Protection of Metallic Monuments from Biodeterioration | 2003-04

Harvard University

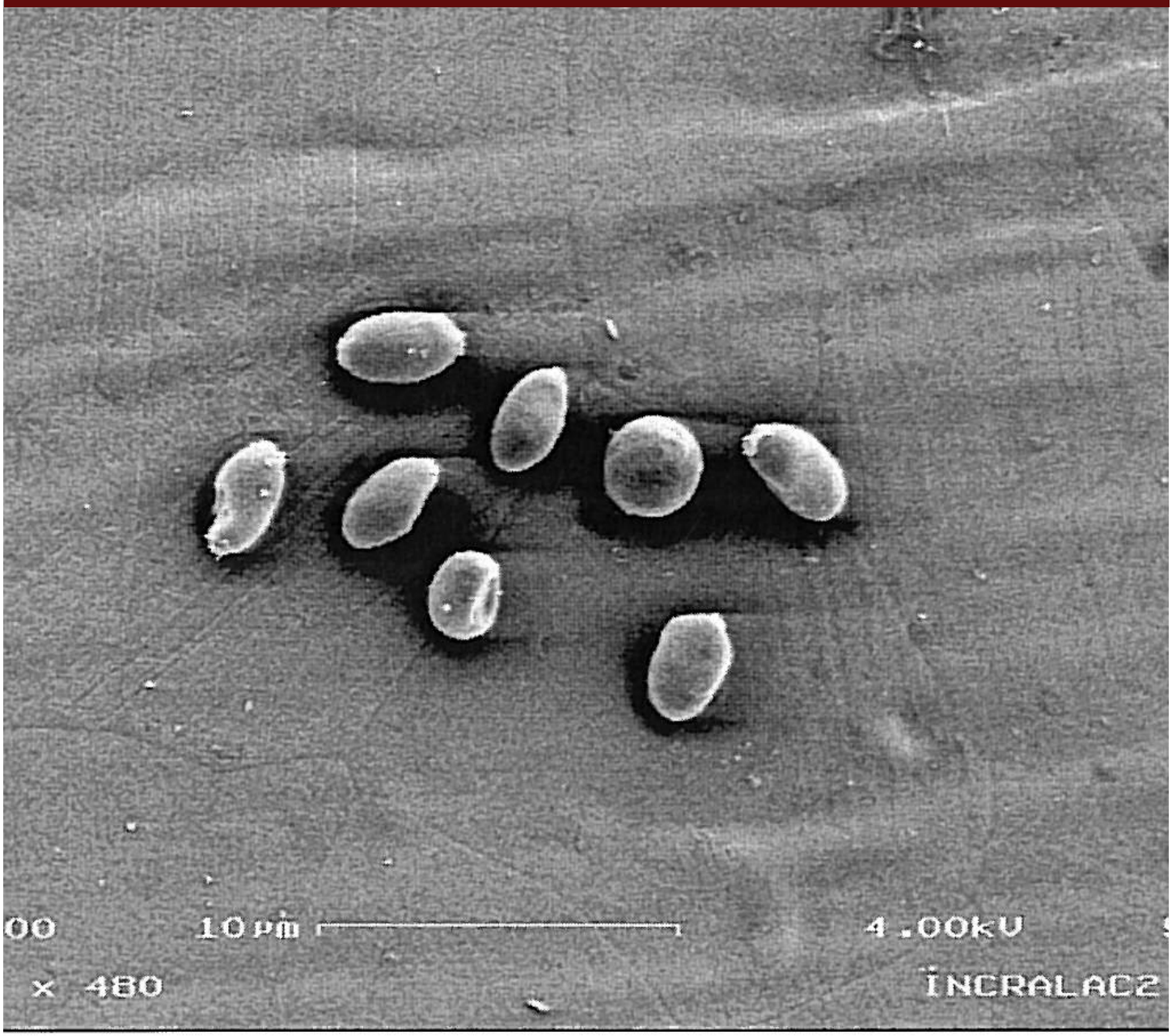

Figure 7. SEM of isolate GWM1 on Incralac coated 316 stainless steel.

National Park Service
U.S. Department of the Interior

National Center for Preservation Technology and Training

Final Report

Protection of Metallic Monuments from Biodeterioration

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To

Harvard University

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Division of Engineering & Applied Sciences

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SUMMARY

In this study we determined the susceptibility of monument coatings to degradation by the activity of the indigenous microflora. We found that microorganisms normally present in the air were capable of utilizing both beeswax and Incralac as sources of energy, and growing on these coatings. Both bacteria and fungi grew well on either Incralac or beeswax. We isolated and grew in pure culture a number of microorganisms capable of growing on these coatings. A common yeast was particularly aggressive on beeswax. This organism grew actively within four weeks. Growth on Incralac was slower.

