



The corrosion of mild steel : a new interpretation of ac impedance spectra of corroding mild steel
by Michael Lee Morrison

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Chemistry

Montana State University

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Abstract:

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A model for the evaluation of the spectra collected under various conditions was developed. This model was applied to spectra from highly controlled to uncontrolled experiments. It was found to accurately model the experimental spectra in all cases.

The model assumed, that the passivated surface and pitting region could be treated as two separate electrodes with their spectra superimposed onto each other. This was a new approach because it allowed the separation of the chemical process effects for the first time.

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of a thesis submitted by

Michael Lee Morrison

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Montana State University

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ABSTRACT

The use of ac impedance for the study of corrosion has been around for a long time. The method of interpreting the data was based on equivalent circuits that mimic the experimental ac impedance spectrum. The models developed to date all try to use the strict logic of electronic circuitry. The reaction chemistry occurring at the electrode surface has been ignored. This has caused problems when the information at frequencies greater than 10kHz are evaluated.

The effects of a biofilm on ac impedance spectra have never been evaluated. The nature of the bacteria in the biofilm and the nature of the extracellular polymer does have an effect on the spectra.

It was proposed that the information above 10kHz was a result of the formation and propagation of a pit on the surface of the 1018 mild steel coupons tested. A technique to control the location and size of a pit was developed to test this hypothesis. With this controlled pitted coupon, the effects of different conditions were examined.

A model for the evaluation of the spectra collected under various conditions was developed. This model was applied to spectra from highly controlled to uncontrolled experiments. It was found to accurately model the experimental spectra in all cases.

The model assumed that the passivated surface and pitting region could be treated as two separate electrodes with their spectra superimposed onto each other. This was a new approach because it allowed the separation of the chemical process effects for the first time.

INTRODUCTION

What Started The Process.

Experiments carried out to study the effects of microbial induced corrosion of mild steel under a mixed culture biofilm using ac impedance gave a pattern that was worth while in pursuing. AC impedance spectrum was taken from 65kHz down to 0.001Hz periodically on coupons of 1018 mild steel that had a biofilm growing on the surface.

This biofilm was formed by inoculation of the reactor with aerobic, fermenting, and sulfate reducing bacteria (SRB). The design of the mixed culture was to allow a study of the effects of SRB on mild steel in an aerobic environment. SRB's are anaerobic bacteria that metabolize sulfates to produce hydrogen sulfide. The biofilm's aerobic and fermenting bacteria reduced the oxygen concentration at the metal surface to a point where SRB's became active. This area of reduced oxygen concentration was localized under the bacteria colonies. Once the SRB becomes active they would produce hydrogen sulfide next to the surface of the coupon. The presence of H_2S accelerate the localized corrosion rate of the metal.

The ac impedance spectrum obtained during these experiments repeatedly showed a response (defined as the high frequency peak) in the range of frequencies greater than 10kHz in the Nyquist, the phase angle, and Bode plots. This response was only visible after pits began to form on the surface of a coupon. Pit formation confirmed on coupons removed from the solution to be studied with SEM/EDAX. This response continued to grow and shift to lower frequencies as the pit grew.

A method was needed to control the pit size and location on the coupon surface to evaluate these phenomena. The method would show if the peak that was observed in the high frequency range was due to the pit as we suspected, or some other cause. If it was due to the pit formation and propagation, then a better technique of monitoring the corrosion of mild steel could be developed.

The literature used the Nyquist plot to evaluate the impedance spectra. The phase angle gives more information at a glance compared to a Nyquist plot. The Nyquist plot is the plot of real impedance versus imaginary impedance. The plot of the frequency response of a simple parallel RC circuit is a semicircle with the origin as the highest frequency point. The unit most derived from the plot was the polarization resistance (R_{po}). This value could be

determined by looking at the span of the semicircle. The R_{po} value was twice the radius of the circle.

The phase angle gives more information than the Nyquist plot. The phase angle plot gives the capacitance and resistance at a glance. The position of the peak in relation to the frequency gives the capacitance. The peak at a lower frequency gave a greater capacitance value. The resistance is related to the height of the peak. The greater the height was the larger the resistance. The peak that shows up at frequencies greater than 10kHz had a capacitance of about 10^{-8} F. This capacitance was ignored until recently.

