers such as protozoa and algae grazers such as snail can be directly affected (U.S. EPA, 1986). Removal of protozoa can result in slower decomposition of organic detritus as indicated by the significantly higher concentration of total and suspended organic carbon in the PR water column, reduced turnover of inorganic nutrients, interruption of the food chain link, accumulation of aged bacterial cells (Sherr and Sherr, 1984) and smaller fish diversities as previously detected by Schacher (1988) in studies conducted in PR. The absence of snails from the microbial communities recovered from the attached metal surfaces exposed to the PR (Díaz, on site observation) and the decrease of arachidonic acid on the surfaces exposed to the PR confirm the interruption of the food chain link and the possible damages caused by the concentration of copper in the PR. The signigifcantly higher concentration of PUFA observed on the SS than on the MS can be explained in terms of the possible toxic effects of the corrosion products released by the MS surfaces. However, further studies under controlled situations are required to explain why this *in situ* behavior was observed.

ISO & ANTEISO BRANCHED SATURATE & MONOENOIC PHOSPHOLIPID FATTY ACIDS

The presence of significantly higher concentration of monoenoic and iso and anteiso branched saturate PELFA on the MS, in contrast with the SS surfaces at all three sites emphasizes the substrata effect on the type of microbial community that developed in nature. The presence of corrosion products observed only on the MS surfaces and the decreased of protozoa as previously indicated could have reduced the concentration of eukaryotic biomass and increased the bacterial biomass, respectively. The decreased in zooplankton, which predate on the bacterial community, can result in an increased bacterial biomass as detected on all the surfaces exposed to the PR. However, high concentration of zooplankton can increase

the microbial biomass through the release of nutrients from their feces (Amy and Hiatt, 1989) as indicated by the 16s rRNA OEP and PELFA results measured from the MS collected surface samples exposed to the NR.

BACTERIAL LIPID BIOSYNTHESIS AND TRAN/CIS RATIO

The results of these two biological parameters for the surfaces exposed to the three different sites resulted in no significant differences.

COMMUNITY STRUCTURE

The application of three different techniques to assess the total biomass collected from two different metal surfaces exposed to three different aquatic communities described satisfactorily the complex communities that developed on the metal surfaces (Berk et al., 1981). The recovery of odd numbers and iso and anteiso branched-chain fatty acids, which are mainly found in gram positive bacteria with the exception of *Pseudomonas maltophilia, Legionella* and *Bacteroides* (Tornabene, 1985), in contrast with the even number of straight-chain and cyclopropyl fatty acids from the metal surfaces, which are mainly present in gram-negative bacteria (Mackie et al., 1991; Rezanka, 1992; Zelles et al., 1992). The recovery of these PELFA in conjunction with the recovery of PUFA, which are not produced by bacteria except for filamentous cyanobacteria strains (Tornabene, 1985) and by an isolate from the family of *Beggiatoaceae*, genus *thiotrix*, recently detected by Temara et al. (1991), indicate the formation of complex microbial communities on the exposed metal surfaces. The recovery of PELFAs *18:1w7c* and *16:1w7c*, which are end products of the bacteria anaerobic desaturase pathway (Nickels et. al 1981; White et al., 1991), *Cy19:0(w7,8)*, known as lactobacillic acid (Mackie et al., 1991), and iso and anteiso saturated 15, 16 and 17 which are typical biomarkers of anaerobic bacteria (Mikell et al., 1987; Mackie et al., 1992; Rajendran et al., 1992) reaffirm the complex structures that developed on the surfaces exposed to oxygenated and low-oxygenated environments.

Differences in microbial communities have been reported by Nickels et al. (1981) in seawater studies containing different O_2 concentrations while using natural and artificial surfaces. Bobbie et al. (1978) reported similar results while exposing natural and artificial surfaces to estuarine water. This trend

has also been reported by White et al. (1991), who found different microbial communities at different depths within the sedimentary column. The differences in microbial communities, which have been reported here for the different sites, could have been caused by surface abrasion (Nickels et al., 1981) such as changes in water velocity. However, the differences between surfaces exposed to the same site could have been caused because changes in growth conditions (Mikell et al., 1987) within the biofilm induced by the decrease in nutrient and energy, favoring K-selected species (Odum, 1985) and differences in the surface energy of the substrata such hydrophobic or hydrophilic surfaces (Mittleman, 1991). K-selected species are those species that utilize most of their scarce resources toward their growth and dedicate low portion of it toward offspring production (Odum, 1989),

The principal component results confirmed the separation of the three different aquatic sites based on the influenced exerted by the significant concentration of PELFAs *16:1w13t, 21:0* and *18:2w6*, which were found in higher concentrations on those surfaces exposed to the NR than on those exposed to the PR. The former PELFA, which has been associated with green algae (Guckert et al., 1991), was not detected on the surfaces exposed at the reservoir. Recovery of PELFA longer than 19 carbon such as *21:0* and PUFA, *18:2w6* and *22:5w3*, indicates the presence of microeukaryotes because bacteria fatty acids are mainly characterized by one double bond or chain length no longer than 19 carbon (Harwood and Russell, 1984), emphasizing that the Nolichucky microbial community consisted mainly of microeukaryotes. The detection of PUFAs, *20:2w3, 22:2w6* and *22:5w3*, only on those surfaces exposed to the NR confirmed the presence of microeukaryotes. However, the PR microbial communities, as described by the results of PC, were mainly differentiate by the presence of iso and anteiso *17:1* which were also found on the MS surfaces of DR. The detection of *10Me16:0* from only those surfaces exposed to the PR in conjunction of the iso and anteiso *17:1* confirm that the SRB played a major role in the differentiation of the PR microbial community from the other two sites.

The microbial community developed on the MS surfaces exposed to DR differentiated from the SS surfaces exposed to the sme site by the presence of the monoenoics *15:1w6c, 17:1w6c, 18:1w7t, 15:0* and *a15:0*, which were only detected on the former surface. The recovery of monoenoic with unsaturation

at w7 indicates the presence of bacterial anaerobic desaturase pathway (Mikell et al., 1987), suggesting that the MS community consisted mainly of bacteria. However, based on PC 2 both type of surfaces differentiate from each other by the presence of *16:1w7c* and *20:4w6*. Both geometric configurations for the former PELFA were found to be in significant concentrations on the MS than on the SS surfaces regardless of the site. However, PUFA *20:5w3* and *20:4w6* showed opposite behavior with the exception of the latter PUFA which was found on both surfaces exposed to the NR. These results indicate that the microbial community structure is influenced by the type of metal surface.

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PART IV

MICROBIAL CORROSION

1. INTRODUCTION

On-line non destructive monitoring techniques such as scanning vibrating electrode technique (SVET) (Franklin et al., 1991a), electrochemical impedance spectroscopy (EIS) (Franklin et al.,1988; Dowling et al.,1988a,b), attenuated total reflection infrared spectroscopy (ATR/FT-IR) (Bremer and Geesey, 1991; Nivens et al., 1992) and open circuit potential (OCP) (Mittelman et al., 1992) have been widely employed in laboratory settings monitoring viable bacteria in microbiological influenced corrosion (MIC) investigations. Electrochemical methods such as direct current polarization methods (i.e., Tafel polarization (Booth and Tiller, 1960), potentiodynamic sweep techniques (Dexter and Gao, 1988), polarization resistance (Obuekwe et al., 1981)), electrical resistance probes (Cooper, 1986), split-cell measurements (Little and Wagner, 1986), alternating current (Franklin et al.,1988,1991; Dowling et al.,1988a,b) and electrochemical noise (Dawson et al., 1983) have been applied with the sole intent to comprehend the corrosion mechanism that single bacterium and complex microbial consortium can effect on exposed surfaces.

Polarization resistance provides general corrosion information but does not distinguish between uniform or localized corrosion (Walsh et al., 1992). This technique is suitable for uniformly corroding surfaces (Little et al., 1989). It can detect and monitor changes in corrosion rates but does not provide sufficient information to explain them (Dexter et al., 1991). Nevertheless, this fast technique can provide statistically precise and reproducible information for different metal surfaces (Mansfeld, 1976; Walter, 1977). This chapter contains the polarization resistance (P_R) values measured while exposing 1010 mild steel (MS) surfaces to two different electrolytes collected from previous study inflows addressed in Part III, Materials and Methods section. The objectives of this study were to: 1) compare the corrosion qualities of the electrolytes while controlling some of the *in situ* parameters such as temperature, water flow, etc. and 2) compare the corrosion rates of similar metallurgical materials exposed to *in situ* conditions.

2. MATERIALS AND METHODS

SITE DESCRIPTION

The sites for which the corrosion rates were obtained in nature were previously described in Part III, Materials and Methods. Water collected from similar sites, with the exception of the reservoir, were evaluated in the laboratory using the standard test method for corrosiveness of water in the absence of heat transfer (ASTM D 2776-79^{E1}) and standard practice for conducting potentiodynamic polarization resistance measurements (ASTM G 59-78) (ASTM, 1989).

ENVIRONMENTAL PARAMETERS

The temperature, pH, redox, conductivity and dissolved oxygen of the water column for the *in situ* measurements have already been described in Part III, Materials and Methods. The pH and temperature of the water collected at the inflows on February 23, 1992 are shown in table 2.1. The pH and temperature of the collected water was measured in triplicate with a Porta/pH 3 meter (American Scientific Product, Atlanta, GA) and immersion thermometer, respectively.

Table 2.1. Average (AVG) values for the pH and temperature parameters with its respective standard deviations (STD) of the water collected at the inflows on February 23,1992.

Physical Parameters	Nolichucky River AVG	STD	Pigeon River AVG	STD
Temperature (°C)	14.5	0.1	12.8	0.1
pH (unit)	8.05	0.15	7.66	0.2

SAMPLE SIZE

The sample sizes for the *in situ* measurements were previously described in Part III, Materials and Methods. The sample size for the polarization resistance measurements consisted of triplicate measurements using a MS coupon with a 16 mm diameter (Metal Samples Inc. Munford, AL).

METAL COMPOSITION

The metal samples for *in situ* testing were previously described in Part III, Materials and Methods. The MS sample composition used for the electrochemical testing of the corrosiveness of the *in situ* water chemistry are contained in Table 2.4. of Materials and Methods section of Part III.

PREPARATION OF THE SURFACES AND ELECTROCHEMICAL TEST SYSTEM

The cleaning procedures for the sample exposed to *in situ* conditions have been described in Part III, Materials and Methods. The MS samples used for the polarization resistance and potentiodynamic tests were prepared as described in standard practice for preparing, cleaning, and evaluating corrosion tests specimens (ASTM G 1-88) (ASTM, 1989). The MS surface was polished with a uniform 600 grid paper and embedded in epoxide resin mix with epoxide hardener (Bueltzer Company, Lake Bluff, IL) (5:1 w/w), exposing only one side to the tested electrolyte. The exposed side of the working electrode was rinsed three times with 18 mohm-cm water, followed by degreasing with acetone. Upon cleaning the working electrode surface, the sample was suspended in 500 ml of the collected water for one hour to allow acclimation. The electrochemical cell contained a MS working electrode, a titanium counter electrode, a saturated calomel reference electrode connected via a lugging probe containing 3% NaCl. The equipment used for performing the electrochemical measurements consisted of the Zplot software (Scribner Associate Inc.), a Solartron 1255 HF frequency response analyzer and a potentiostat/galvanostat model 273 (EG&G Princeton Applied Research).

CORROSION PRODUCT CLEANING PROCEDURE

The procedure to clean the corrosion products from the metal samples exposed to *in situ* conditions has been previously described in Part III, Materials and Methods. The samples exposed to the laboratory tested electrolyte were reused and cleaned as described above before each measurement.