We used electrochemical impedence spectroscopy to analyze the degradation of the coatings by the indigenous microorganisms. Impedence analysis demonstrated that during growth of the microflora on Incralac and beeswax, the coatings were being degraded. Electron microscopy was used to determine the growth of the aggressive microorganisms into the coatings, when they were spread on metal surfaces. Our electron micrographs confirmed the formation of biofilms on both the beeswax and the Incralac. We concluded that these biofilms were responsible for degradation of the coatings.

INTRODUCTION

Outdoor metals are highly susceptible to corrosion in many environments. Consequently, organic coatings are widely used to protect the metals from the processes of corrosion (1). A wide range of polymers are used for this purpose, including laquers, waxes, and natural resins.

Most polymeric coatings are highly susceptible to microbial attack (2). A variety of microorganisms, including bacteria, fungi, and algae, are capable of growing on coatings and causing deterioration. These microorganisms form biofilms on the coating surface. Acid and enzyme production beneath the biofilm can result in selective leaching of coating components (3), leading to failure of the coating.

The effect of microbial biofilms can frequently be seen on organically coated metals. Microbial activity on coatings leads to blistering or cracks, delamination, and changes in porosity. If left unchecked, the ultimate result is degradation of the underlying metal.

Natural and synthetic waxes havce frequently been used to protect metallic monuents. They produce a barrier that excludes moisture and oxygen from the metal, and they are often used in conjunction with acrylic coatings as a further protective layer.

Many studies have examined the effectiveness of various coatings for the protection of metals, but few have addressed the microbial degradation of these coatings. In this study, we have examined the susceptibility of two coatings, Incralac and Beeswax, to biodeterioration by microorganisms.

Materials and Methods

Isolation of Microorganisms

Microorganisms were collected by swab sampling in April 2001 from the George Washington Monument, New York City. Samples were inoculated into sterile minimal salt medium (l^{-1}: 0.22 g $(NH_4)_2SO_4$, 1.20 g KH_2PO_4, 0.23 g $MgSO_4 \cdot 7H_2O$, 0.25 g $CaCl_2$, 0.024 g yeast extract) containing cured pieces of Incralac. These enrichment cultures selected for microorganisms capable of using the coating as the sole source of carbon and energy. After 10 days, media from the enrichment cultures was plated on nutrient agar and isolates were collected.

To insure that isolates were capable of growth on Incralac and not simply dormant organisms revived on nutrient agar, the isolates were re-inoculated into flasks of minimal salt medium with cured pieces of Incralac. For those isolates capable of growth (determined visually by turbidity of cultures), the Gram Reaction and morphology of isolates was determined.

Growth Curves

Beeswax was pressed into glass wells approximately 1.5 cm in diameter and 0.5 cm deep. Incralac was poured into the wells and allowed to cure overnight. The incralac and beeswax were removed from the wells, sterilized with 80% ethanol and placed into flasks containing 150 ml of minimal salt medium. Flasks were inoculated with yeast isolate GWM1, isolated from the George Washington Monument and shaken (100 rpm) at room temperature. Samples were collected from flasks periodically and preserved with 1% formaldehyde. Yeast cells were stained for 5 min with 1.0 µg/ml of the nucleic acid stain 4',6-diamidino-2-phenylindole (DAPI), concentrated by filtration (15 kPa vacuum) onto a 0.22 µm pore size black polycarbonate membrane (Poretics, Livermore, California), and rinsed with 1.0 ml deionized water (4). Cells were enumerated using epifluorescence microscopy.

Electrochemical Impedance Spectroscopy

Biodeterioration of Incralac and beeswax was analyzed using electrochemical impedance spectroscopy (EIS). In order to obtain a thin coating, Incralac was mixed to a final concentration of 10% with toluene and then applied with a brush to one side of a 316 stainless steel coupon (5.0 cm x 5.0 cm). The Incralac was cured overnight at room temperature. Beeswax was rubbed into the coupon and then heated over low heat until melted to ensure the entire surface was covered. A 5.0 cm long acrylic tube (3.2 cm I.D., 3.8 cm O.D.) was then adhered to the coated coupon using Amercoat 90HS (Ameron International, Alpharetta, GA) resin and cure in a ratio of 4:1. The Amercoat adhesive was dried overnight at room temperature and cured at 37°C for 72-96 hrs. Cells were

surface sterilized using UV irradiation in a laminar flow hood, and filled with minimal salt medium. Cells were then either inoculated with isolate GWM1, or maintained as an uninoculated control.

