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Original article

# Biodeterioration of Incralac used for the protection of bronze monuments

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**Abstract**

Outdoor bronze sculptures are highly susceptible to corrosion in many environments and organic coatings are widely used for their protection. The purpose of this study was to determine the susceptibility of the commonly used coating Incralac to biodeterioration by microorganisms. A yeast was isolated from a bronze statue treated with Incralac and its ability to degrade Incralac was determined using growth curves, scanning electron microscopy (SEM), and electrochemical impedance spectroscopy (EIS). The organism grew slowly on Incralac in liquid culture, but SEM images demonstrated its ability to adhere to Incralac coated metal. Additionally, the yeast caused a rapid drop in the low frequency impedance of Incralac coated metal that was not observed under sterile conditions, indicating that the organism accelerated deterioration of the coating. The potential for microbial growth to accelerate deterioration of Incralac should be considered when developing a maintenance strategy for the protection of outdoor metal monuments.


*Keywords:* Microorganisms; EIS; Coatings; Growth curves; SEM

## 1. Research aims

Outdoor metallic monuments are highly susceptible to corrosion in many environments and organic coatings are widely used for their protection [1]. A wide range of polymers are used for this purpose, including lacquers, waxes, and natural resins [2]. Many studies have examined the effectiveness of various coatings for the protection of metals from physical and chemical weathering processes (e.g., see [1,3,4]), but few have addressed the microbial degradation of these coatings [5,6]. Therefore, the purpose of this study was to determine the susceptibility of a commonly used coating, Incralac, to biodeterioration by microorganisms.

## 2. Introduction

Most polymeric coatings are susceptible to microbial attack [6]. Microorganisms, including bacteria, algae, and fungi, adhere to the coating surface and form biofilms [7]. Acid and enzyme production beneath the biofilm can result in selective leaching of coating components, leading to failure of the coating [8]. 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 [7].

Incralac is an ethyl methacrylate and butyl acrylate copolymer containing toluene, ethanol, benzotriazole, and epoxidized soybean oil [4]. Incralac is the most widely used coating for outdoor bronze monuments and is reported to provide protection for 2–3 years (or longer when applied with a wax topcoat) [18]. Biodeterioration of Incralac was measured using electrochemical impedance spectroscopy (EIS). EIS uses a low magnitude polarizing voltage that cycles from peak anodic to cathodic values over a range of alternating current frequencies to provide information about coating properties. EIS is a useful technique for the evaluation of the corrosion behavior of polymer coated metals [9,10]. Furthermore, EIS has been used to analyze the biodeterioration of polymers and organic coatings, including polyimides [11,12], fiber reinforced polymeric composites [13], and polyurethane coatings [14].

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## 3. Experimental

### 3.1. Isolation of microorganisms

Microorganisms were collected by swab sampling in April 2001, from the bronze monument of George Washington at Federal Hall, New York City. The bronze statue has been treated numerous times with a protective layer of Incralac. Samples were inoculated into sterile minimal salt growth medium (0.22 g $l^{-1}$ $(NH_4)_2SO_4$, 1.20 g $l^{-1}$ $KH_2PO_4$, 0.23 g $l^{-1}$ $MgSO_4{\cdot}7H_2O$, 0.25 g $l^{-1}$ $CaCl_2$, 0.024 g $l^{-1}$ yeast extract) containing cured pieces of Incralac. These enrichment cultures selected for microorganisms capable of using Incralac as a source of carbon and energy. After 10 days, growth medium from the enrichment cultures was plated on nutrient agar and microbial isolates were collected.

To insure that these microbial isolates were capable of growth on Incralac and not simply dormant organisms that had revived on nutrient agar, the microbial isolates were re-inoculated into flasks of minimal salt growth medium containing cured pieces of Incralac. Isolates capable of growth (determined visually by turbidity of cultures) were then examined using light microscopy.