A second reason for the investigation of the higher frequency information was to determine how a biofilm affects the ac impedance spectrum taken at lower frequencies. Anomalies noted in the use of impedance data to calculate the corrosion rate did not correspond to that calculated from weight loss experiments on the same coupon.

The Methods That Might Work.

It was decided to try to find a way to passivate a metal coupon reproducibly. The problem with this was that the passive film needed to be of a similar material as what

naturally forms on the coupon surface exposed to air. The first attempt was to form an oxide coating on the surface by heating it in a flame. The resulting oxide coating was not strong enough to control all pitting on the surface. It was then decided to try to use a muffle furnace. The muffle furnace succeeded in forming an oxide that was stable enough to control where the pit formed. Many coupons were tested to determine what was happening in relation to the frequencies greater than 10kHz.

The Hypothesis.

The purpose of this study was to determine if the frequency range above 10kHz contained any useful information. It was believed that the peak at frequencies above 10kHz was the formation of localized corrosion on the surface of the coupon. A method of testing this hypothesis needed to be developed. The relation of the peak to the localized corrosion would permit the construction of a model that could explain the spectra.

A second area of interest was the effects of a biofilm on the impedance spectra. A method of testing just the biofilm was needed. This was to show what the biofilm did to the spectrum without the presence of corrosion products.

The byproduct of this work would be a method of detecting the viability of the bacteria in the biofilm.

HISTORY

Basic Method of Modeling Equivalent Circuits.

The use of ac impedance as a tool for the study of electrochemistry began shortly after radio frequency technology came into existence. It was used to study biological samples from as early as 1910. It has been used for electrochemical analysis from the twenties. The use of impedance for the study of corrosion became prevalent in the literature starting in the 1970's.

The basic method of developing the equations to calculate the impedance spectrum was to replace the components in the circuit with their equivalent mathematical formula. The formulas used are

$$Z_R = R \quad \text{Equation 1}$$

for resistance,

$$Z_C = \frac{1}{\omega C} \quad \text{Equation 2}$$

for capacitance. The method of calculating the parallel circuit is to use the formula

$$W = \frac{1}{Y_0 \sqrt{j\omega}} \quad \text{Equation 3}$$

for the Warburg's impedance. The Warburg's impedance is an impedance that takes into account the diffusion rates of the reactants and product. This appears as a type of resistance in the circuit that is frequency dependent and causes a phase shift at low frequencies. The term Y_0 in the Warburg's equation is the value that is entered for the calculation. It is the admittance associated with the Warburg's impedance. Admittance is the inverse of the impedance. The frequency in radians was ω , and j is -1 .

The method of applying the ac signal to the sample is to apply a potential to the coupon that is equal to the open circuit potential¹. This locks the electrode at the equilibrium potential preventing it from drifting. The ac signal is then superimposed on the applied potential. The ac signal is normally a sine wave with a peak to peak voltage of 10 mV. The properties of this ac signal affect the oxidation/reduction reactions that cause the open circuit potential.

The applied signal is then collected at the counter electrode and the current is amplified in the potentiostat. This signal as well as the applied signal is passed to a frequency response analyzer (FRA). The FRA then determines the magnitude of the current signal and the amount of phase

shift between the applied and the collected signals. The phase shift is reported as the phase angle. The output from the FRA is the real and imaginary impedance and the phase angle.

Randles' Work in 1947.

J.E.B. Randles was the first to propose a model for the interface between metal and solution as an equivalent circuit similar to that used today². The model was for the impedance of an electrochemical cell constructed of an amalgam of mercury and Cd/Cd^{2+} , (with many other compounds tested as well). The solution was 10^{-3}M Cd^{2+} with a 1M KNO_3 supporting electrolyte. The equivalent circuit put forth in his paper is in Figure 1. The R_c was the solution resistance, C_r and R_r were the capacitance and resistance, respectively, associated with the chemical reaction, and C_{dl} was the double layer capacitance. The electrical components C_r and R_r are not actual components in a circuit but the way the chemical reaction affects the impedance signal. The symbols used in all of the equivalent circuits are the same as that used by the author.