The EIS analytic system consisted of a Schlumberger 1250 frequency response analyzer with a Schlumberger 1286 electrochemical interface (now manufactured by Solartron Analytical, Houston, TX). Z-Plot (Scribner Associates Inc., Southern Pines, NC) was used to control the instruments and analyze the data. EIS cells were held at their open circuit potential and a sinusoidal perturbation of 20 mV was applied. The impedance response was measured over a range of frequencies from 65 KHz to 1 mHz. A trielectrode system was used in this study: a saturated calomel reference electrode, platinum mesh counter electrode, and the stainless steel coupon as the working electrode (Fig. 1). All measurements were made in a laminar flow hood to prevent contamination of the cells.

Scanning Electron Microscopy

Samples were dehydrated using an ethanol series (40% ethanol in water replaced stepwise with 100% ethanol). Samples were critical point dried, gold/palladium sputter coated and observed with a scanning electron microscope (LEO 982, Elektronenmikroskopie GmbH, Oberkochen, Germany).

RESULTS

Five isolates capable of growth using both Incralac and beeswax as sole carbon and energy sources were isolated. Three of the isolates were identified as yeasts and the remaining two isolates were unidentified bacteria. One of the yeast strains, designated GWM1, appeared to grow substantially faster than the other isolates and was used in subsequent experiments.

Yeast isolate GWM1 grew rapidly in media containing beeswax as the only carbon source. The population in the flasks began to increase after 10 d and reached a maximum size of 1.5×10^5 cells/ml (Fig. 2). In contrast, GWM1 grew slowly on Incralac. One day after incoculation there were 5.4×10^4 cells/ml. After 27 days, the cell density had only increased to 5.8×10^4 cells/ml.

Electrochemical impedance spectroscopy was used to determine if GWM1 was able to degrade beeswax and Incralac. In the absence of GWM1, beeswax showed a gradual decline in impedance over the initial 30 d of the experiment. After 30 d the impedance remained stable at ca. 1×10^5 (Fig. 3A). The inoculated cell showed a large drop in impedance within 10 d of inoculation (Fig. 3B). After 10 d, there was a gradual decline in the impedance of the beeswax. The electrochemical response of Incralac was similar to that of beeswax. A gradual decline in impedance was observed in the uninoculated cells (Fig. 4A) while a sharp drop in the impedance of cells inoculated with GWM1 was observed in the first 10 d of the experiment (Fig. 4B).

Scanning electron microscopy was used to examine GWM1 growing on beeswax or Incralac coated 316 stainless steel (316 SS). Figure 5 shows the beeswax coated metal. Dark spots are visible in the surface. These are consistent with the size and shape of GWM1 and may be points at which yeast cells had attached to the surface. Preparation of the surface for SEM may have dislodged cells loosely attached to the beeswax. Figures 6 and 7 show GWM1 on the surface of the Incralac coated 316 SS.

CONCLUSIONS

1. A variety of microorganisms, both bacteria and fungi, were capable of growth using beeswax or Incralac as sole sources of carbon and energy. Of these organisms, the yeast isolate designated GWM1 appeared to be the most aggressive.

2. GWM1 was capable of rapid growth on beeswax (within 1 month), but grew more slowly on Incralac.

3. Electrochemical impedance spectroscopy indicated that GWM1 was able to rapidly degrade and cause a drop in the impedance of both beeswax and Incralac.