### 3.2. Growth curves

Incralac was poured into the wells (1.5 cm in diameter and 0.5 cm deep) of a ceramic spot plate and allowed to cure overnight. The Incralac was removed from the wells, surface sterilized with 80% ethanol and placed into flasks containing 150 ml of minimal salt medium. Flasks were inoculated with a yeast isolate, designated GWM1, 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, CA), and rinsed with 1.0 ml deionized water [15]. Cells were enumerated using epifluorescence microscopy (BX60 Microscope with BX-FLA Reflected Light Fluorescence Attachment, Olympus, Melville, NY; 100 W Mercury Lamp, Chiu Technical Corp., Kings Park, NY; DAPI Filter cube 31000, Chroma Technology, Rockingham, VT).

### 3.3. Electrochemical impedance spectroscopy

Biodeterioration of Incralac was analyzed using electrochemical impedance spectroscopy (EIS). Stainless steel was used in our EIS cells instead of bronze or copper because of its resistance to corrosion. In order to obtain a thin coating for EIS analysis, Incralac was mixed to a final concentration of 10% with toluene and then applied with a brush to one side of 316 stainless steel coupons (5.0 cm × 5.0 cm) cleaned with 80% ethanol. The Incralac was cured overnight at room temperature. A 5.0 cm long acrylic tube (3.2 cm I.D., 3.8 cm O.D.) was then adhered to the coated coupons 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 h. EIS cells were surface sterilized using UV irradiation in a laminar flow hood, and filled with minimal salt medium. Two cells were inoculated with isolate GWM1, and two were maintained as uninoculated controls.

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 and Zview (Scribner Associates Inc., Southern Pines, NC) were used to control the instruments and collect and analyze 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 tri-electrode 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.

### 3.4. Scanning electron microscopy

Small stainless steel coupons (1.0 cm × 1.0 cm) were coated with Incralac as described above and placed in flasks of minimal salt medium. Flasks were inoculated with isolate GWM1 and shaken at 100 rpm at room temperature. After 17 days, coupons were removed for scanning electron microscopy analysis. Coupons were dehydrated using an ethanol series (40–100% ethanol at 10% increments), critical point dried (Autosamdri-815, Tousimis, Rockville, MD), gold/palladium sputter coated (Desk II Sputtering Unit, Den-

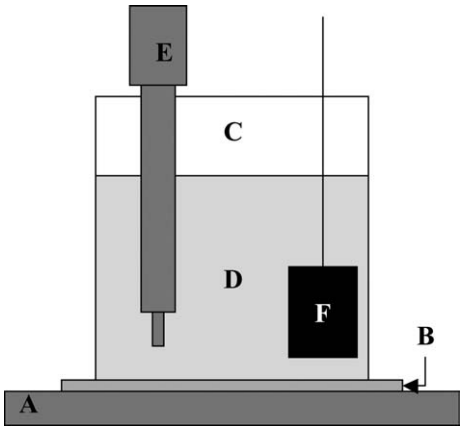


Fig. 1. Schematic diagram of an electrochemical impedance spectroscopy cell used for measuring the biodeterioration of Incralac. Cells consisted of a stainless steel coupon (A) coated with Incralac (B) and an acrylic tube adhered to the surface (C). The cell was filled with minimal salt medium (D). A tri-electrode system was used in which saturated calomel reference electrode (E) and platinum mesh counter electrode (F) were inserted into the medium and the stainless steel coupon (A) functioned as the working electrode.

ton Vacuum, Moorestown, NJ) and observed with a scanning electron microscope (LEO 982, LEO Electron Microscopy Inc., Thornwood, NY).

## 4. Results and discussion

Five microorganisms capable of growth using Incralac as a carbon and energy source were isolated. Three of the isolates were identified as yeasts and the remaining two isolates were bacteria. One of the yeast strains, designated GWM1, was used in subsequent experiments. Flasks containing Incralac as the carbon source were inoculated with ca. $5.0 \times 10^4$ cells/ml, and after one month the cell density had increased to $5.8 \times 10^4$ cells/ml, indicating a slow rate of growth in liquid culture.

Despite the failure to grow rapidly using Incralac in flasks, scanning electron microscopy demonstrated the ability of the isolate to attach and grow on the metal coated with Incralac. At low magnification (Fig. 2A), cells can be seen scattered across the surface. At high magnification (Fig. 2B), dark areas were visible surrounding attached cells indicating degradation of the coating by the yeast.