WEIGHT LOSS MEASUREMENTS

Each metal sample was weighed in triplicate prior to exposure at *in situ* conditions and after 24 hours of drying period in the desiccator at room temperature. The weight loss measurements were made with a Metler balance AE 100 to 4 significant decimal figures. The P_R values for the sample tested in the laboratory were not converted to the actual weight loss values because the actual tafel slopes for each electrolytes were not measured.

LOCALIZED CORROSION MEASUREMENTS

A total of 10 fields for each 304 stainless steel (SS) surface were examined using a 100X

magnification for a total area of .01 cm^2 using an Olympus BH-2 microscope. The pitting evaluation consisted of counting the pits present in 10 randomly selected areas. The average pit density for each exposed surface was added, resulting in an N=9 for all the three sites with the exception of the Pigeon River where two samples were lost. The average pitting count for the non-exposed surface was 22.67 pits, with a standard deviation of 4.73 which could have been produced by the chemical treatment, cold scratches and/or by the manufacturer. No testing of the SS coupon was performed by neither of the electrochemical applications previously described.

SAMPLE COLLECTION

The collection of the exposed samples has been described in Part III, Materials and Methods. The water collected for laboratory testing consisted of 4 L of the inflow waters collected in sterilized brown bottles to avoid photoreaction. The bottles were kept in ice during transportation to the laboratory and at room temperature in the laboratory. The collected water was evaluated within 12 days after collection.

CHEMICAL ANALYSIS OF THE WATER COLUMN

Evaluation of the water composition consisted of filtering 125 ml of water collected at the same depth at which the metal samples were suspended. The water was filtered through a 0.45 μm filter (Millipore Corporation, Bedford, MA) and transferred to a 125 ml brown bottles provided by Tennessee Valley Authority (TVA). Also, 125 ml of unfiltered water was directly collected into a 125 ml brown bottle and analyzed for unfiltered content. The water samples were kept on ice and transported to the TVA laboratories located in Chattanooga where the testing was performed. TVA performed the analysis of the water column samples for total organic carbon (TOC) and suspended organic carbon (SOC) as described in the Environmental Protection Agency (EPA) method 415.1. The chloride and sulfate content was performed as described in EPA method 325.2. The metals were evaluated as described by the EPA method MTB (Mr. R. Stansbury., per phone conversation). The chemical content of the water used for the polarization resistance measurements was not evaluated.

BACTERIAL COUNTS

Acridine orange direct counts (AODC) of the water tested with the above electrochemical

techniques were performed by serial dilutions as described previously in Part III, Materials and Methods section. The average results and their standard deviation are shown in table 2.2.

Table 2.2. Number of cells/ml measured in the collected waters from the previous studied inflows by the AODC method.

INFLOWS	AODC (x 10^6)	
	AVG	STD
PIGEON	3.8	1.5
NOLICHUCKY	1.3	0.96

STATISTICAL ANALYSIS

The polarization resistance measurements taken with the previous electrochemical applications and the corrosion rate measurements were evaluated with the student T-test procedure provided by the SAS with an α=0.05. Statistical analyses for the chemistry of the water column was performed by comparing the three measured values collected for each site, using an ANOVA procedure with an α=0.05.

3. RESULTS

UNIFORM CORROSION DETERMINED BY THE WEIGHT LOSS METHOD

The corrosion rate values measured on the MS surfaces exposed at the Pigeon River (PR) (n=11, one metal sample lost) were significantly higher than the MS surfaces exposed at the Nolichucky River (NR) (n=12) ($p<0.006$) (Figure 3.1). Similar comparison of the SS surfaces within the two inflows resulted in significant higher corrosion rate for the samples exposed to the PR than the ones exposed to the NR ($p<0.02$) (Figure 3.1). However, the weight loss method *per se* does not provide an acceptable and reliable evaluation of the damage caused by localized corrosion (Ringas and Robinson, 1988). Nevertheless, this method provides critical data in terms of the metallurgical behavior when exposed to the *in situ* microbial community.

LOCALIZED CORROSION DETERMINED BY PIT DENSITY

Statistical evaluation of the SS surfaces by enumerating the pits formed on the SS surfaces provides a reliable evaluation when the data is compared to unexposed surfaces (control). The pit density determined on the control SS surfaces resulted in no significant differences when compared to the PR, NR and DR, resulting in $p<0.18$, $p<0.49$ and $p<0.66$, respectively (Appendix F.1). However, direct observation of the pit formed on the SS surfaces suggest that an additional evaluation of the pit must be performed, such as pit depth, before any further conclusion is reached.

Appendix F.2. and F.3 show the pit density observed on the surfaces exposed to the three aquatic sites. The presence of pits on the SS surface exposed to the aquatic environments (see Appendix F.2.B. and F.3.A-B) when compare to the control surface (Appendix F.2.A.) demonstrates that the pit initiation occurred randomly throughout the exposed surfaces.

LOCALIZED CORROSION DETERMINED BY POLARIZATION RESISTANCE

Evaluation of MS surface in the presence of water collected from the PR and NR by the standard test method for corrosiveness of water in the absence of heat transfer and standard practice for conducting potentiodynamic polarization resistance measurements (ASTM, 1989) resulted in no significant differences as indicated by the probabilities of $p<0.15$ and $p<0.15$ (Appendix G.1 and G.2), respectively.

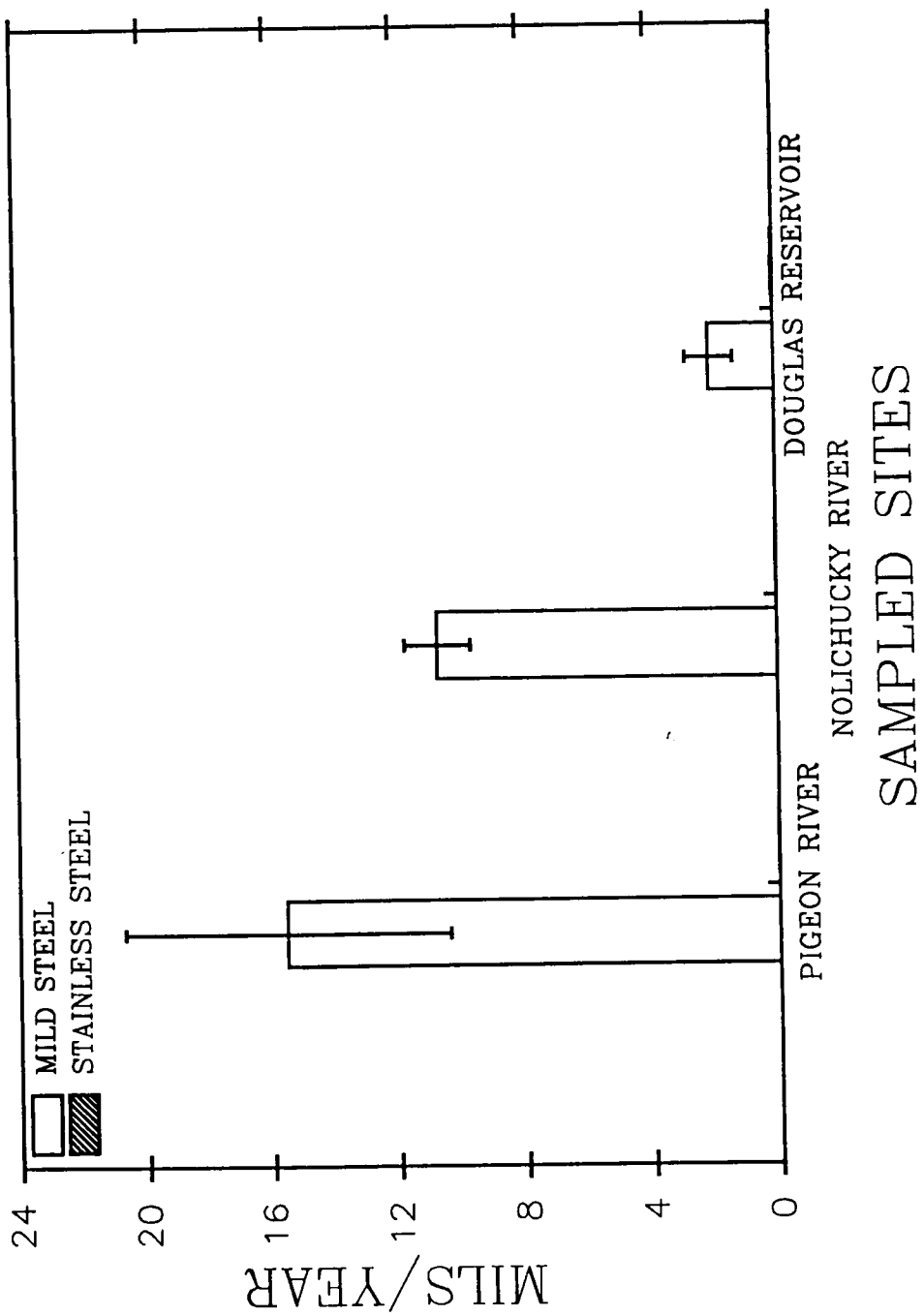

Figure 3.1. Corrosion rate results for MS surfaces exposed at three sites.

CHEMISTRY OF THE WATER COLUMN

The differences in water composition measured in the water column of the sites previously mentioned are shown in Table 3.1. The average (AVG) value measured for each parameter and its standard deviation (STD) are included for an n=3 subsites. The column labeled x contains the statistical results determined by ANOVA with an α=0.05.

TABLE 3.1. Chemistry of the water column where the metal samples were suspended.

ELEMENTS	DOUGLAS RESERVOIR		NOLICHUCKY RIVER		PIGEON RIVER		X
	AVG	STD	AVG	STD	AVG	STD	
Aluminum^T	210	45.8	283.3	89.6	160	52	@
Iron^T	1766.7	461.9	850	511	1053	273	@
Manganese^T	273.3	134.3	10	8.7	8.7	6.4	D
Calcium^T	13.3	0.6	30.7	11	17.7	8.6	N
Magnesium^T	2.9	0.2	7.2	2.6	3.37	0.1	N
Zinc^T	< 10	0	< 10	0	< 10	0	@
Silicon^T	4400	360.6	4167	152.8	4467	58	@
Copper^T	< 10	0	< 10	0	< 10	0	@
Iron*	56.7	20.8	23.3	15.3	110	10	P
Manganese*	266.7	145.7	7	2	10.3	1.2	D
Copper*	11.7	2.9	106.7	5.8	126.7	21	A
SOC	2.4	0	1.6	0.1	4.8	0.1	P
TOC	2.47	0.12	1.7	0.1	4.8	0.1	P
SO_4^{2-} in Water	12	2	44.7	10.8	20	0	N
Chloride in Water	11.33	1.15	6.7	1.2	32	0	A
CaMg Hardness	45.33	2.31	106.3	38.6	58	1.7	N

The symbols in column X stands for; @=No significant differences at a $p<0.05$, D=Douglas Reservoir value significant higher than the two inflows at a $p<0.05$, N=Nolichucky River value significant higher than the two other sites at a $p<0.05$, P=Pigeon River value significant higher than the other 2 sites at a $p<0.05$ and A=all sites were significantly different from each other at a $p<0.05$. The unit for all the measured elements is µg/L except for Ca, Mg, SOC, TOC, Chloride and SO_4^{2-} which are expressed as mg/L. CaMg is expressed as µg/L $CaCO_3$. The following abbreviations in column labeled elements stand for; SOC=suspended organic carbon, TOC=total organic carbon, T=total in water and *=total dissolved.