4. Scanning electron microscopy provided evidence for the attachment of GWM1 to both beeswax and Incralac.

5. Beeswax and Incralac are susceptible to rapid biodeterioration by microorganisms.

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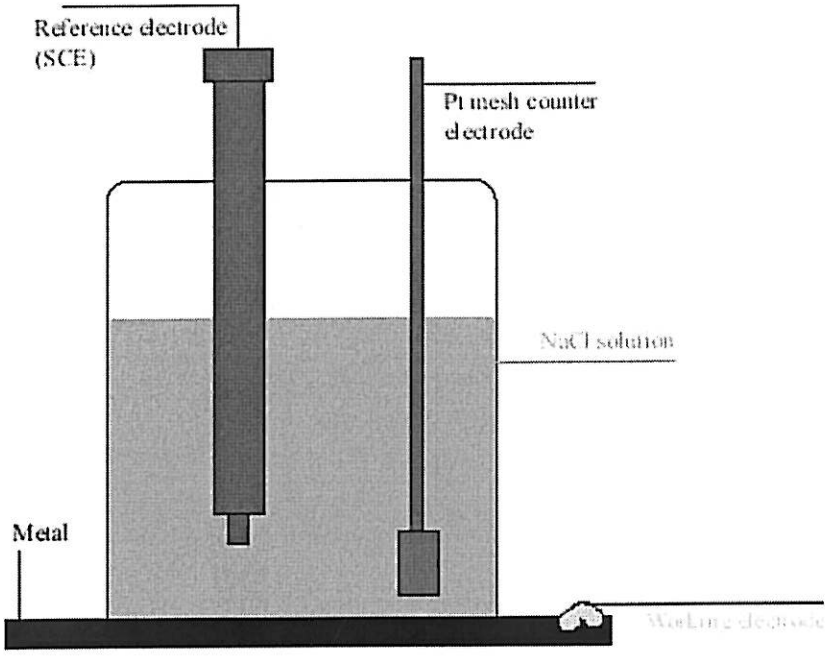

Figure 1. Schematic of EIS cell.

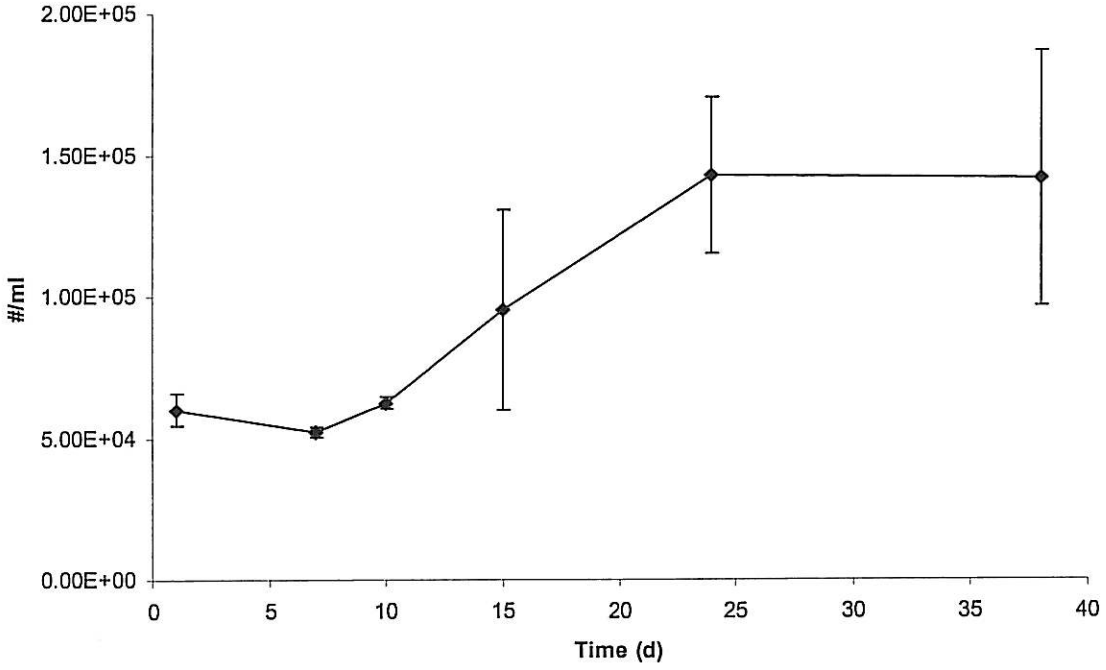

Figure 2. Number of DAPI-stained cells in media containing beeswax as sole carbon source.