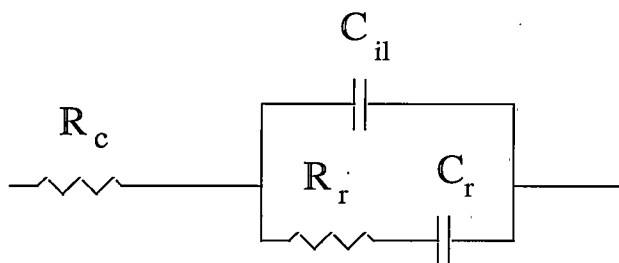


Figure 1. Randles' equivalent circuit for the impedance interpretation of a simple electrode interface.

This representation of the interface between the metal and the electrolyte split the ac impedance spectrum into two regions. The first was due to the double layer effects and the second for the chemical reaction with the charge transfer at the metal/solution interface. The reaction properties (R_r and C_r) were separated from the total ac impedance spectrum and analyzed. Randles used the data to calculate the rate constant of several metal/ion interactions at the mercury surface.

Randles derived equations for the relation of the resistance and capacitance to the chemistry occurring at the electrode's surface. The first was for capacitance in equation 4,

$$C_r = \frac{n^2 F^2 A C}{RT} \sqrt{\frac{D}{2\omega}} \quad \text{Equation 4}$$

and for resistance in equation 5,

$$R_r = \frac{RT}{n^2 F^2 A C} \left(\sqrt{\frac{2}{\omega D}} + \frac{I}{k} \right) \quad \text{Equation 5}$$

where R is the gas law constant, T is the temperature in Kelvin, n is the number of charges transferred, F is Faraday's constant, A is the area of the electrode surface, C is the concentration of the analyte, ω is the frequency used in radians, D is a diffusion coefficient for the reactants, I is the current, and k is the rate constant.

Haruyama's Initial Work.

It was not until the 70's that ac impedance was applied to the corrosion of metals seriously. Some work was done with ac impedance that led to the models now used^{3,4,5,6}. Haruyama and co-workers developed a method to monitor the corrosion by measuring ac impedance data at two frequencies⁷. He used these measurements to determine the

polarization resistance and correlate it to corrosion rate. There was discussion of problems associated with this technique in the literature⁸. The method of evaluating the data gave only the value associated with the polarization resistance. There were differences between the impedance technique and conventional methods unless the corrosion rate was determined and used to calibrate for a given system.

Mansfeld Early Work.

Mansfeld began to use ac impedance techniques to measure corrosion reactions and corrosion rates in low conductivity media in the late 1970's^{3,9}. He showed that the use of ac impedance can determine the polarization resistance and double layer capacitance data simultaneously. In a review article published in 1981³, he explains in detail how the impedance of a coated metal sample can be broken down into a set of components.

The First Equivalent Circuits Developed by Mansfeld.

The equivalent circuit is shown in Figure 2 where R_o was the resistance associated with all resistance's not part of the polarization resistance, C was the double layer capacitance, and R_p was the polarization resistance. In

this early equivalent circuit, he gave the double layer capacitance as the only capacitor in the circuit.

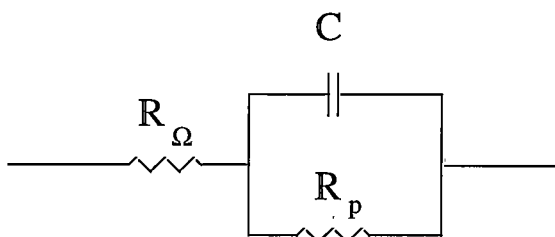


Figure 2. Mansfeld's first equivalent circuit

The fact that there was only a single capacitance in the circuit assumes that there was no chemistry occurring at the electrode/solution interface. The single resistor in parallel with the capacitance was the total resistance associated with the electrode/solution interface.