Electrochemical impedance spectroscopy (EIS) was used to quantitatively estimate the capacity of the organism to degrade Incralac. In the sterile EIS cells, Incralac showed a small decline in low frequency impedance magnitude ($|Z|_{lf}$) over the course of the experiment (Fig. 3A). In contrast, the inoculated cells initially showed a small increase in $|Z|_{lf}$ followed by a large drop in impedance within 14 d of inoculation (Fig. 3B). After the large decline in $|Z|_{lf}$ the impedance remained stable for the remainder of the experiment. A decline in the impedance magnitude of a polymer coated metal system is indicative of deterioration of the coating [11]. Our data indicate that the yeast GWM1 accelerated the deterioration of Incralac, compromising the coating within 14 days. After 14 days there was no further significant change in $|Z|_{lf}$ of the inoculated cells, suggesting that the coating had failed completely. Brostoff [18] conducted an accelerated weathering test of Incralac with a wax topcoat and examined coating performance with EIS. The accelerated physical and chemical weathering reduced Incralac performance after 70 days and resulted in complete failure after 140 days. Brostoff [18] indicated that 15 d of accelerated weathering were equivalent to one year of outdoor exposure, suggesting that rates of physical and chemical deterioration of Incralac are potentially much slower than the microbial deterioration reported in this study.

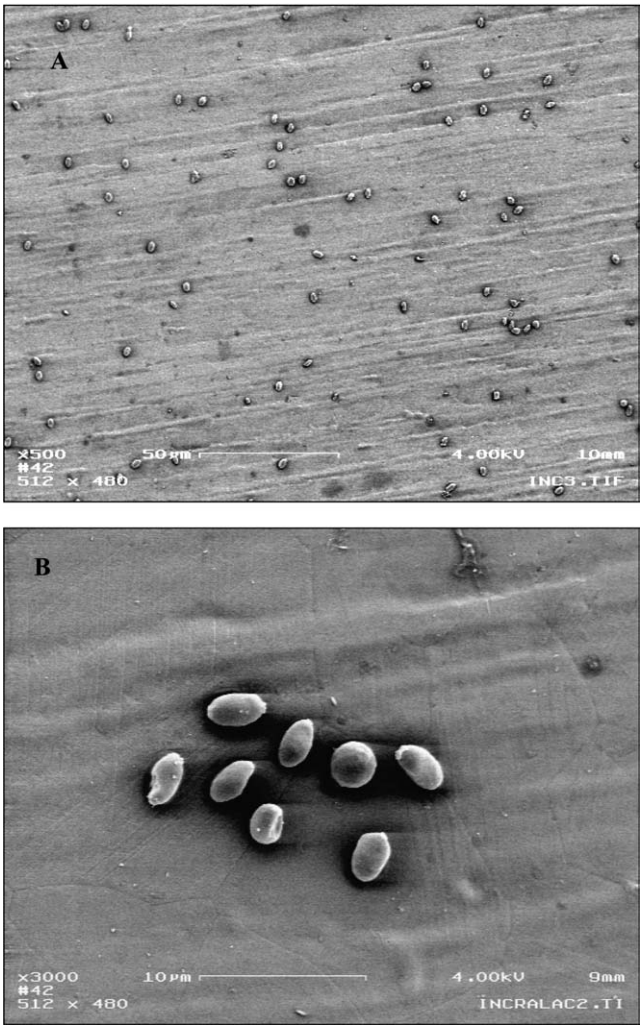


Fig. 2. Scanning electron micrograph of isolate GWM1 on the surface of Incralac coated stainless steel at 500× (A) and 3000× (B) magnification.