4. DISCUSSION AND CONCLUSION

The corrosion rates measured for uniform and localized corrosion experienced by the MS and SS surfaces, respectively, indicated that the MS surfaces exposed to the waters from the PR, mile 5.5, experienced significantly higher corrosion rates than the MS surfaces exposed to the waters from the NR, mile 10.3. The significantly higher concentration of total dissolved iron, chloride ions and total dissolved copper in the water column of the PR than in the other two sites, suggests a complex interaction between the microbial biofilm and these elements, enhancing the corrosion rates observed *in situ* for the MS and SS surfaces exposed to the PR.

The significantly higher concentrations of total dissolved iron, which were measured in the water column of the PR than in the other two sites, have been reported to interfere with the formation of a stronger protective film (e.g., siderite), forming a poorly protective film such as mackinawite (Cristófaro et al., 1986; McNeil and Little, 1990) and causing higher corrosion rates (Iverson, 1987; Weimer et al. 1988). The above factor can increase the corrosion rates as measured in the MS surfaces exposed to the PR, mile 5.5.

As indicated by Reedy (1973), the presence of high dissolved O_2 in soft water and Cl^- and SO_4^{2-} ions could block the formation of calcium carbonate protective film on steels causing an increase in the corrosion rates. Similarly, calcium, magnesium and iron can influence the stability of the biofilm (Lappin-Scott and Costerton, 1989). While these ions were not measured in the collected biofilm and CP, their measured concentrations in the water column provides a reasonable explanation to the corrosion rates measured *in situ*. The significantly higher concentration of total dissolved iron and significantly lower concentration of calcium and magnesium measured in the PR water column, mile 5.5, than in the NR water column, mile 10.3, and the significantly lower concentration of Cl^- ions measured in the NR water column than in the PR water column indicate that the MS surfaces exposed to NR could have developed a protective film while the opposite might have occurred to the surfaces exposed to the Pigeon River. The presence of a protective film would have caused lower corrosion rates on the exposed metal surfaces as measured on the MS surfaces exposed to the NR. The corrosion rates measured for MS surfaces exposed to the PR

agreed with the results obtained by Reedy (1973) while exposing similar type of MS surfaces at two treatment water plants containing different chloride and sulfate concentrations.

Evaluation of the polarization resistance results suggested that the water chemistry of both inflows by itself was not the major contributor to the corrosion rates observed *in situ*. The results obtained within laboratory setting by the above methods cannot be extrapolated to the *in situ* results. This test did not permit the formation of biofilm, which can perform different functions (i.e., shielding, patchiness exposing), resulting in the development of biofilm/bacterial/electrolyte/surface interactions. The tested surface was not exposed for more than two hours to the electrolyte and did not experience changes in water velocity, temperature, and conductivity. These changes, which the surfaces faced *in situ*, could have increased the corrosion rates of the exposed surfaces. Nevertheless, this test confirmed that the electrolyte composition was not the main cause for the corrosion rates measured in nature. The high corrosion rates measured for the MS under oxic conditions, which were greater than 5 mpy (Reedy, 1973), suggest that this type of material must be avoided in any application where the presence of O_2 will be evident and changes in temperature and water velocity are expected.

As indicated in the result section, the stainless steel surfaces exposed to the aquatic sites did not experience significant increase in pitting density when compared to the control surfaces. However, at no time the depth of the pit was evaluated suggesting that no conclusive results can be extrapolated for the behavior of this type of metallurgical material until further investigation is performed under controlled situations.

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APPENDIXES

APPENDIX A

Sample Name		P7M1	P7M2	P7M3	P8M1	P8M2	P8M3	P9M1	P9M2	P9M3
Total mole%		100	100	100	100	100	100	100	100	100
Total Picomoles		119406	32062	95484	38449	122601	231422	93085	123511	40399
Total pmoles/cm2(3)		3701.60	993.92	2960.02	1191.93	3800.65	7174.08	2885.65	3828.85	1252.37
PLFA (1)	ECL (2)	P7M1	P7M2	P7M3	P8M1	P8M2	P8M3	P9M1	P9M2	P9M3
i14:0	13620	0	0	0	0	0	0.45	0.23	0.22	0
14:0	14000	1.98	0	1.72	0	0.80	2.72	2.44	1.92	0
i15:0	14623	1.26	0.90	1.01	0.81	1.95	2.70	1.28	1.15	0
a15:0	14704	0.42	0	0.31	0	0.96	1.44	0.47	0.40	0
15:0	15000	0.53	0	0.45	0	0.43	0.59	0.53	0.43	0
16:3w4	15582	0.67	1.57	0.43	0	0.26	0.50	0.79	0.59	0
i16:0	15640	0	0	0	0	0.73	1.11	0.63	0.62	0
16:1w9c	15697	0.75	0	0.66	0	0.99	1.45	0.66	0.68	0
16:1w7c	15748	41.03	35.20	41.41	29.93	33.25	25.77	26.36	24.68	24.55
16:1w7t	15795	0.74	0	0.85	1.10	1.00	0.71	0.37	0.38	0.43
16:1w5c	15848	1.10	1.14	1.11	1.28	2.33	3.85	1.40	1.24	0.77
16:1w13t	15922	0	0	0	0	0.30	0.52	0.61	0.52	0
16:0	16000	25.75	24.67	24.89	25.47	25.53	22.37	20.40	20.36	20.81
i17:1	16347	0	0	0	0	0.46	0.43	0	0	0
10Me16:0	16438	0	0	0	0	0.63	0.76	0	0	0
a17:1w6c	16470	0	0	0	0	0	0.18	0	0	0
i17:0	16634	0	0	0	0	0.45	0.47	0.31	0.28	0
a17:0	16718	0	0	0	0	0.52	0.72	0.42	0.43	0
17:1w6c	16800	0	0	0	0	0	0.18	0.16	0	0
Cy17:0	16823	3.89	3.30	4.59	5.17	5.34	2.90	0.77	1.48	3.06
17:0	17000	0.46	0	0.45	0.72	0.41	0.55	0.69	0.58	0.83
18:3w6	17422	0	0	0	0	0.25	0.26	0.35	0.32	0
18:4w3	17470	0.87	1.48	0.61	0	0.85	0.89	1.05	1.06	0.86
18:2w6	17603	1.49	2.69	1.05	3.28	1.14	2.64	4.38	3.40	3.93
18:3w3	17646	1.30	3.38	1.08	1.21	0.99	1.56	5.51	7.47	2.97
18:1w9c	17693	1.96	3.22	1.50	5.67	2.35	3.93	7.16	8.30	8.31
18:1w7c	17753	10.31	11.96	10.78	17.53	12.34	9.63	8.18	8.63	15.55
18:1w7t	17805	0	0	0	0	0.27	0.20	0	0	0
18:1w5c	17865	0	0	0	0	0.40	0.74	0	0.23	0
19:0	18000	1.81	3.00	1.98	3.99	1.29	2.06	4.37	4.34	0.85
br19:1?	18044	0	0	0	0	0.45	0.40	0.24	0.24	0
10Me18:0	18401	0	0	0	0	0	0.16	0	0	0
Cy19:0(w7,8)	18831	0	0	0	0.84	0.82	0.86	0.18	0.19	0
20:4w6	19178	0.78	1.52	0.92	1.48	0.94	1.69	1.60	1.51	2.00
20:5w3	19221	2.91	5.96	3.28	1.43	1.49	2.96	8.45	8.32	5.55
20:4w3/20:3w6	19430	0	0	0	0	0	0	0	0	0
20:3w3/i20:0	19665	0	0	0.93	0	0	0.47	0	0	0
20:0	20000	0	0	0	0	0	0.22	0	0	0
21:0	21000	0	0	0	0	0	0.22	0	0	0
22:4w6	21166	0	0	0	0	0	0.73	0	0	3.53

A.1. Phospholipid ester-linked fatty acids (PELFA) recovered from the MS surfaces exposed to the PR, n=9.

Sample Name		P7S1	P7S2	P8S1	P8S2	P8S3	P9S1	P9S2	P9S3
Total mole%		100	100	100	100	100	100	100	100
Total Picomoles		78076	184709	177784	50801	7862	118400	20885	37231
Total pmoles/cm2(3)		2420.37	5725.99	5511.30	1574.84	243.74	3670.41	647.43	1154.17
PLFA (1)	ECL (2)	P7S1	P7S2	P8S1	P8S2	P8S3	P9S1	P9S2	P9S3
i14:0	13620	0.28	0.24	0	0	0	0.20	0	0
14:0	14000	6.03	7.32	0	1.00	0	2.42	3.44	0.78
i15:0	14623	1.28	0.90	0	1.28	0	1.01	1.16	0.59
a15:0	14704	0.41	0.31	0	0.48	0	0.37	0	0
15:0	15000	0.30	0.33	0	0.41	0	0.31	0	0.22
16:3w4	15582	1.39	1.36	0	0.88	0	0.97	2.27	0.97
i16:0	15640	0.65	0.63	0	0.83	0	0.55	0.91	0.66
16:1w9c	15697	0.95	0.75	0	1.18	0	0.61	0.83	0.62
16:1w7c	15748	27.51	25.56	29.10	14.71	6.41	12.88	18.20	11.28
16:1w7t	15795	0.24	0.16	0	0	0	0.10	0	0
16:1w5c	15848	1.79	1.06	0	2.92	0	1.25	1.28	1.18
16:1w13t	15922	1.52	1.80	0	0.49	0	0.71	0.57	0.53
16:0	16000	28.57	28.38	35.89	19.07	14.74	16.81	21.59	15.87
i17:1	16347	0	0.08	0	0	0	0.14	0	0
10Me16:0	16438	0	0	0	0	0	0.16	0	0
a17:1w8c	16470	0	0	0	0	0	0.08	0	0
i17:0	16634	0.21	0.14	0	0.64	0	0.38	0	0.39
a17:0	16718	0.27	0.25	0	0.74	0	0.55	0	0.46
17:1w6c	16800	0	0	0	0	0	0.08	0	0
Cy17:0	16823	0.50	0.17	0	0.53	0	0.21	0	0.38
17:0	17000	0.27	0.21	0	0.84	0	0.78	0.69	1.00
18:3w6	17422	0.44	0.59	0	0.50	0	0.37	0	0.45
18:4w3	17470	1.42	1.27	0	1.38	0	0.99	1.58	1.44
18:2w6	17603	2.42	2.67	0	4.83	6.88	4.70	3.95	5.20
18:3w3	17646	3.77	4.30	0	6.39	9.36	9.78	5.38	9.28
18:1w9c	17693	1.65	1.71	0	13.01	21.45	10.86	6.21	12.55
18:1w7c	17753	4.58	3.19	25.58	7.26	11.24	4.71	8.85	8.19
18:1w7t	17805	0	0	0	0	0	0.04	0	0
18:1w5c	17865	1.13	1.20	0	0.53	0	0.82	0	0.41
18:0	18000	1.42	1.27	9.42	5.35	10.63	6.15	5.83	7.81
br19:1?	18044	0.29	0.17	0	0.32	0	0.18	0	0.20
10Me18:0	18401	0	0	0	0	0	0	0	0
cy19:0(w7,8)	18831	0.56	0.26	0	0.54	0	0.19	0	0.50
20:4w6	19178	1.54	1.92	0	2.80	3.96	2.35	3.38	2.90
20:5w3	19221	8.62	10.55	0	11.07	15.34	16.35	11.42	15.92
20:4w3/20:3w6	19430	0	0.28	0	0	0	0.30	0	0.23
20:3w3/i20:0	19665	0	0.25	0	0	0	0.45	2.47	0
20:0	20000	0	0.10	0	0	0	0.28	0	0
21:0	21000	0	0.28	0	0	0	0.34	0	0
22:6w3	21123	0	0	0	0	0	0.09	0	0
22:4w6	21186	0	0.10	0	0	0	0.34	0	0
24:0	24000	0	0.24	0	0	0	0.17	0	0

A.2. PELFA recovered from the SS surfaces exposed to the PR, n=8 because the loss of one coupon.

