Leptothrix discophora SP-6 : effects of biofilms on passive film chemistry of 316L stainless steel and modeling of growth

by Nurdan Yurt

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemical Engineering
Montana State University

Abstract:
This thesis examined effects of manganese oxidizing bacteria, Leptothrix discophora SP-6 biofilms, on passive film chemistry and pitting corrosion of 316L stainless steel..

Biofilms of manganese oxidizing bacteria attach to the passive films of metals and deposit manganese oxides on the surfaces. These oxides, being in equilibrium with manganese ions in water, shift the corrosion potential of the passive metals in the noble direction, causing a phenomenon known as "ennoblement". Ennoblement is of interest because it increases corrosion potential, which may initiate localized forms of corrosion of passive metals, pitting corrosion. The mechanism of pit initiation on the ennobled coupons still is not completely known. For localized corrosion to occur, the passive film has to be broken down, and it has been speculated that the biofilms of manganese oxidizing bacteria have a direct effect on the chemistry of the passive films.

The effect of ennoblement of 316L stainless steel by biomineralized manganese deposits on chemistry of passive films was studied using surface-sensitive analytical techniques and cyclic polarization. Depth profiles of elements in the passive films on the ennobled coupons were analyzed using x-ray photoelectron spectroscopy (XPS), and distribution of metal elements were examined by time-of-flight secondary ion mass spectroscopy (ToFSIMS). The results showed that oxide layers on the ennobled coupons were thinner than those on the control coupons.. Cyclic polarization curves showed that ennobled 316L stainless steel indicated, significant loss of passivity. It was concluded that metabolic activity of manganese oxidizing bacteria, Leptothrix discophora SP-6 degraded the quality of the passive film on stainless steel coupons by locally reducing its thickness and lowering the pitting potential.

To relate the kinetics of ennoblement to the rate of deposition of manganese oxides on metal surfaces, growth kinetics of Leptothrix discophora SP-6 were quantified in biofilms and in planktonic form. In planktonic form, double-substrate growth kinetics, using Monod growth kinetics for pyruvate and Tessier growth kinetics for oxygen, showed the best agreement with the experimental data. Monod model of microbial growth kinetics adequately represents the growth of Leptothrix discophora SP-6 biofilms.

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APPROVAL

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This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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NOMENCLATURE

B_i Constant in Contois model for substrate *i* (mg substrate/mg microorganism)

D Dilution rate (1/h)

D_{eff} Effective diffusion coefficient of oxygen in biofilm (m^2/s)

i Subscript refers to the growth model, M for Monod and T for Tessier

K_{sM} Monod half rate coefficient (g/m^3)

K_{sMi} Monod half saturation constant for substrate i

K_{sMZi} Moser constant for substrate i

K_{sT} Tessier coefficient (g/m^3)

K_{sTi} Tessier saturation constant for substrate i

L_f Biofilm thickness (m)

m maintenance coefficient for oxygen (mg microorganism/mg oxygen)

m_i Maintenance factor for limiting substrate *i*, (mg microorganism/mg substrate *i*)

m_M maintenance coefficient for oxygen when growth is described according to Monod kinetics (mg microorganism/mg oxygen)

m_T maintenance coefficient for oxygen when growth is described according to Tessier kinetics (mg microorganism/mg oxygen)

M_M $= \dfrac{m_M L_f^{\,2} X_f}{D_{eff} S_s}$

M_T $= \dfrac{m_T L_f^{\,2} X_f}{D_{eff} S_s}$

N Number of experiments (integer)

Q Volumetric flow rate (L/h)

S Oxygen concentration in biofilm (g/m^3)

S* Dimensionless oxygen concentration in biofilm ($=S/S_s$)

S_{bottom} Oxygen concentration at the bottom of biofilm (g/m^3)

S^*_{bottom} Experimentally measured dimensionless oxygen concentration at the bottom of biofilm ($=S_{bottom}/S_s$)

$S^*_{experimentally\_measured}$ Experimentally measured dimensionless oxygen concentration in the biofilm