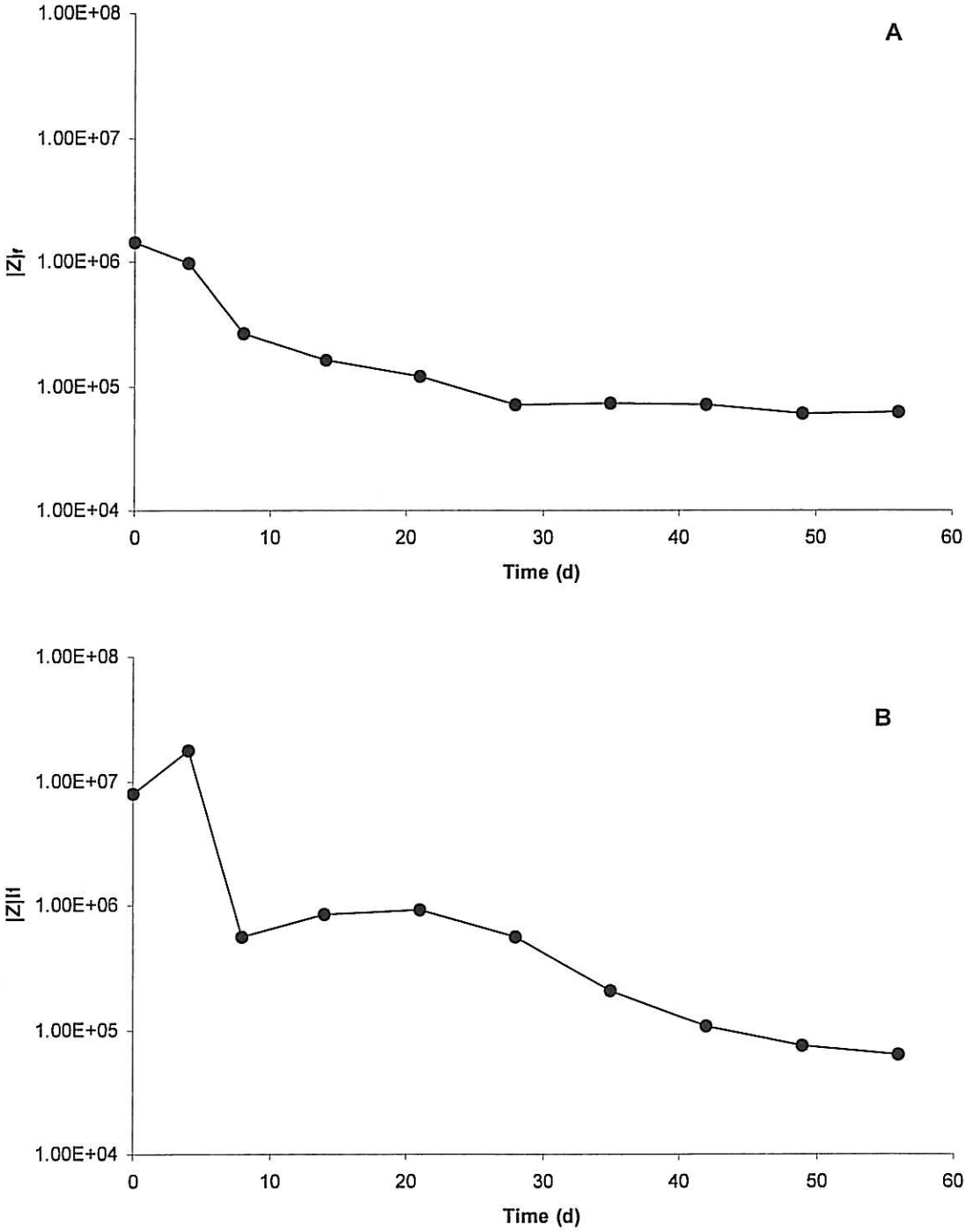

Figure 3. Beeswax low frequency impedance ($|Z|_{lf}$): inocualted (A) and uninoculated (B).

