



Influence of the distribution of the manganese oxidizing bacterium *Leptothrix discophora*, on ennoblement of 316L stainless steel  
by Scott Cameron Campbell

A thesis submitted in partial fulfillment Of the requirements for the degree Of Masters of Science In Microbiology  
Montana State University  
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**Abstract:**

Corrosion of metals enhanced by microorganisms stems from the modification of the near-surface chemistry due to microbial attachment or microbial metabolism. The modification of the near-surface water chemistry by microorganisms ultimately affects the electrochemistry of the metal. To further our understanding of microbially influenced corrosion, a greater level of microbial activity at the metal surfaces needs to be achieved. Measurements of the potential across the stainless steel surface was done to monitor ennoblement. Next, Fluorescent In Situ Hybridization was applied to locate the activity of a manganese oxidizing bacterium in a multi-species biofilm. Finally, oxygen microelectrodes were then used to measure oxygen concentrations through the biofilm. Type 316L stainless steel (SS) ennobled to an open circuit potential (OCP) of +323 mVscE within a 5-day period due to the deposition of manganese oxides on the metal surface by a monospecies biofilm of *L. discophora*. However, the same metal experienced only partial ennoblement, achieving a maximum potential of +143 mVscE during the same period when colonized by a 3-species biofilm containing *L. discophora*, and even this level of ennoblement by the mixed-species biofilm was only transient since the potential decreased to +122 mVscE shortly after achieving the maximum potential.

The mixed-species biofilm was significantly thicker (>200  $\mu\text{m}$ ) than the *L. discophora* monospecies biofilm (120  $\mu\text{m}$ ). Using 16s rRNA probes specific for *L. discophora*, fluorescent in-situ hybridization revealed cell activity was heterogeneously distributed throughout the monospecies biofilm. In the 3-species biofilm, 16s rRNA probes revealed a homogeneous layer of *L. discophora* activity that resided proximal to the biofilm-bulk solution phase. At the most distal position from the biofilm-bulk solution phase near the glass-biofilm interface there was very little to no activity of *L. discophora*.

Microelectrode studies revealed the presence of oxygen (3.85 to 4.35 mg/L) at the monospecies-glass substratum interface in 50% of the areas of the substratum assayed.

No oxygen was detected at the 3-species biofilm-glass interface where the thickness of the overlying biofilm exceeded 200  $\mu\text{m}$ . This study demonstrates that the distribution of the activity of the manganese oxidizing bacterium *L. discophora* in a biofilm is influenced by the presence or absence of oxygen, and this distribution of microbial activity could possibly have profound consequences for on the corrosion of steel.

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BACTERIUM *LEPTOTHRIX DISCOPHORA*, ON ENNOBLEMENT OF 316L  
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A thesis submitted in partial fulfillment  
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Of

Masters of Science

In

Microbiology

MONTANA STATE UNIVERSITY – BOZEMAN  
Bozeman, Montana

August 2003

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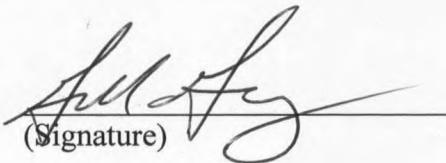
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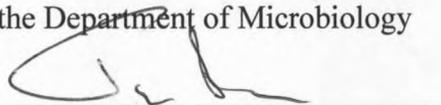
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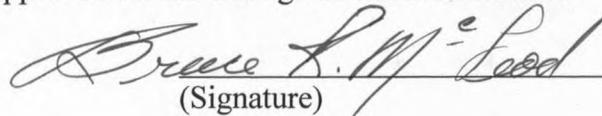
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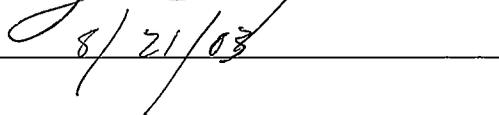
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This work is dedicated to my Mom and Dad who gave me the means to finish what I started, my wife, Ilona, who gave me the hope and support to finish, and my kids Alexandria, Tyler, and Brianna, who gave me the passion to want to finish.

Thanks to all!

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