$S^*_{predicted}$ Calculated dimensionless oxygen concentration from the solution of the model in biofilm

S_{ei} Substrate concentration in effluent stream (mg/L)

S_{fi} Concentration of substrate *i* in influent stream (mg/L)

S_i Concentration of substrate *i* (mg/L)

SOUR Specific oxygen uptake rate (mg oxygen/mg microorganism/h)

S_s Oxygen concentration at biofilm – air interface (g/m^3)

SSD: Sum of squares of difference

V Reactor volume (L)

x Distance from the bottom of the biofilm (m)

X	Microorganism concentration in chemostat (mg/L)
x^*	Dimensionless distance from the bottom ($=x/L_f$)
X_f	Biofilm density (g/m^3)
$Y_{X/i}$	Yield coefficient for limiting substrate *i* (mg microorganism/mg limiting substrate)
$Y_{x/o}$	Yield coefficient for oxygen (g microorganism produced/g oxygen consumed)

<u>Greek letters</u>

β_M	$=\frac{K_{sM}}{S_s}$
β_T	$=\frac{K_{sT}}{S_s}$
Φ	Thiele modulus (Φ_M or Φ_T)
Φ_M	$=\sqrt{\frac{\mu_{max}L_f^2X_f}{Y_{x/o}D_{eff}S_s}}$
Φ_T	$=\sqrt{\frac{\mu_{max}L_f^2X_f}{Y_{x/o}D_{eff}S_s}}$
λ_i	Moser coefficient for substrate *i*
μ	Specific growth rate of microorganism, (1/h)

$\mu_{experimental}$ Experimentally determined specific growth rate of microorganism from (μ=Q/V), (1/h).

μ_i Specific growth rate of microorganism for limiting substrate *i*, (1/h)

μ_{max} Maximum specific growth rate (s^{-1})

μ_{model} Specific growth rate of microorganism predicted from the models, (1/h)

Subscripts

i Substrate

p Pyruvate

o Oxygen

n NH^{4+}

ABSTRACT

This thesis examined effects of manganese oxidizing bacteria, *Leptothrix discophora* SP-6 biofilms, on passive film chemistry and pitting corrosion of 316L stainless steel.

Biofilms of manganese oxidizing bacteria attach to the passive films of metals and deposit manganese oxides on the surfaces. These oxides, being in equilibrium with manganese ions in water, shift the corrosion potential of the passive metals in the noble direction, causing a phenomenon known as "ennoblement". Ennoblement is of interest because it increases corrosion potential, which may initiate localized forms of corrosion of passive metals, pitting corrosion. The mechanism of pit initiation on the ennobled coupons still is not completely known. For localized corrosion to occur, the passive film has to be broken down, and it has been speculated that the biofilms of manganese oxidizing bacteria have a direct effect on the chemistry of the passive films.

The effect of ennoblement of 316L stainless steel by biomineralized manganese deposits on chemistry of passive films was studied using surface-sensitive analytical techniques and cyclic polarization. Depth profiles of elements in the passive films on the ennobled coupons were analyzed using x-ray photoelectron spectroscopy (XPS), and distribution of metal elements were examined by time-of-flight secondary ion mass spectroscopy (ToFSIMS). The results showed that oxide layers on the ennobled coupons were thinner than those on the control coupons. Cyclic polarization curves showed that ennobled 316L stainless steel indicated significant loss of passivity. It was concluded that metabolic activity of manganese oxidizing bacteria, *Leptothrix discophora* SP-6 degraded the quality of the passive film on stainless steel coupons by locally reducing its thickness and lowering the pitting potential.

To relate the kinetics of ennoblement to the rate of deposition of manganese oxides on metal surfaces, growth kinetics of *Leptothrix discophora* SP-6 were quantified in biofilms and in planktonic form. In planktonic form, double-substrate growth kinetics, using Monod growth kinetics for pyruvate and Tessier growth kinetics for oxygen, showed the best agreement with the experimental data. Monod model of microbial growth kinetics adequately represents the growth of *Leptothrix discophora* SP-6 biofilms.