A.3. PELFA recovered from the MS surfaces exposed to the Noliehucky River (NR), n=9.

Sample Name		N4M1	N4M2	N4M3	N5M1	N5M2	N5M3	N6M1	N6M2	N6M3
Total mole%		100	100	100	100	100	100	100	100	100
Total Picomoles		113756	258369	189955	152737	253557	181941	128220	126032	285777
Total pmoles/cm2(3)		2971.29	6748.57	4961.60	3989.46	6622.88	4752.28	3349.10	3291.94	7464.46
PLFA (1)	ECL (2)	N4M1	N4M2	N4M3	N5M1	N5M2	N5M3	N6M1	N6M2	N6M3
i14:0	13622	0.46	0.26	0.25	0.26	0.20	0	0.76	0	0.16
14:0	14000	7.52	11.17	7.15	6.81	7.40	3.13	3.03	1.99	6.45
i15:0	14626	2.25	0.99	0.85	1.18	0.90	0.73	4.16	1.54	0.89
a15:0	14705	1.12	0.37	0.32	0.42	0.29	0.28	3.66	0.90	0.33
15:0	15000	0.47	0.34	0.35	0.34	0.32	0.28	0.93	0.43	0.31
16:3w4	15581	1.15	0.71	0.96	0.79	0.76	0.67	0.40	0.80	1.43
i16:0	15631	0.71	0.37	0.47	0.53	0.46	0.42	1.49	0.77	0.67
16:1w9c	15697	0.92	0.65	0.68	0.83	0.66	0.69	1.48	0.79	0.57
16:1w7c	15748	26.64	15.72	20.07	22.97	20.11	18.63	22.05	25.28	24.38
16:1w7t	15791	0.52	0.12	0.11	0.36	0.26	0.27	0.93	0.63	0.23
16:1w5c	15847	1.65	1.08	0.78	2.11	1.59	1.73	3.63	1.81	0.71
16:1w13t	15917	1.25	1.93	1.40	0.92	0.92	0.92	0.65	1.47	2.44
16:0	16000	23.80	21.44	22.63	22.98	20.62	19.88	21.05	25.21	27.74
i17:0	16634	0.41	0.29	0.31	0.35	0.31	0.40	0.93	0.45	0.14
a17:0	16720	0.38	0.34	0.43	0.46	0.42	0.47	1.37	0.60	0.23
17:1w6c	16800	0	0	0	0	0.08	0	0.60	0.19	0
Cy17:0	16825	0.97	0.19	0.31	0.85	0.67	0.65	1.84	0.98	0.34
17:0	17000	0.41	0.42	0.62	0.56	0.60	0.69	0.65	0.44	0.18
18:3w6	17422	0.55	0.67	0.70	0.58	0.66	0.72	0.40	0.68	0.98
18:4w3	17465	0.82	0.75	1.05	1.10	0.94	1.21	0.89	1.10	1.19
18:2w6	17605	3.15	7.41	5.12	5.35	6.22	6.93	2.43	2.64	3.02
18:3w3	17648	1.87	2.32	4.89	4.78	5.05	5.23	1.40	3.08	5.51
18:1	17696	3.11	7.58	6.54	4.95	5.76	6.27	4.37	3.82	2.56
18:1w7c	17755	6.02	5.53	5.30	6.48	5.65	7.33	9.63	9.01	3.83
18:1w7t	17803	0	0	0	0	0	0	0.32	0	0
18:1w5c	17867	0.71	0.49	0.52	0.41	0.25	0.35	1.37	1.09	0.89
18:0	18000	2.36	2.82	4.19	3.93	4.97	5.31	1.83	2.06	0.93
br18:1?	18044	0.25	0.16	0.15	0.23	0.16	0.21	0.72	0.39	0.12
19:1w12c	18669	0	0	0	0	0	0	0	0	0
a19:0	18711	0	0	0	0	0	0	0	0	0
cy19:0(w7,8)	18829	0.39	0.13	0.16	0.32	0.24	0.32	1.40	0.72	0.17
20:4w6	19180	3.45	5.42	3.81	2.68	3.47	4.32	2.12	3.83	4.25
20:5w3	19222	8.27	8.40	9.70	5.95	8.21	8.80	2.71	6.26	8.72
20:4w3/20:3w6	19434	0	0.14	0	0	0.19	0	0	0	0.20
20:2w3	19611	0	0.17	0	0	0	0	0	0	0
20:3w3/i20:0	19668	0.42	0	0	0.27	0.43	0.86	0	0	0
20:1w7c	19764	0	0	0	0	0	0	0	0	0
20:0	20000	0	0.14	0.20	0.23	0.36	0.36	0	0	0
21:0	21000	0	0.75	0	0	0.52	0.55	0.44	0	0
22:6w3	21048	0	0.59	0	0	0.35	0.46	0.37	0	0.28
22:4w6	21173	0	0	0	0	0	0	0	1.04	0.18
22:5w3	21212	0	0.16	0	0	0	0.92	0	0	0

Sample Name		N4S1	N4S2	N4S3	N5S1	N5S2	N5S3	N6S1	N6S2	N6S3
Total mole%		100	100	100	100	100	100	100	100	100
Total Picomoles		26506	31194	124168	272041	225557	257959	95747	75287	138686
Total pmoles/cm2(3)		692.33	814.77	3243.26	7105.67	5891.52	6737.85	2500.90	1966.48	3622.47
PLFA (1)	ECL (2)	N4S1	N4S2	N4S3	N5S1	N5S2	N5S3	N6S1	N6S2	N6S3
i14:0	13622	0	0	0.21	0.13	0.34	0.30	0.16	0	0.08
14:0	14000	0	0	5.29	4.55	7.62	8.42	6.04	1.61	4.50
i15:0	14626	0	0	0.65	0.75	1.09	0.89	0.73	0.49	0.81
a15:0	14705	0	0	0.28	0.27	0.39	0.33	0.36	0.21	0.25
15:0	15000	0	0	0.28	0.28	0.33	0.32	0.29	0.17	0.26
16:3w4	15581	0.84	0.77	0.74	0.62	0.80	0.61	1.44	1.23	0.82
i16:0	15631	0	0	0.32	0.46	0.45	0.51	0.50	0.47	0.74
16:1w9c	15697	0	0.48	0.56	0.56	0.76	0.76	0.39	0.44	0.67
16:1w7c	15748	17.38	13.05	13.24	15.82	17.04	18.05	25.53	19.64	21.15
16:1w7t	15791	0	0	0	0.17	0.18	0.17	0.32	0.36	0.19
16:1w5c	15847	0.64	0.73	0.77	1.22	1.44	1.09	1.26	1.50	0.70
16:1w13t	15917	0.64	0.85	0.81	0.84	1.21	0.94	2.44	1.80	2.06
16:0	16000	24.25	20.55	18.47	20.77	20.55	23.45	26.76	25.66	33.31
i17:0	16634	0	0.39	0.34	0.33	0.34	0.28	0.14	0.20	0.14
a17:0	16720	0	0	0.37	0.43	0.50	0.42	0.17	0.25	0.23
17:1w6c	16800	0	0	0	0.06	0.09	0.05	0	0	0
Cy17:0	16825	0	0	0.39	0.17	0.21	0.15	0.28	0.42	0.19
17:0	17000	0.82	0.77	0.69	0.63	0.60	0.59	0.20	0.30	0.20
18:3w6	17422	0.62	0.73	0.47	0.59	0.62	0.46	0.58	0.70	0.80
18:4w3	17465	1.47	1.29	0.75	0.82	0.87	0.70	1.03	1.18	1.03
18:2w6	17605	5.82	8.88	10.24	7.46	6.79	7.12	2.63	4.33	4.91
18:3w3	17648	4.73	4.42	4.44	6.29	5.92	6.06	2.53	4.36	9.02
18:1	17696	4.61	7.85	10.00	7.90	5.77	6.28	2.22	3.85	2.19
18:1w7c	17755	6.92	9.49	3.86	4.75	5.02	3.77	3.08	5.40	4.85
18:1w7t	17803	0	0	0	0	0.04	0	0	0	0
18:1w5c	17867	1.54	0.82	0.88	0.24	0.56	0.31	1.42	1.08	1.60
18:0	18000	4.52	6.40	5.49	5.68	4.82	4.50	1.24	2.70	0.95
br19:1?	18044	0	0	0.14	0.13	0.14	0.12	0	0.20	0.15
19:1w12c	18669	0	0	0.08	0.08	0.07	0.06	0	0	0
a19:0	18711	0	0	0	0.04	0	0	0	0	0
Cy19:0(w7,8)	18829	0	0	0.05	0.20	0.18	0.16	0.22	0.45	0.26
20:4w6	19180	5.63	6.74	5.51	4.47	3.66	3.10	3.51	5.05	1.98
20:5w3	19222	19.56	14.32	11.94	9.99	8.23	8.12	12.53	11.37	5.01
20:4w3/20:3w6	19434	0	0	0.08	0.10	0.11	0.16	0.36	0.29	0.15
20:2w3	19611	0	0	0.12	0.12	0.10	0.06	0	0	0
20:3w3/20:0	19668	0	0.93	0.46	0.64	0.62	0.30	0.33	0.73	0
20:1w7c	19764	0	0	0	0.05	0.06	0	0	0	0
20:0	20000	0	0	0.29	0.36	0.32	0.30	0	0	0
21:0	21000	0	0.54	0.56	0.65	0.64	0.36	0	0.16	0
22:6w3	21046	0	0	0.37	0.22	0.48	0.30	0.20	0.21	0.15
22:4w6	21173	0	0	0	1.03	0	0.12	0.56	2.73	0.33
22:5w3	21212	0	0	0.15	0	0.91	0.15	0	0	0
22:2w6	21322	0	0	0.72	0	0.04	0.07	0	0	0
22:0	22000	0	0	0	0.05	0	0	0	0	0
24:0	24000	0	0	0	0.09	0.10	0.09	0.45	0.47	0.33

A.4. PELFA recovered from the SS surfaces exposed to the NR, n=9.