This circuit gives an impedance plot in the Nyquist configuration (Figure 3), as a semicircle formed from the response of the real and imaginary impedance. The shift of the center of the semicircle to the right is the result of the solution resistance. The radius of the semicircle is the result of the capacitance and the polarization resistance. Equation 6 gives the relationship of frequency to the capacitance and polarization resistance.

$$\omega_{Max} = \frac{1}{CR_p}$$

Equation 6

The frequency at which the highest value of the imaginary impedance reached (the mark at the top of the semicircle) was given the symbol ω_{Max} . The terms for this

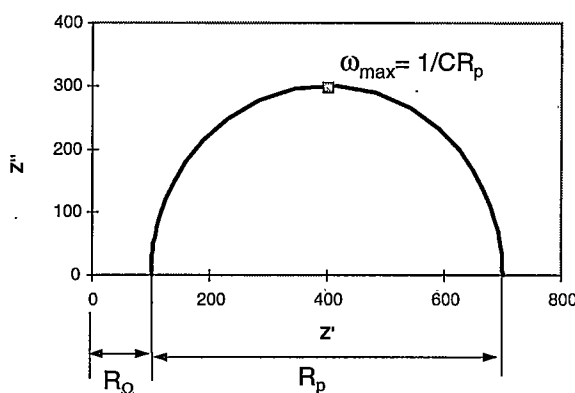


Figure 3. Nyquist plot of Mansfeld's initial equivalent circuit model.

equation are ω_{Max} is the frequency at which the imaginary impedance is maximum, C is the capacitance, and R_p is the polarization resistance. Equation 7 gives the relation of R_p to the phase angle

$$R_p = 2|Z| \tan \delta_{\text{max}} \quad \text{Equation 7}$$

where $|Z|$ is the combined impedance (both real and imaginary), and δ_{max} is the maximum value of the phase angle.

Interpretation of an ac impedance spectrum in this manner allows for the direct determination of the polarization resistance. A benefit of this analysis was the double layer capacitance. An ac impedance spectrum allows

the phase angle and the real and imaginary impedance to be calculated directly. With equation 6 and 7, the values can be calculated for C , and R_p by fitting the theoretical ac impedance spectra to the experimental spectra. This is done by inserting values into the equations derived from the equivalent circuit. The values for the components are changed until the best obtainable fit is obtained between the experimental and theoretical impedance spectra.

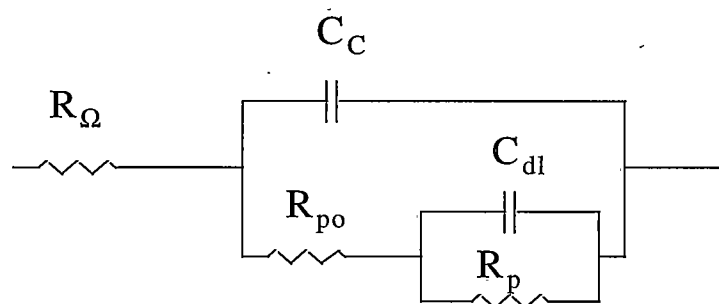


Figure 4. Mansfeld's final equivalent circuit.

Mansfeld et al. presented a better model as an equivalent circuit in 1982 (Figure 4)¹⁰. This circuit and the equation derived from it gave a cleaner fit to the data. This circuit is the basis for the analysis of the ac impedance spectra of a coated metal. The circuit is still used for the basis of all models involving corrosion of coated steels. It is the basis of models for the corrosion of aluminum as well.

Warburg's impedance was added to the equivalent circuit to account for the shape of ac impedance spectra at lower frequencies¹¹. In the Nyquist plot at frequencies below 1Hz, the response begins to rise at a constant angle of either 45 or 22.5°. The Warburg's impedance in series with the R_{po} resistor, shown in Figure 5, models this rise in the data accurately.

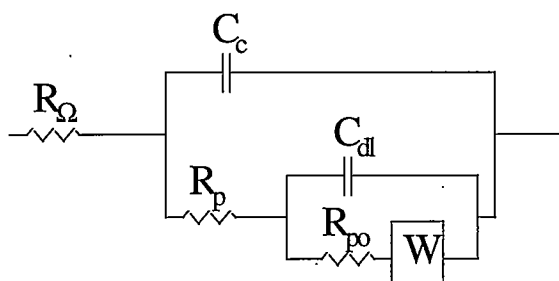


Figure 5. Mansfeld's final model corrected for the low frequency response of the data.

There were many scientists who have used this model as a basis for their work^{12,13,14}. The model gives a good explanation of the process at frequencies below 10kHz. It begins to fail when High frequency data at frequencies greater than 10kHz is included.

The Break Point Theory.