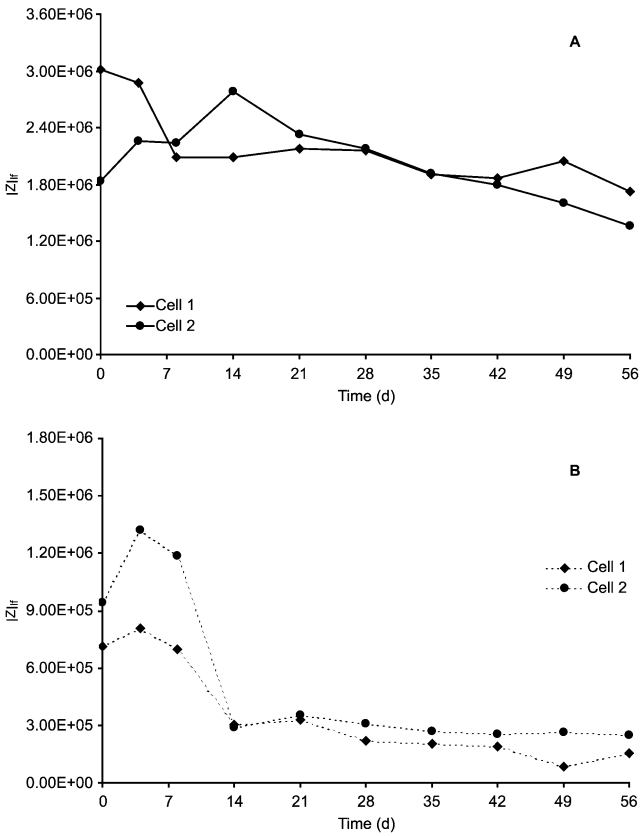


Fig. 3. Incralac low frequency impedance ($|Z|_{lf}$) response: control (A) and inoculated (B).

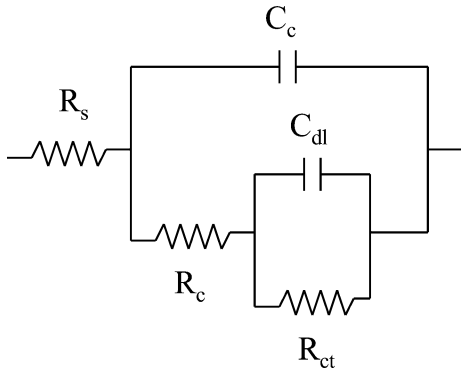


Fig. 4. Diagram of the circuit used to describe polymer coated metal systems. The circuit is composed of the solution resistance ($R_s$), coating resistance ($R_c$), coating capacitance ($C_c$), charge transfer resistance ($R_{ct}$), and double layer capacitance ($C_{dl}$).

The circuit shown in Fig. 4 describes a polymer-coated metal system [16]. In this system, the circuit is composed of the solution resistance ($R_s$), coating resistance ($R_c$), coating capacitance ($C_c$), charge transfer resistance ($R_{ct}$), and double layer capacitance ($C_{dl}$). Using our data, $R_c$ was determined for Incralac using the equivalent circuit function in Zview. Initial $R_c$ values for the Incralac coated coupons were on the order of $10^4$ ohms/cm$^2$ and had dropped to values as low as $10^3$ ohms/cm$^2$ at the conclusion of the experiment. Coatings are thought to provide poor protection once $R_c$ falls below $10^6$ ohms/cm$^2$ [8], suggesting that the protective characteristics of Incralac were relatively poor. In cases of accelerated weathering tests and in common conservation practice, the performance of Incralac is increased by the use of a wax topcoat [18]. This sacrificial layer may also improve the performance of Incralac with regards to biological degradation as it would initially form a barrier between Incralac and colonizing microorganisms. However, the wax topcoat may also provide an additional substrate for microbial growth. More research is needed to determine the extent to which wax coatings may protect Incralac from microbial biodeterioration.

## 5. Conclusions

Studies of coating degradation have shown that Incralac performs relatively well to protect metallic substrates from the effects of weathering [17,18]. However, unlike the effects of acid rain or light exposure on organic coatings, biological organisms have not commanded the same attention as other degradative factors. Our study has shown that microorganisms are capable of accelerating the deterioration of Incralac. An understanding of the limitations of the protection provided by a coating necessitate a regular maintenance program. The biodeterioration of Incralac should be considered along with the effects of weathering when developing strategies for the protection of outdoor metal monuments.

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