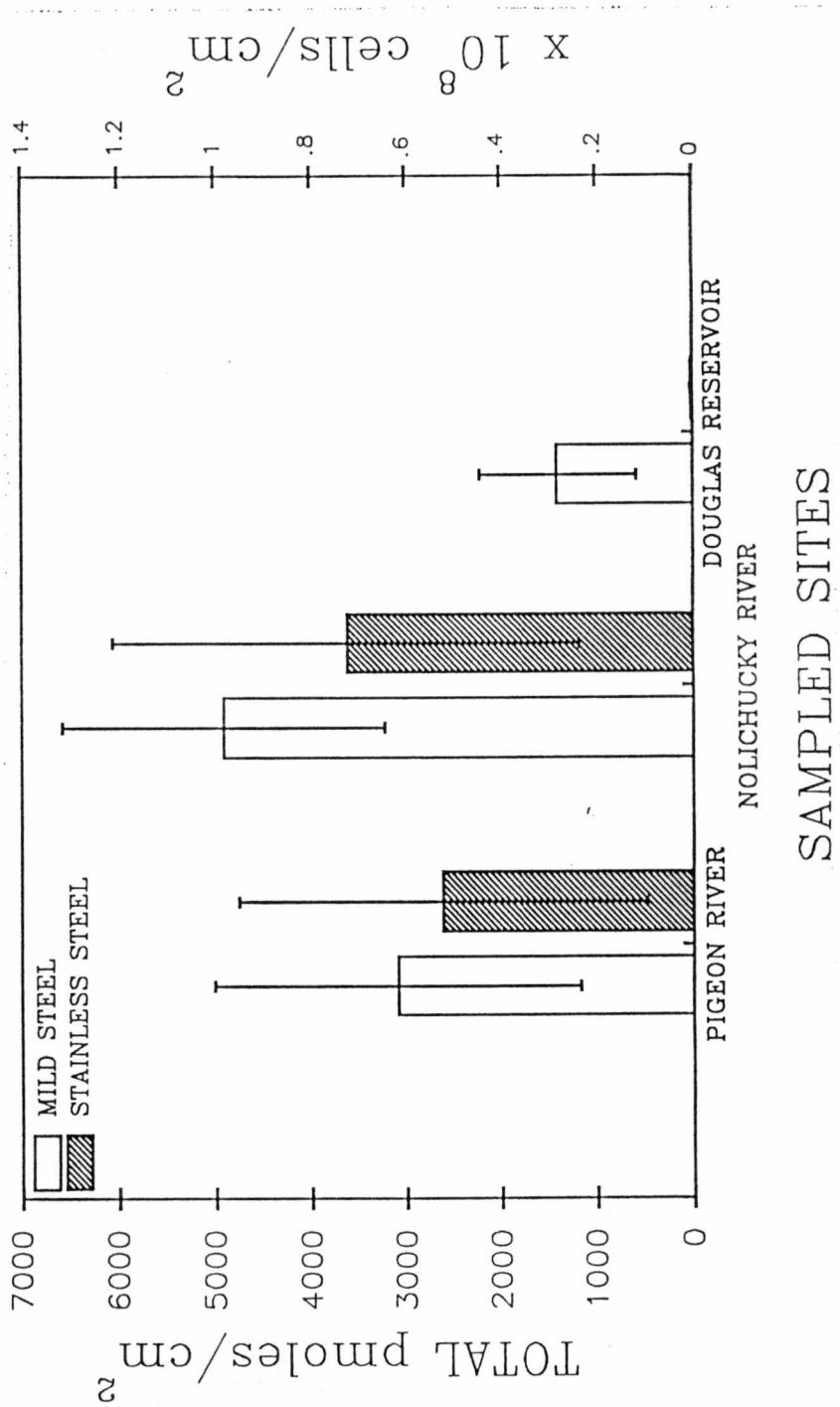
Sample Name		D1M1	D1M2	D1M3	D2M1	D2M2	D2M3	D3M1	D3M2	D3M3
Total mole%		100	100	100	100	100	100	100	100	100
Total pmoles/cm2(3)		1287.98	1385.09	1114.93	365.37	225.79	1273.58	2623.15	2364.29	2008.98
PLFA (1)	ECL (2)	D1M1	D1M2	D1M3	D2M1	D2M2	D2M3	D3M1	D3M2	D3M3
i14:0	13621	0.15	0.09	0	0	0	0.13	0.09	0.05	0
14:0	14000	1.33	0.75	0.51	0.96	1.20	1.29	1.00	0.71	0.49
i15:0	14624	1.37	1.09	0.77	1.80	1.59	1.28	1.39	1.34	0.70
a15:0	14703	0.85	0.63	0.54	0.95	0.80	0.81	0.63	0.52	0.29
15:1w6c	14792	0.20	0.11	0.10	0	0	0.20	0.06	0.06	0
15:0	15000	0.45	0.45	0.31	0.38	0	0.43	0.30	0.28	0.19
i16:0	15625	0.20	0.17	0.20	0	0	0.24	0.13	0.12	0.10
16:1w9c	15698	0.74	0.58	0.65	0.42	0	0.69	0.19	0.22	0.18
16:1w7c	15759	51.40	50.62	47.72	54.00	54.24	50.29	56.88	53.85	54.37
16:1w7t	15792	1.15	1.28	1.04	1.83	2.14	1.11	1.09	1.16	0.87
16:1w5c	15841	2.35	2.13	2.62	1.71	1.54	2.34	1.49	1.64	1.76
16:0	16000	24.46	24.27	25.56	26.19	28.61	24.57	24.28	24.04	26.04
i17:1	16343	0.30	0.22	0.31	0	0	0.28	0.16	0.18	0.16
a17:1/10Me16:0	16437	0.31	0.26	0.33	0	0	0.31	0.13	0.13	0.12
i17:0	16631	0.23	0.20	0.27	0	0	0.24	0.11	0.15	0.13
a17:0	16717	0.37	0.29	0.33	0	0	0.36	0.16	0.18	0.14
17:1w6c	16797	0.57	0.64	0.64	0	0	0.58	0.20	0.20	0.39
Cy17:0	16823	0.46	0.46	0.52	0	0	0.45	0.75	0.53	0.43
17:0	17000	0.37	0.42	0.41	0	0	0.41	0.17	0.27	0.22
18:3w6	17465	0.22	0.19	0.28	0	0	0.25	0	0.11	0
18:4w3	17540	0.14	0	0.16	0	0	0	0	0	0
18:2w6	17604	0.49	0.34	0.49	0	0	0.78	0	0.33	0.10
18:3w3	17646	0.43	0.35	0.50	0	0	0.70	0	0.32	0
18:1w9c	17694	0.59	0.54	0.79	0.79	0	0.68	0.11	0.78	0.15
18:1	17696	0.29	0.25	0.43	0	0	0	0	0	0
18:1w7c	17755	8.61	11.64	11.93	10.04	8.38	8.56	9.91	10.82	12.03
18:1w7t	17803	0.13	0.17	0.17	0	0	0.09	0.08	0.12	0.10
18:1w5c	17860	0.31	0.53	0.56	0	0	0.33	0.14	0.20	0.24
18:0	18000	0.57	0.58	0.84	0.92	1.49	0.86	0.34	0.65	0.58
br19:1?	18049	0.12	0.14	0.16	0	0	0.12	0.07	0.19	0
Cy19:0	18826	0.15	0.17	0.20	0	0	0.16	0	0	0
20:4w6	19177	0.20	0.20	0.31	0	0	0.46	0.06	0.42	0.09
20:5w3	19220	0.25	0.23	0.36	0	0	0.89	0.10	0.43	0.13
20:0	20000	0.22	0	0	0	0	0.11	0	0	0

A.5. PELFA recovered from the MS surfaces exposed to the DR, n=9.

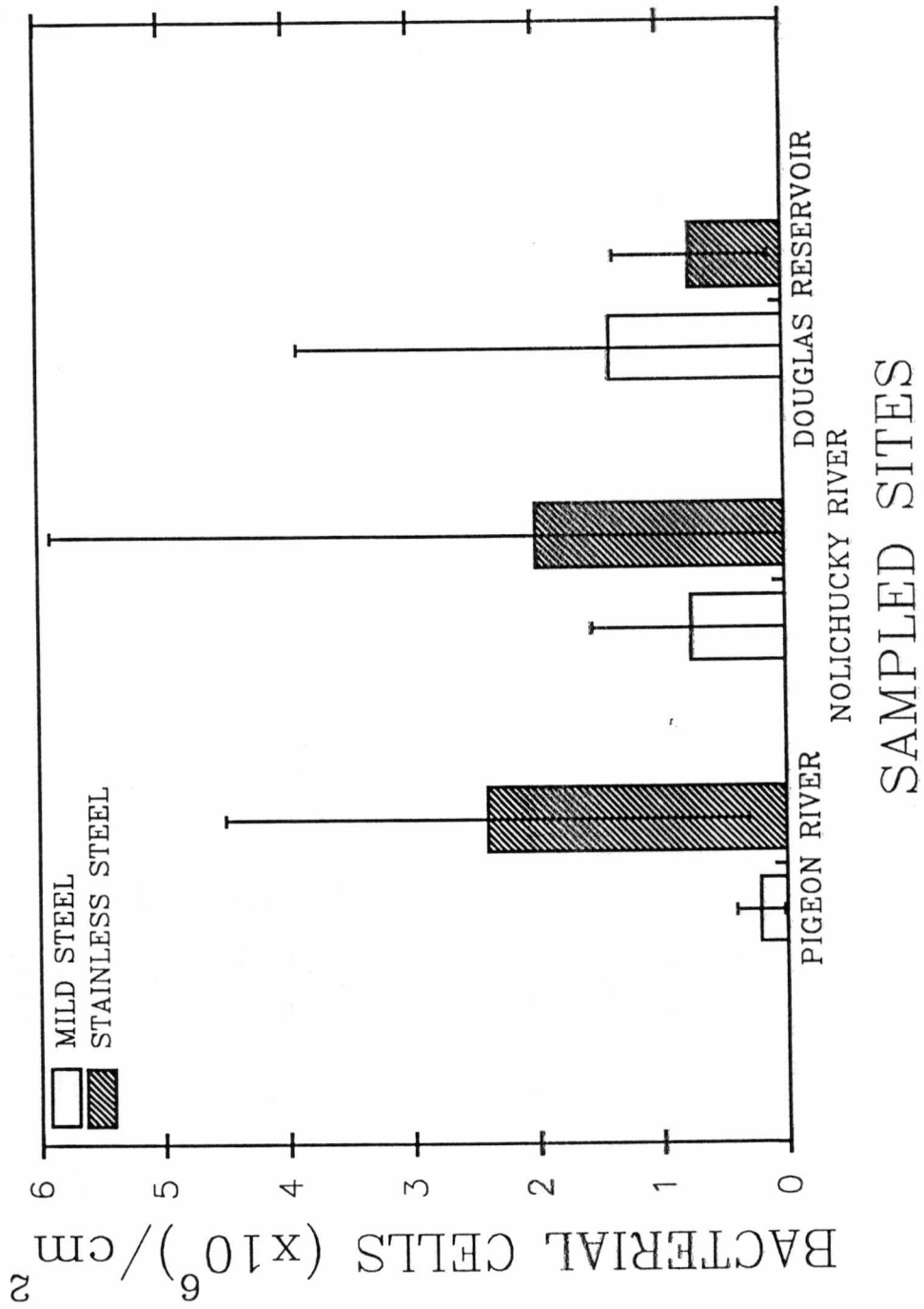
Sample Name	D1S1	D1S2	D1S3	D2S1	D2S2	D2S3
Total mole%	100	100	100	100	100	100
Total pmoles/cm2(3)	4.09	11.53	7.05	4.35	33.92	4.73
PLFA (1)	ECL (2)	D1S1	D1S2	D1S3	D2S1	D2S2	D2S3
i14:0	13621	0	0	0	0	0	0
14:0	14000	0	0	0	0	1.36	0
i15:0	14624	0	0	0	0	1.61	0
a15:0	14703	0	0	0	0	0	0
15:1w6c	14792	0	0	0	0	0	0
15:0	15000	0	0	0	0	0	0
i16:0	15625	0	0	0	0	0	0
16:1w9c	15698	0	0	0	0	4.35	0
16:1w7c	15759	0	8.68	11.69	0	21.98	7.69
16:1w7t	15792	0	0	0	0	1.90	0
16:1w5c	15841	0	0	0	0	8.75	0
16:0	16000	19.80	30.32	34.15	12.91	17.53	11.99
i17:1	16343	0	0	0	0	0	0
a17:1/10Me16:0	16437	0	0	0	0	0	0
i17:0	16631	0	0	0	0	0	0
a17:0	16717	0	0	0	0	0	0
17:1w6c	16797	0	0	0	0	0	0
Cy17:0	16823	0	6.49	0	0	2.23	0
17:0	17000	0	0	0	0	1.08	0
18:3w6	17465	0	0	0	0	2.41	0
18:2w6	17604	0	5.92	0	8.95	2.68	0
18:3w3	17646	0	3.22	0	0	2.03	0
18:1w9c	17694	12.98	9.88	5.34	13.70	3.41	10.96
18:1	17696	0	0	0	0	1.65	0
18:1w7c	17755	26.93	10.45	10.32	25.26	9.00	17.02
18:1w7t	17803	0	0	0	0	0	0
18:1w5c	17860	0	0	0	0	0	0
18:0	18000	20.72	15.02	13.18	21.97	4.62	16.61
br19:1?	18049	0	0	0	0	0	0
Cy19:0	18826	0	0	0	0	0	0
20:4w6	19177	9.26	5.18	6.24	8.02	5.58	16.88
20:5w3	19220	10.30	4.85	7.98	9.19	7.84	18.85
20:0	20000	0	0	11.10	0	0	0

A.6. PELFA recovered from the SS surfaces exposed to the DR, n=6.