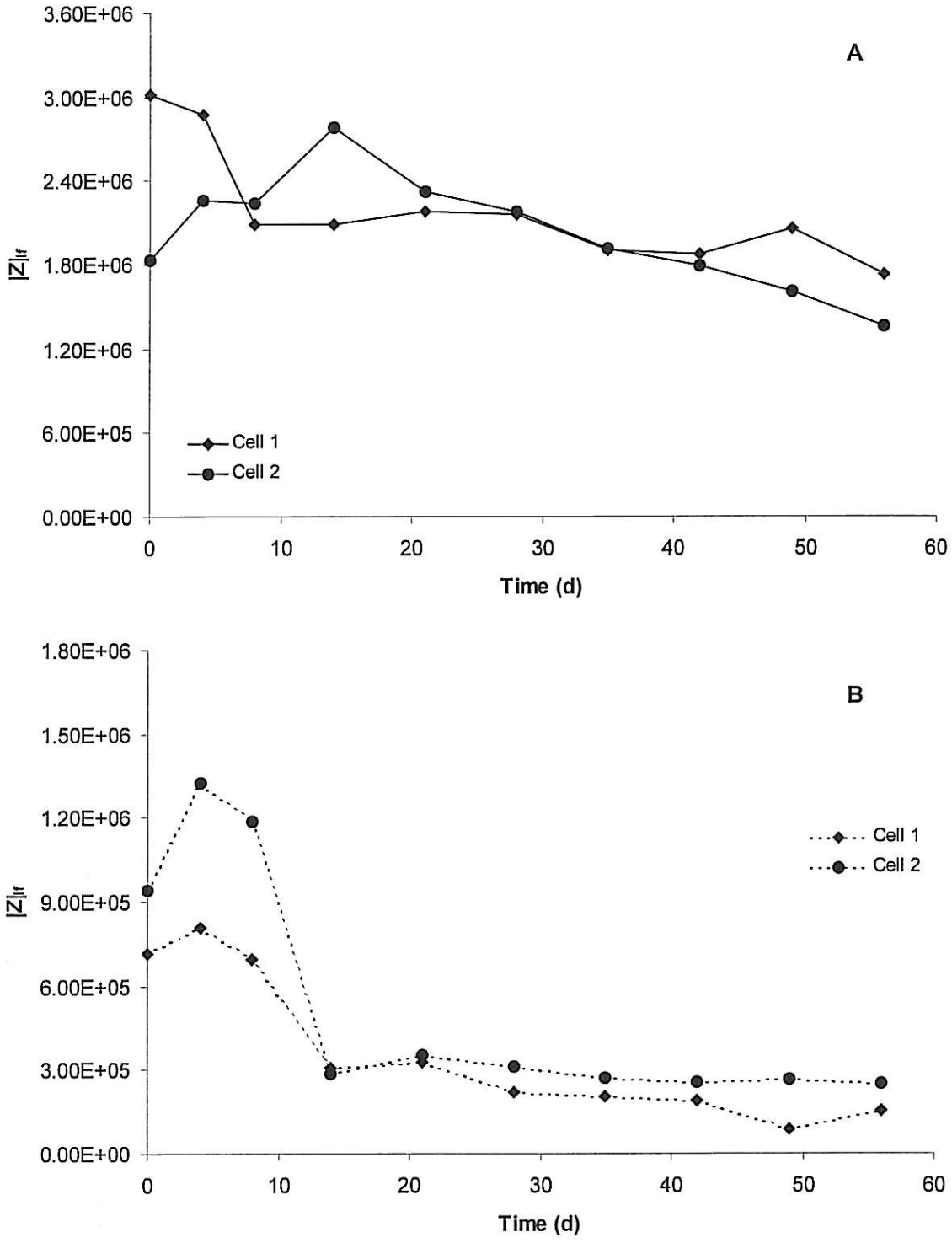

Figure 4. Incralac low frequency impedance (|Z|lf): inoculated (A) and uninoculated (B).