Haruyama and Hirayama put forth a theory using the break point frequency of the Bode plot to determine the area of delamination of a coated metal coupon^{15, 16}. This was the first report of an attempt to include the high frequency information as a separate component. The break point frequency was defined as the frequency at which the impedance begins to change direction.

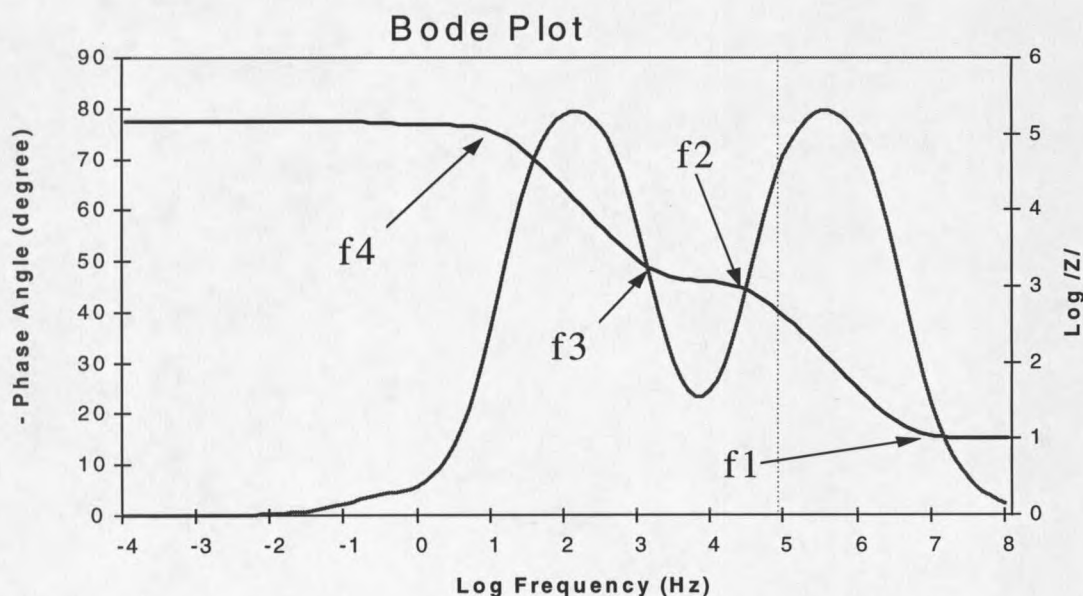


Figure 6. Theoretical data set demonstrating the break point theory.

Figure 6 shows four break points as indicated in the Bode plots of a set of theoretical data based on his equivalent circuit (Figure 7). The break point has been

defined as the point at which the phase angle crosses 45° . This occurs four times in the diagram. The break point that is of interest is f_2 . This break point of the higher frequency peak was the point from which the resistance associated with the delaminated surface was calculated.

The resistance associated with the break point f_2 and f_4 were related to the delamination of the coupons under study. A general method to determine the delamination area was not reported at the time the paper was published. The use of this technique was limited by the need to run tests with the parameters for a given set of solution, temperature and material conditions. The parameters could not be transferred from one set of conditions to the next. There was no general theory that would fit data from several different systems.

The Equivalent Circuit.

The equivalent circuit used by Haruyama is in Figure 7. The coating capacitance is C_f . The solution resistance is R_{sol} . The coating resistance is R_f . The double layer capacitance C_{dl} , and R_c is the resistance associated with the substrate/coating interface. The Warburg's impedance factor is W . The R_p is the total ionic migration through the pores.

C_{dlp} and R_{cp} are the double layer capacitance and charge transfer resistance at the bottom of a pore.

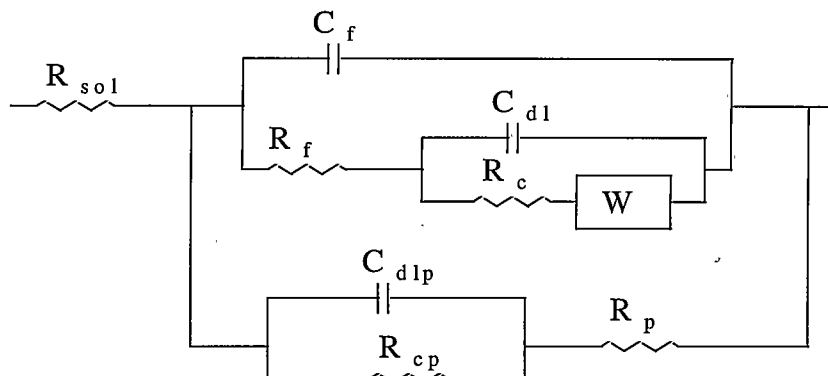


Figure 7. Haruyama's break point equivalent circuit.