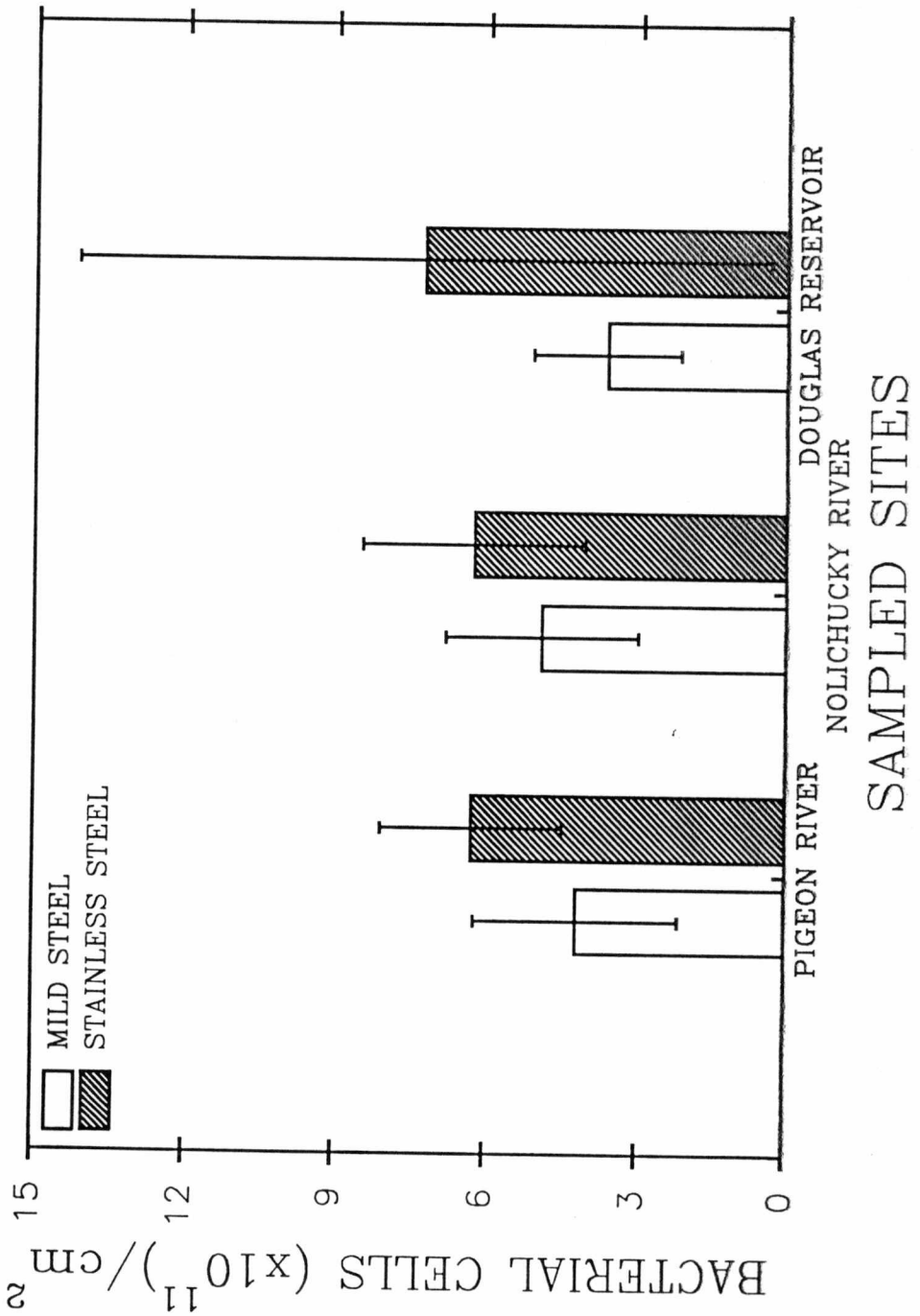
APPENDIX B



Appendix B.1. Total biomass measured by the recovery of phospholipid ester-linked fatty acids from two different surfaces exposed at three aquatic sites. N=9 except for the stainless steel (SS) values from the Pigeon River (PR) and Douglas Reservoir (DR). The N value for the former is 8 and for the latter is 6 because the loss of one coupon and 3 values below the detection limit, respectively. The vertical line indicates the sample standard deviation.



Appendix B.2. Total bacteria counts estimated by the 16s rRNA oligonucleotide eubacteria probe collected from two different surfaces exposed at three aquatic sites. N=9 except for the SS values from PR which n=8 because the loss of one coupon. The vertical line indicates the sample standard deviation.

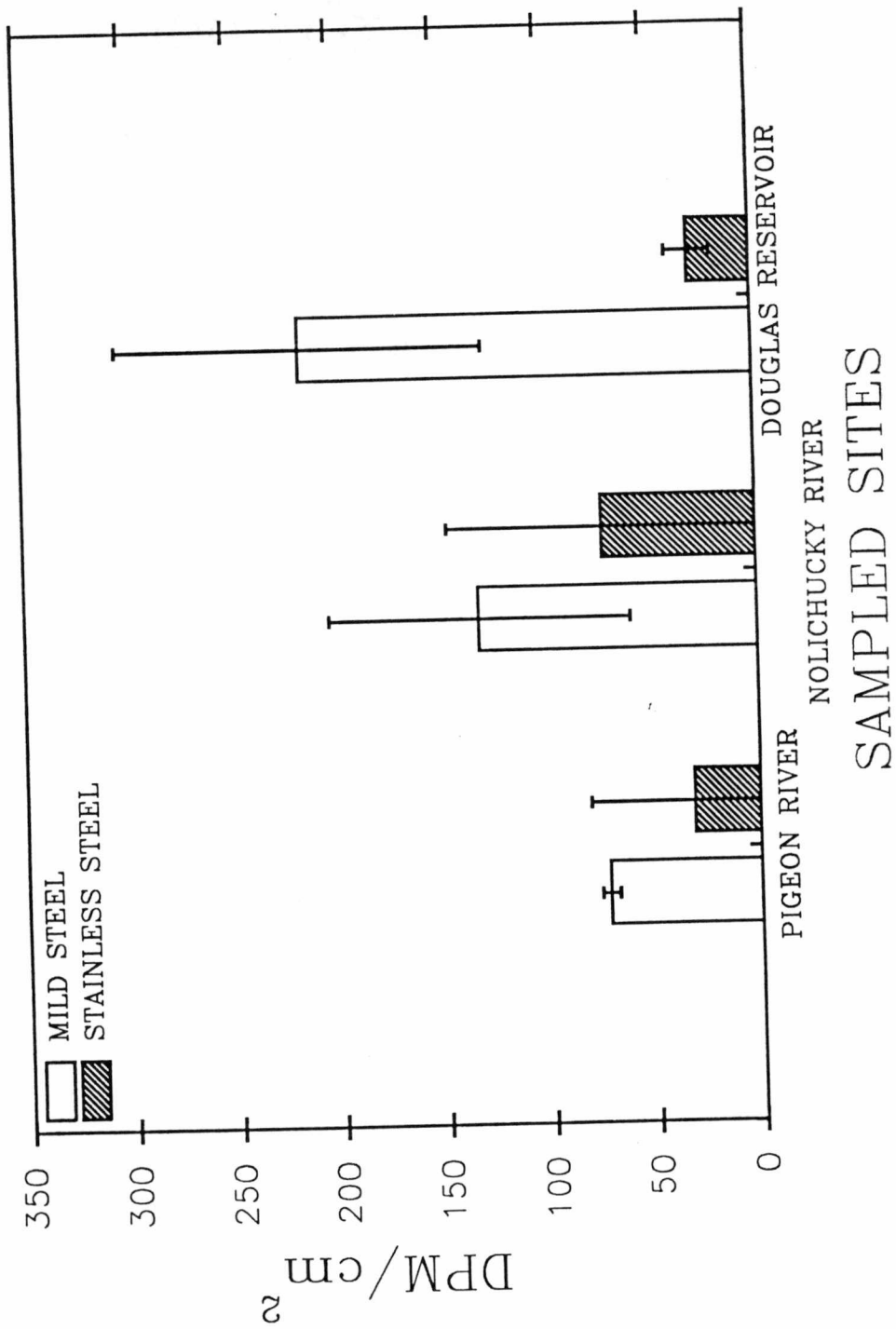


Appendix B.3. Total biomass enumerated by acridine orange direct counts (AODC) collected from two different surfaces exposed at three aquatic sites. N=9 with the exception of the SS values from PR which n=8 because the loss of one coupon. The vertical line indicates the sample standard deviation.