CHAPTER 1

INTRODUCTION

This thesis examines several effects of growth and attachment of *Leptothrix discophora* SP-6 in biofilms. The effects of biofilms of these manganese oxidizing bacteria on passive film chemistry and on the pitting potentials of 316L stainless steel were investigated. Also, the growth kinetics of *Leptothrix discophora* SP-6 were determined for planktonic and biofilm growth. This introductory section briefly describes the content of the thesis, presents research questions and hypotheses to be addressed, and shows the organization of the thesis.

A research group at the Montana State University (MSU) is systematically studying the ennoblement of stainless steel by microbially deposited manganese oxides. It has been shown that biofilms of *Leptothrix discophora* SP-6 grown on 316L stainless steel (SS) increase the open circuit potential to values of 300 – 400 mV_{SCE} by depositing manganese oxides (Olesen et al., 2000a,b; Dickinson et al., 1996). This phenomenon, which increases the open circuit potential, is called ennoblement. The manganese deposition mechanism and ennoblement on stainless steel coupons have been well described by the MSU research group (Shi et al., 2002; Olesen et al., 2000a,b; Dickinson et al., 1996). The ennoblement increases risk of pitting corrosion by lowering pitting potential of stainless steel in the presence of active ions such as chloride (Olesen et al., 2001; Amaya and Miyuki 1997; Linhardt 1996; Suleiman et al., 1994). One of the possible mechanisms that could lower pitting potential is that the passive film is degraded by ennoblement caused by manganese deposition. Continuing the efforts at MSU, the first part of this thesis investigates passive film chemistry, explores these processes, presents previous literature and states research goals (section 1.1). The research correlating passive film chemistry and pitting potential of stainless steel ennobled by biomineralized manganese was prepared as a research paper and is presented in chapter 2 (Yurt et al., 2002a). The effect of biomineralized manganese on pitting corrosion of type 304L stainless steel are examined, and the basic approach to measure pitting corrosion of stainless steel 304L used in this thesis, and tests results of pitting potentials for clean and ennobled coupons, are presented in the appendix as a published paper (Olesen, B. H., Yurt, N., Lewandowski, Z. 2001).

For ennoblement studies, the model microorganism used at MSU has been *Leptothrix discophora* SP-6, which grows in bulk liquid, attaches to stainless steel surfaces, grows as biofilms and then deposits manganese and causes ennoblement. The second part of this thesis explores quantification of the growth kinetics of *Leptothrix discophora* SP-6 in planktonic and biofilm forms. Discussion of microorganism growth in planktonic form and in biofilms along with a literature review and research goals are presented in section 1.3. The growth kinetics of *Leptothrix discophora* SP-6 in planktonic form is presented as a research paper in chapter 3 (Yurt et al., 2002b). Since there was not any adequate published method to calculate biokinetic parameters in biofilms, I developed an algorithm to extract biokinetic parameters of microorganism growth in *Leptothrix discophora* SP-6 biofilms. The developed algorithm and biokinetic parameters for *Leptothrix discophora* SP-6 biofilms are presented as a research paper in chapter 4 (Yurt et al., 2002c). The developed algorithms to calculate biokinetic parameters for planktonic growth and biofilm growth are integrated into MATLAB programs. These MATLAB programs were added to corresponding sections as an appendix to make them available for other researchers, since they are not available in the literature.

The experimental methods and instruments used in the thesis are presented in section 1.4 for future reference.

The hypotheses addressed in this thesis are:

1. Manganese oxidizing activity of *Leptothrix discophora* SP-6 in a biofilm leads to ennoblement of stainless steel and causes changes in the passive film chemistry of the steel.

2. The presence of biomineralized manganese oxides on the surface of 316L stainless steel initiates pitting corrosion in a low-chloride aqueous environment.

3. The statistically optimum model to represent *Leptothrix discophora* SP-6 growth in the planktonic form can be determined by an algorithm, developed by the author, which optimizes parameters for the four major kinetic models.