The components that they used for the determination of the amount of delamination are R_p , R_{cp} , and C_{dlp} . This was the circuit that they added to their model to account for the same high frequency peak that was studied for this report. The model was developed from the basis that the current through the pit continues to affect the low frequency spectrum. This parallel circuit's values can be calculated from the frequency at which the break occurs in the Bode plot at f_2 . This model would not fit the data collected for the mild steel experiments.

Current Work with The Break Point Theory.

Mansfeld published a paper at the same time that the break point theory was updated by Haruyama¹⁷. In this paper Mansfeld reaffirms the break point theory as a method of determining the delamination area. He used an equivalent circuit in Figure 4 (page 14) as the basis for his evaluation of the theory.

Mansfeld states that the time dependency of R_{po} could be used to characterize the corrosion resistance. The corrosion resistance between different coatings was large. The reasoning for the large differences was the accumulation of corrosion products on the coating produced mechanical pressure on the coating that induced additional defects and decreased the value for R_{po} .

Deflorian et al. evaluated the break point technique¹⁸. Their evaluation of the technique stated that there were some difficulties associated with the theory. Experimental data indicated that the break point was related more to the coating defects and the porosity of the coating than the delamination area. They stated that the application of the theory would not work when the values of the coating capacitance and double layer capacitance are close.

McIntyre et al. used the break point theory to evaluate the degradation of a steel substrate protected with an aluminum alloy pretreatment covered by a polyurethane¹⁹. They reported that the break point theory gave them reliable predictions of the performance of the different coatings studied. The changes in the R_{po} correlated well with the performance of the coating.

A paper by Garrard supports the break point theory²⁰. In his report, he found that the performance of the metal and coating determined by the break point theory agreed with the pitting data obtain by separate means. There were some problems in that he stated that the break point technique gave only the uniform corrosion rates and not the pitting rate.

Problems with The Current Methods.

The two articles published by Mansfeld and Hirayama in 1991 were followed by another article²¹. This article was written by Kendig in response to Mansfeld's article. In this critic, Kendig shows the delamination for a totally delaminated surface (free film) was not 1 as assumed by Mansfeld. The delamination value for the free film from Mansfeld's data stays at about 10^{-4} throughout the experiment and did not change as reported. This causes

difficulty in the theory because the assumption of a delamination value of 1 for the free film seems to be in error. If the delamination is not 1 for the free film, then the delamination was referenced to an invalid standard.

One of the major things that was noticed in reviewing these papers is that there were a lot of math and theoretical evaluations of that math. The information was of a theoretical nature and not related back to the experimental data. The ones that gave data limited it to the correlation of pieces of the data and not the whole ac impedance spectrum. During the examination of their theoretical work, the model would not fit the experimental data collected for the entire ac impedance spectrum.

The model of a parallel circuit similar to that given in Haruyama's paper for the break point theory was attempted and could not give a good fit. No values for the parameters could be found that would satisfy the entire ac impedance spectrum. When attempts to reproduce the data that was from the papers, it was found that if calculated exactly as presented it was not the same. The reason for the pursuit of this research was to find a better model to explain the data collected.

The Use of AC Fields for The Study of Bacteria.

The effects of a biofilm on the impedance collected for a biofilm covered coupon has not been examined to date. The bacteria cause some unique problems with the collection and interpretation of ac impedance data. There is no other source of information that bridges the gap between the chemical and the biological aspects of corrosion under a biofilm.

A second reason for trying to evaluate the effect of bacteria was the possibility the impedance spectrum could be used to determine the viability of the bacteria. The viability of bacteria is defined as the ability of the bacteria to respire. This was the definition given by the microbiologist. The reason for this is that bacteria can be severely injured and remain alive, but cannot reproduce. It is believed that some of these bacteria can heal themselves. Once healed, they can reproduce and reintroduce the contamination in the system that was treated.