APPENDIX C

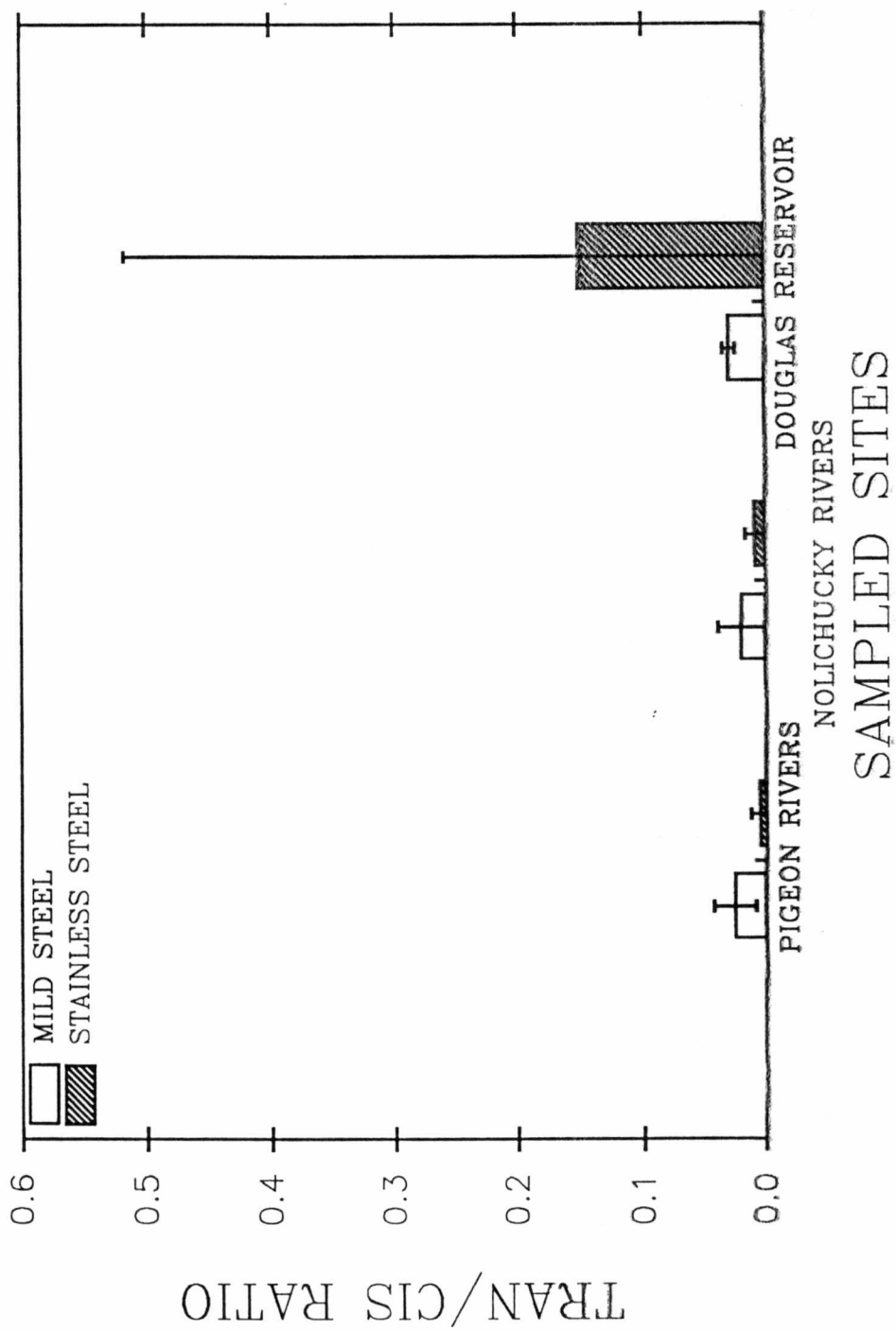
Appendix C. Total SRB (lactate utilizers), measured by MPN technique and recovered from two different surfaces exposed at three aquatic sites. N=9 with the exception of SS surfaces exposed to the PR for which n=8 because of the loss of 1 coupon. The vertical line indicates the sample standard deviation.

APPENDIX D



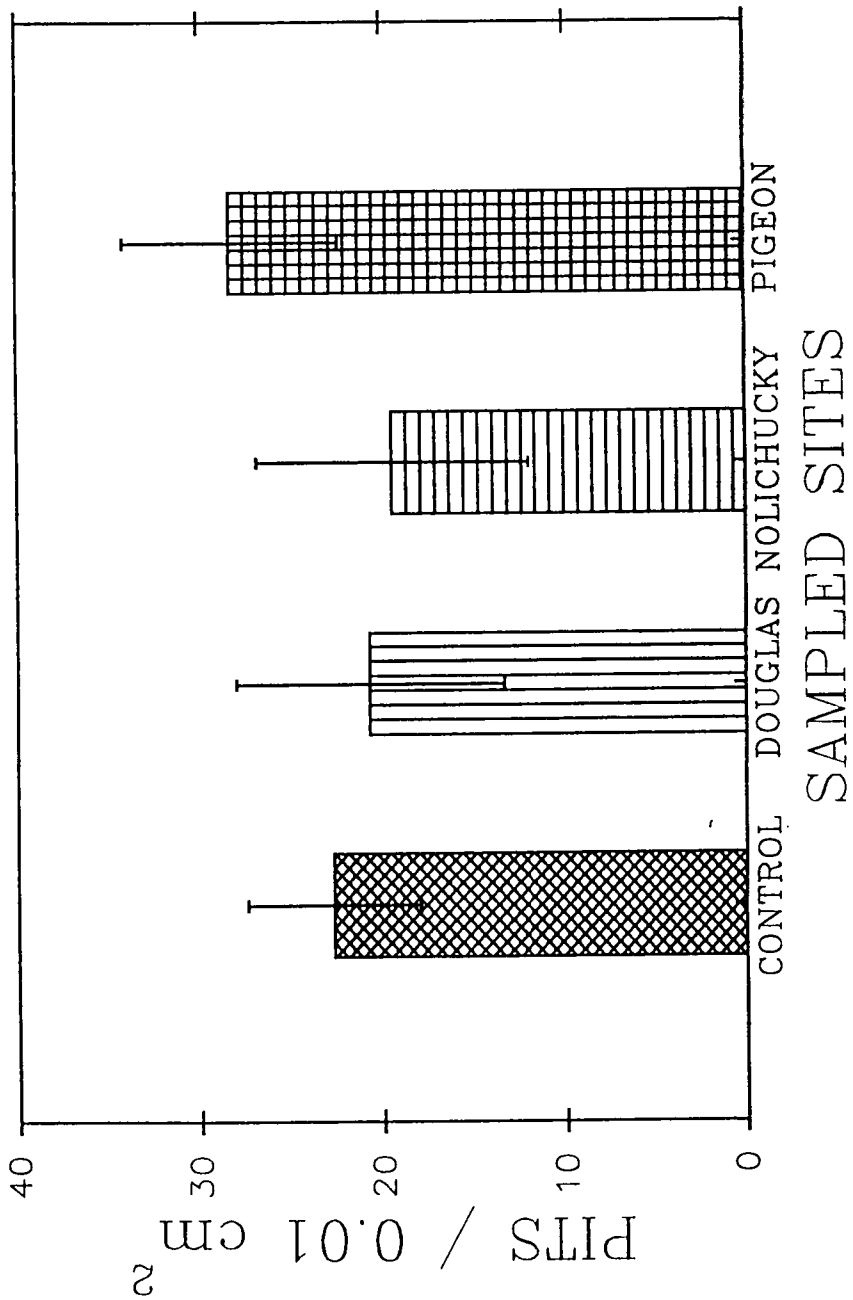
Appendix D. Total lipid biosynthesis measured by the incorporation of ^{14}C-1-acetate in undisturbed biofilm formed on two different surfaces exposed at three aquatic sites. N=3, except for PR which n=2 because the lost of one coupon. The vertical bar for the PR value stands for the range while fro the other two sites stand for the sample standard deviation.

APPENDIX E

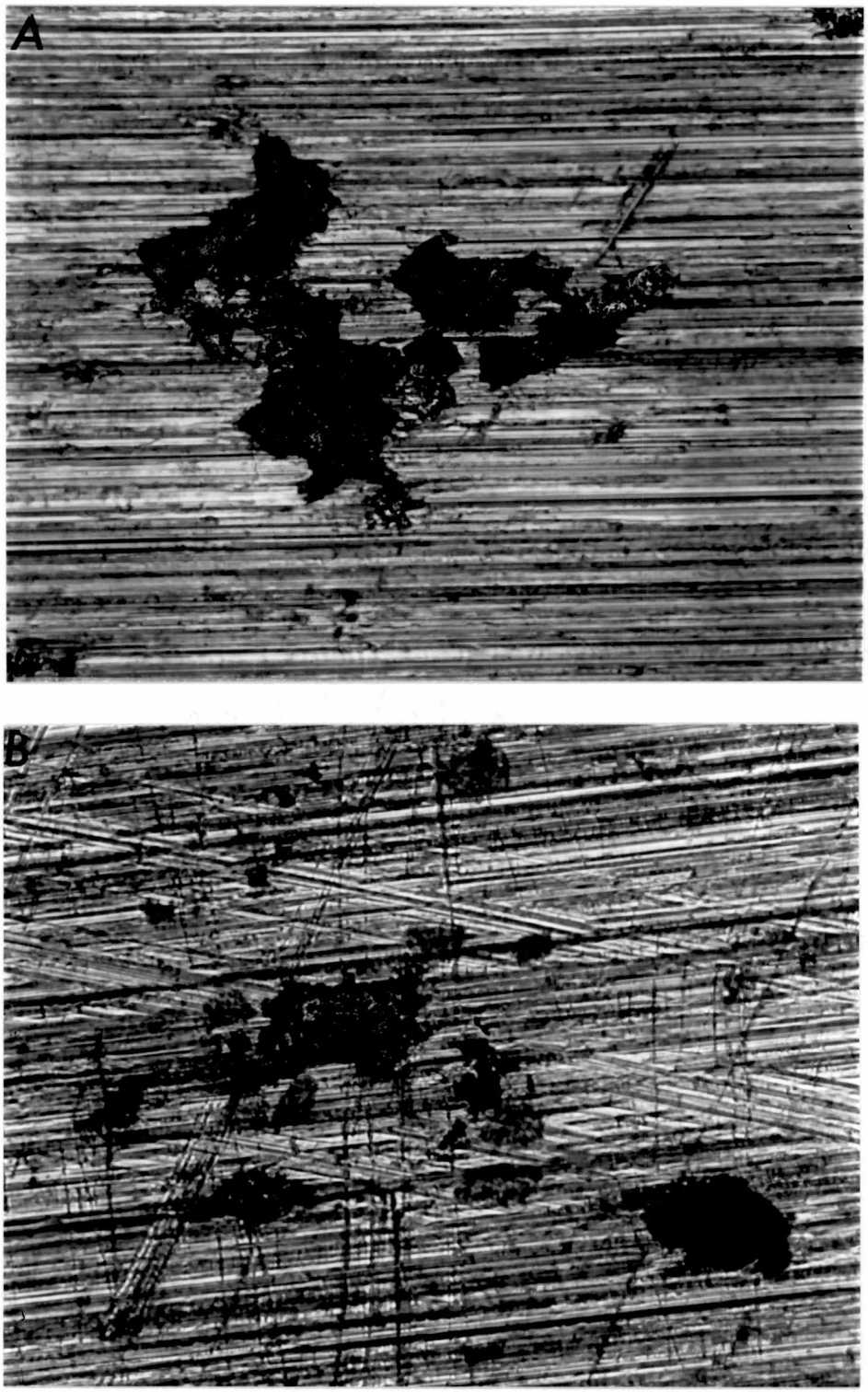


Appendix E. Tran/Cis ratio measured on two different surfaces exposed at three sites. N=9 except for the SS surfaces values from the Pigeon River (PR) and Douglas Reservoir (DR). The N value for the former is 8 and for the latter is 6 because the loss of one coupon and 3 values below the detection limit, respectively. The vertical line indicates the sample standard deviation.