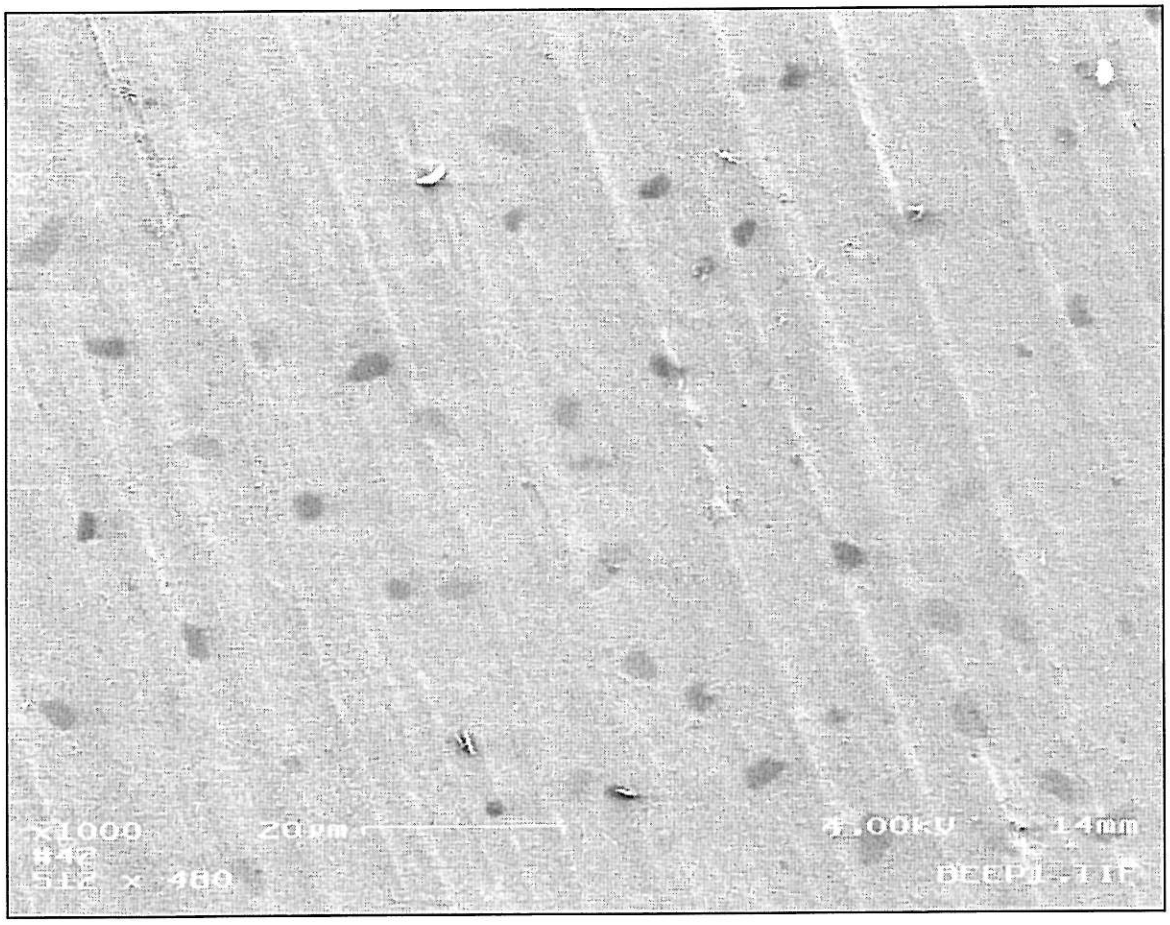

Figure 5. SEM of beeswax after incubation with isolate GWM1.