Current methods for the monitoring of biological activity are chemical mass balance, microbiological techniques, and microelectrodes. The method of chemical mass balance uses the rate of consumption of carbon and/or oxygen to determine biological presence and activity^{22, 23, 24, 25, 26}.

The difference in the soluble organic carbon (SOC) versus the total organic carbon (TOC) is calculated for the influent and effluent of the reactor. This difference can be correlated back to the activity of the bacteria. The greater the difference, the more active the microbes are in the system. A problem with this technique is that it may not be possible to monitor the influent and effluent to acquire the necessary TOC and SOC values. Other problems are that some of the possible changes in the SOC are due to chemical reactions and not biological activity. The changes may be too small for the instrument's resolution to detect a small cluster of biofilm.

Microbiologists use two different techniques as the primary method of detecting biological activity. The first is serial dilution of a sample followed by plate counts or most probable number counts, to find the point at which the microbes no longer grow in a medium²⁷. The second method is to directly stain the cells with a cytochemical or immunological stain²⁸. The first method can become contaminated, is time consuming, and costly. The second technique requires several hours and much sample preparation, can give false readings, and requires a controlled environment for analysis. Staining and counting methods are susceptible to the technicians bias.

A third method of monitoring biological activity is the use of microelectrodes^{29,30,31,32}. For an aerobic biofilm, the oxygen profile through the biofilm can indirectly indicate local activity of the microorganisms within the biofilm film. Problems associated with this technique are that the biofilm is heterogeneous in nature and results in readings that are not consistent across the film. This technique is not possible for field work due to the precise manipulation of the electrodes required and the delicate nature of the electrodes.

The Possible Use of Impedance to Determine Viability.

The use of frequencies greater than 100kHz for the study of biological systems has been in existence for a long time. In 1910, Hoeber applied an ac field to a biological sample³³. He found a difference in the conductivity of erythrocytes at low and very high frequencies. His theory for these phenomena was the structure of an erythrocyte was made up of a membrane surrounding a highly conductive solution. This was the first recorded verification of the theory of cell structure just being developed at the beginning of this century. In the 20's and 30's Cole and Fricke established the use of ac impedance as a tool to probe the living cell^{34,35,36}. They studied the cellular structures of many

different cells. Osterhout described the use of electrical conductivity and permeability to monitor the injury of cells, and the subsequent recovery or death of the injured cells³⁷.

H.P. Shwans Use of Frequencies Between 20GHz and 1Hz.

In the time period between the thirties and the fifties there was a lull of research activity in this area due to limitations in the equipment and manpower available. After World War II, the advances in electronics made it possible to reopen the study of cellular systems with impedance. H. P. Schwan began to evaluate the use of the dielectric permittivity, (the ratio of the capacitance measured for a given medium between two plates to the capacitance measured with a vacuum between the plates), as a method of probing the living cell^{38,39,40}.

They used a four electrode cell to apply the ac field to the sample. The reason for a four electrode cell was to eliminate any chemical effects on the working\counter electrode combination^{41,42,43,44}. Any chemical changes at the working or counter electrode will not be measured by the inner two electrodes at the high frequencies used. This gives the field strength and phase shift across the sample without chemical interference from the solution/electrode interface.

They calculated the dielectric permittivity versus frequency for many different cellular tissues. What they found were three perturbations in the dielectric permittivity. These perturbations were associated with all the various tissues, but were the most pronounced in muscle tissue. The first perturbation at around 100Hz was identified as the outer cell membrane capacitance with significant interference from solution chemistry, solution conductivity, and electrode effects. The middle perturbation, between 100kHz and 10MHz, was a Maxwell - Wagner type relaxation resulting from the charging of the cell membrane or the relaxation of enzyme bound water. The third perturbation occurred in the frequency range associated with dielectric relaxation of free water.

Schwan made it clear that all samples were freshly excised from the host. The reason for this was that the death of the tissue had a profound impact on the measured results. The perturbations that he measured in the tissue were decreased after the sample was 24 hours old or older.

The Use of Impedance to Determine The Viability of Bacteria.