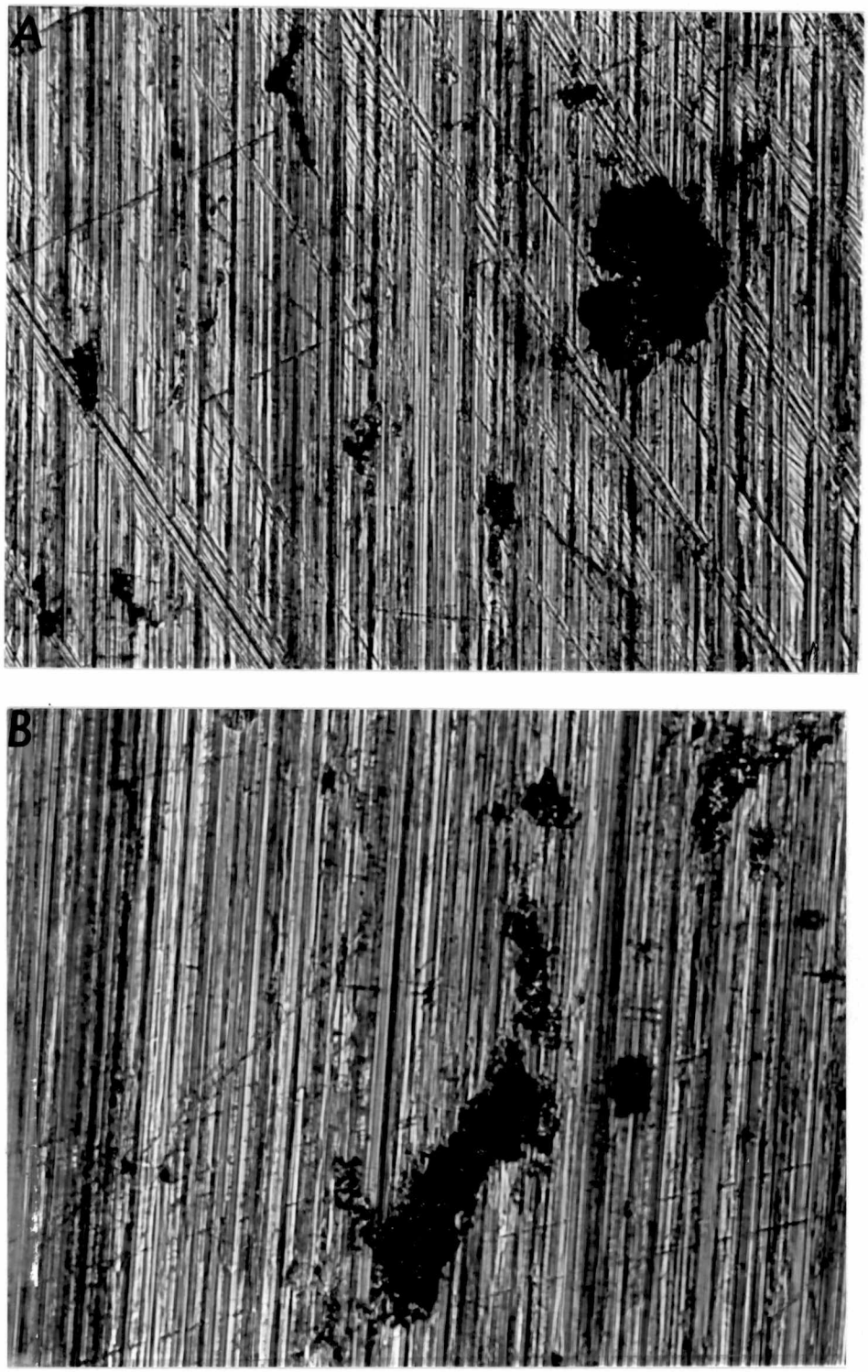
APPENDIX F



Appendix F.1. Total pits counted on SS surfaces exposed to three different environments.

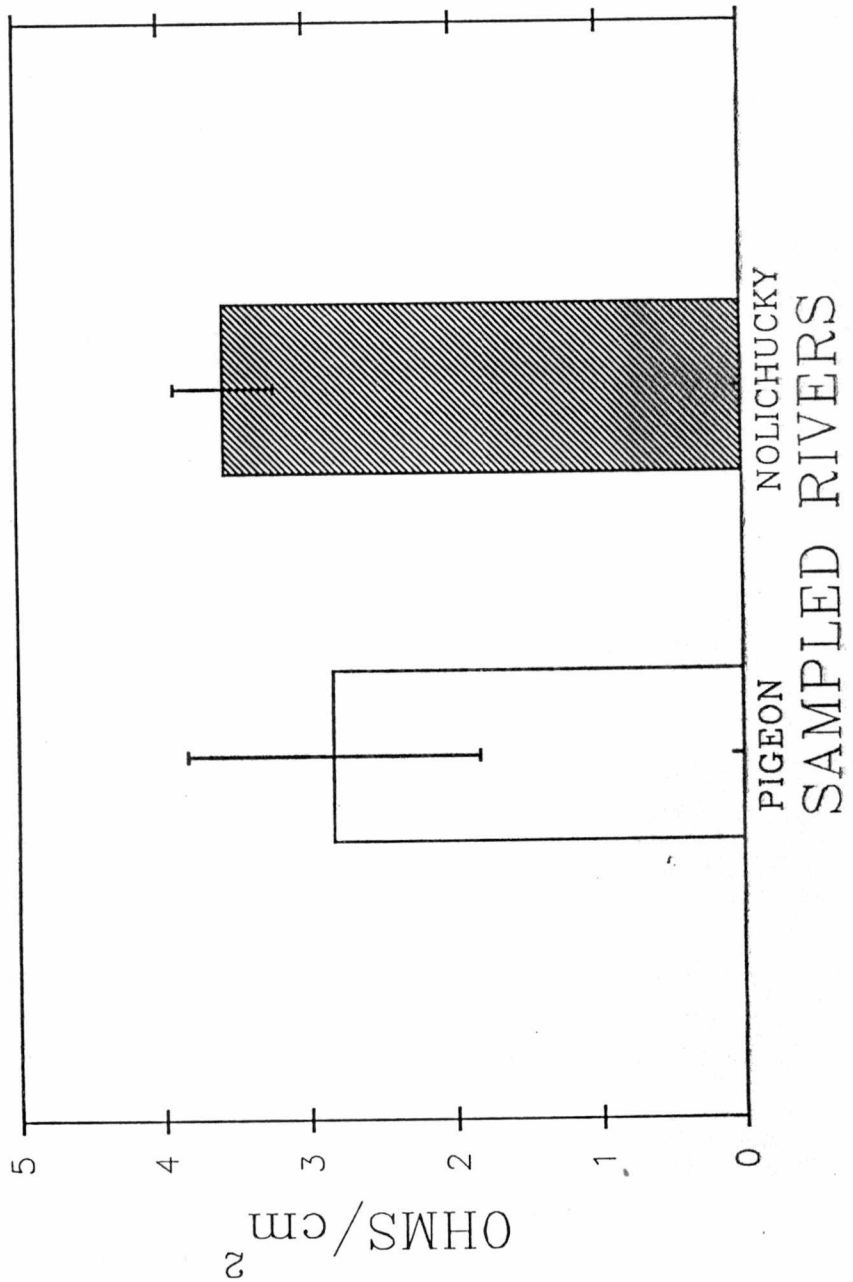


Appendix F.2. Pits observed on the SS metal surfaces. A. Unexposed Surface B. Surface exposed to PR.

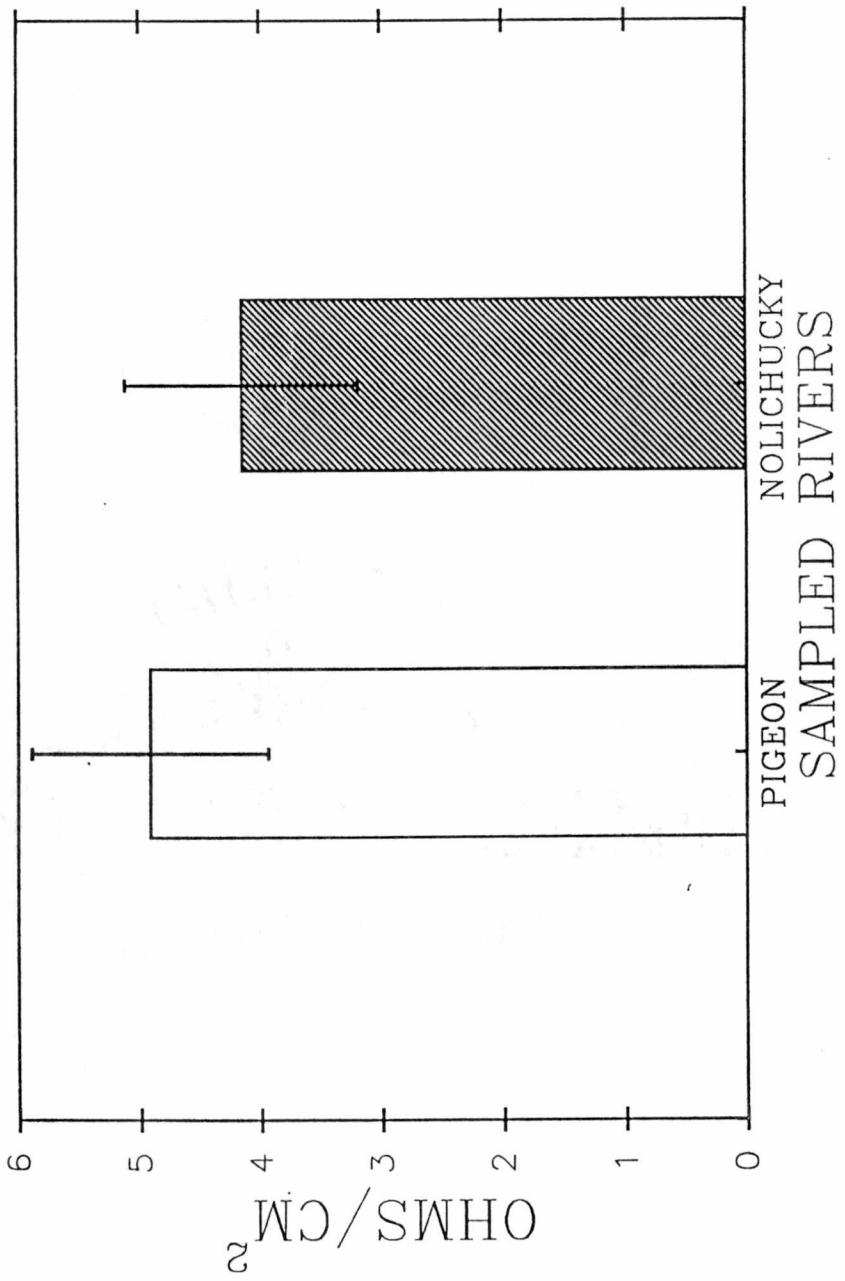


Appendix F.3. Pits observed on the SS metal surfaces. A. SS surface exposed to NR B. SS surface exposed to DR.

APPENDIX G



Appendix G.1. Standard test method P_R results in the absence of heat transfer for MS surfaces exposed to two different electrolytes.



Appendix G.2. Results of the standard practice for conducting potentiodynamic P_R measurements for MS surfaces exposed to two different electrolytes.

VITA

Raúl Orlando Díaz was born in Santurce, Puerto Rico on November 12, 1962. He attended elementary schools in La Milagrosa College (Bayamón) and graduated from La Salle College in May, 1980. The next fall he entered the Natural Science Program at University of Puerto Rico, Río Piedras, Puerto Rico and during his third year he joined the Reserve Officers' Training Corps at the same university. On June 1984 he received the commission of Second Lieutenant in the United States Armed Forces, Army followed by an appointment to active duty for three years. On February 5, 1985, one month after receiving his Bachelor of Science in Biology, he was called into active duty where he spent the next five years serving as Nuclear, Biological and Chemical (NBC) Officer in combat units at Fort Campbell, KY.

Upon resigning to his Captain position as an active duty officer, he entered the Microbiology Graduate Program at University of Tennessee and joined the 489th Civil Affairs Company, Army Reserve. On December 27, 1990, he was called to active duty as part of the task force deployed to Saudi Arabia where he served as NBC Officer and assistant to the Displaced Civilian Officer. On the Summer term of 1991, he rejoined the Microbiology Graduate Program at University of Tennessee, receiving the Master of Science in December 1992.

He is currently tutoring at the Black Cultural Center and the Educational Advancement Office in numerous subjects.