4. The statistically optimum model to represent *Leptothrix discophora* SP-6 growth in the biofilm form can be determined by an algorithm, developed by the author, which optimizes parameters.

5. *Leptothrix discophora* SP-6 has different growth and substrate consumption kinetics in planktonic and biofilm form.

Surface sensitive techniques such as XPS, ToFSIMS were used to determine passive film chemistry and thickness. The anodic polarization technique was used to measure pitting potentials. Hypotheses 1 and 2 are addressed in chapter 2. A chemostat operated at steady state was used to determine the best growth model, along with corresponding biokinetic parameters of *Leptothrix discophora* SP-6, and hypothesis 3 is addressed in chapter 3. Dissolved oxygen microsensors were used to measure concentration profiles in *Leptothrix discophora* SP-6 biofilms. A new method was developed to extract the best representative growth model and corresponding biokinetic parameters of *Leptothrix discophora* SP-6 growth in biofilms. Hypotheses 4 and 5 are addressed in chapter 4. Chapters 2, 3, and 4 are presented in the format of a journal paper.

Manganese Biomineralization on 316L Stainless Steel

Stainless Steels

Stainless steels are iron-chromium alloys with a minimum of 10 wt % chromium. When the steel contains at least that much chromium, a thin, transparent, protective passive film forms on the surface. The passive film forms spontaneously as a result of the reaction between the chromium in the steel and the oxygen in the air. The passive film is a barrier between the steel and the environment and prevents corrosive attack of the underlying steel as long as the passive film remains intact. This protective passive film is quite thin, on the order of nanometers, and its actual composition depends on the alloying elements in the stainless steel and the environment to which it is exposed (Figure 1.1).

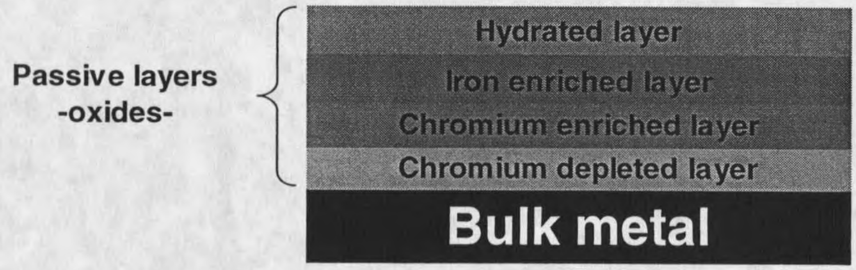

Figure 1.1. Schematic representation of passive layers in the presence of water.

Pitting Corrosion

Pitting is localized attack at a point where the passive film can no longer protect the stainless steel. Theoretically, pitting processes consist of the following stages; 1) passive film breakdown, 2) metastable pit formation, and 3) pit growth. These stages are discussed below.

Passive Film Breakdown. The breakdown of the passive film is the initiation of the pitting process. The breakdown happens rapidly on a very small scale, which makes direct observation difficult. The passive film can be drawn schematically as a simple inert layer covering the underlying metal, blocking access of the environment to the metal (see Figure 1.2). Depending on alloy composition, environment, potential, and exposure history, this film can have a range of thickness, structure, composition, and protectiveness. Typical passive films are quite thin, and support an extremely high electric field (on the order of 10^6-10^7 V/cm). The passage of a finite passive current density is evidence of continual reaction of the metal, with resultant film thickening, dissolution into the environment, or some combination of the two. The view of the passive film as *a dynamic structure*, rather than static, is critical to the proposed mechanisms of passive film breakdown and pit initiation.

Theoretically, passive film breakdown and pit initiation have been categorized according to three main mechanisms; 1) passive film penetration, 2) film breaking and, 3) adsorption.

Figure 1.2 shows the penetration mechanism for pit initiation. It involves the transport of the aggressive anions through the passive film to the metal/oxide interface where accelerated dissolution of metal is promoted (Hoar 1965).