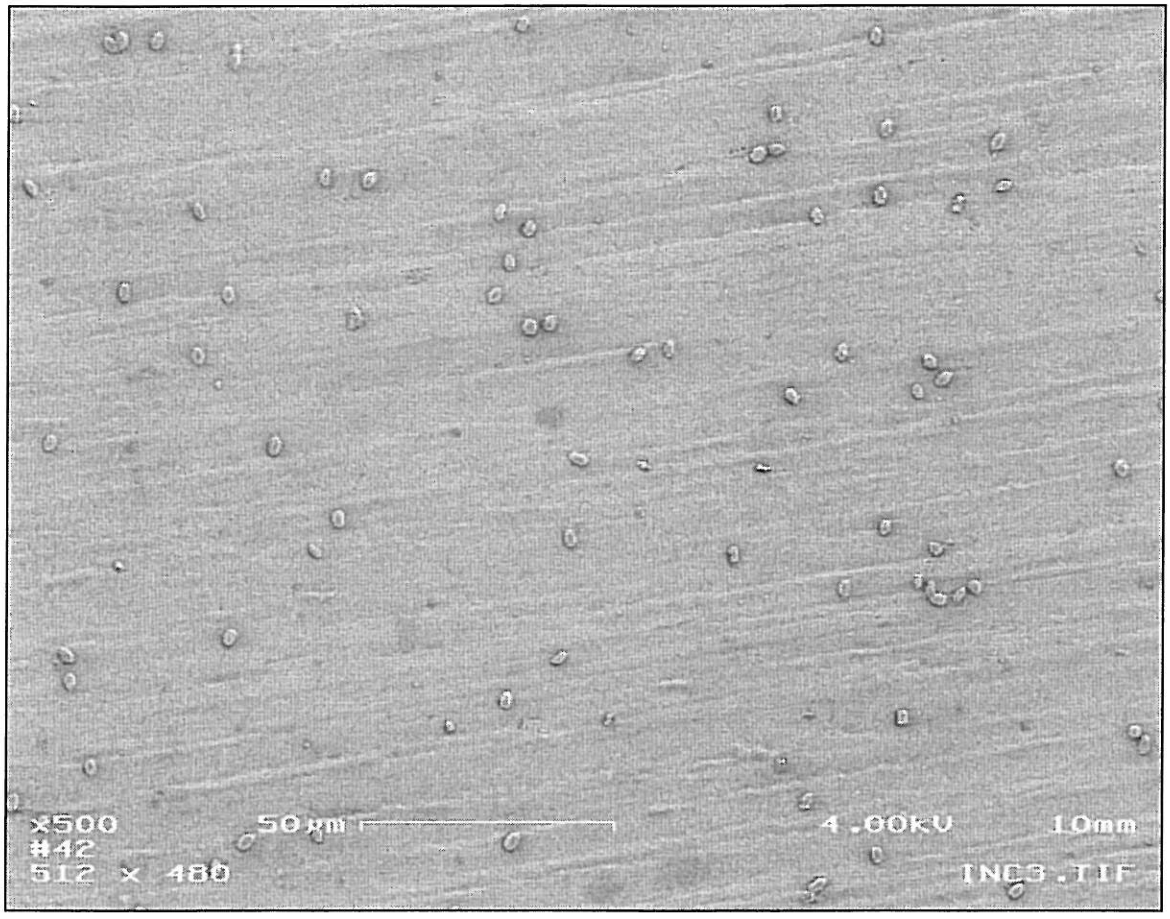

Figure 6. SEM of Incralac after incubation with isolate GWM1.

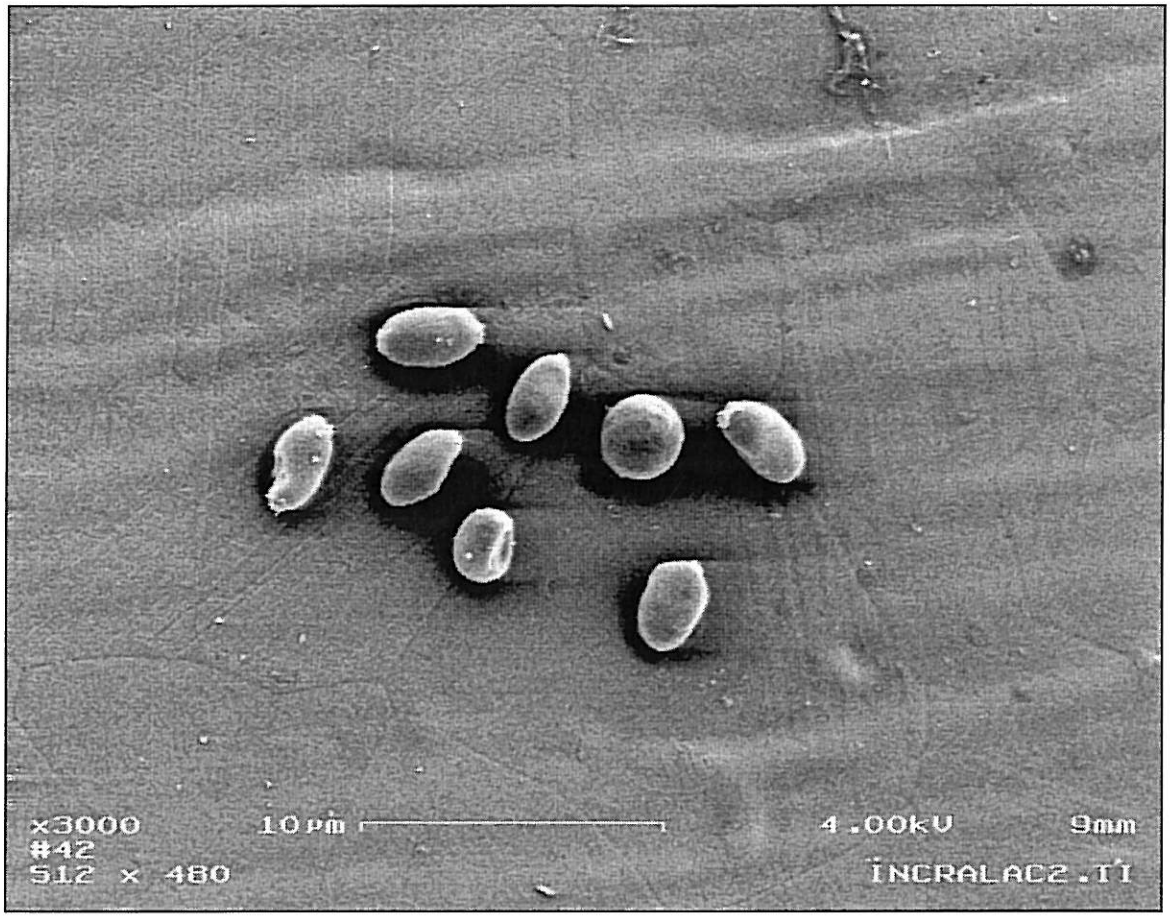

Figure 7. SEM of isolate GWM1 on Incralac coated 316 stainless steel.