The work by Asami et al. on the dielectric permittivity of yeast cells in suspension indicate that the dielectric perturbation in the range of 1MHz was due to the cytoplasmic

membrane^{45, 46, 47}. This is the membrane that controls the transport of nutrients into and waste products out of a cell. They treated the yeast with dodecyl dimethyl benzyl ammonium chloride and sodium dodecyl sulfonate to disperse the cytoplasmic membrane; results showed a marked reduction in the dielectric perturbation after the introduction of the dispersing agents.

Use of ac electric fields to study the viability of microbial cells is a more recent venture. Davey's et al. published a paper that showed that the monitoring of the cell viability is possible by measuring the dielectric permittivity of the cytoplasmic membrane^{48, 49}. They grew a yeast culture in suspension and measured the dielectric permittivity. After the culture was well established, they injected octanol to lyse the cell membrane and measured a drop in the dielectric permittivity. They verified the cells were dead by using currently accepted staining techniques.

Dielectrophoresis as a Tool to Study Bacteria.

A different technique based on the use of ac fields to affect uncharged matter was proposed by H. Pohl. In 1953, Pohl defined what the effects of a non-uniform ac field were on neutral polarizable particles^{50, 51, 52, 53, 54}. The process was given the name of *Dielectrophoresis* (DEP). DEP is the

induction of a dipole within a neutral particle. The fact that a non-uniform field is used makes the dipole uneven. The side that is closest to the point electrode has tighter lines of field than the other side. This causes the charge on the stronger side to be more concentrated and forces the particle to move in the direction of the higher field strength. The side with the highest field strength is always the same regardless of the polarity of the electrodes. This causes a particle with an induced dipole to move towards the point electrode even as the polarity of the electrode changes.

Pohl was the first to use non-uniform ac fields to separate living bacterial cells from dead cells in 1968⁵⁵. Since his first publication, the technique has been used for cell separations, cell sorting, cell characterizations and analysis. Another technique used by Pohl showed that living cells emit radio frequency ac fields^{56, 57, 58}. Small particles of BaTiO_3 are introduced to the solution and if a radio frequency in the right range is applied to them, they will have a dipole induced in them. If a dipole appears then the particles were either attracted to or repelled from the electrodes. BaTiO_4 was added as a control because no dipole could be induced in it at the frequencies under study. Bacteria were observed under a microscope to attract the BaTiO_3 . This was interpreted as the bacterium was emitting an

ac field that induced a dipole and attracted the particles. The emission of ac fields by living bacteria may be a way to determine the concentration of bacteria within a system independent of the conditions in that system.

The Possibility of Measuring Viability with AC Impedance.

The fact that there is a difference between living and dead cells in an electrochemical sense made it possible to theorize that a method of monitoring a culture directly can be developed. A cell count has been shown to be measured directly with the use of impedance measurements. This was for suspended cells only. Living cells can be separated from dead cells of the same species if the right frequency is applied. The use of impedance as a tool to monitor the viability of a biofilm is theoretically possible.

EXPERIMENTAL

The Instrumentation Used.

The instruments used to collect the data were a Schlumberger 1250 or 1255 Frequency Response Analyzer, and a PARC 273A potentiostat/galvanostat from the Princeton Applied Research Corporation. A DEC 386 16MHz computer was used to collect the data. The program used to collect the data was ZPLOT from Princeton Applied Research Corporation. The initial modeling was done with Equivalent Circuit program from the Department of Chemical Technology, University of Twente. Mathcad 5.0 was used to carry the models beyond the Equivalent Circuit program.

The programs used for the study of the viability of bacteria were written by this researcher. A copy of the program is in the **Error! Reference source not found..** The Schlumberger 1255 was modified in accordance with the technical manual to collect ac impedance spectra.

How The Coupons were Prepared.

The coupons were initially prepared by grinding in a series of steps from 320 to 600 grit wet/dry emery paper.

The grinding process was done by moving the coupon in a straight line across the emery paper then lifting it and placing it back at the starting point. This insured that the prepared surface was flat and not rounded on the edges. It also ensured that all striations were linear across the surface. The choice of 600 grit emery paper was from the industrial standards for this type of coupon. These coupons were used in the first trials of the ac impedance technique.

From these experiments, a pattern began to appear that was interesting enough to explore more completely. The literature recommended not to go above 10kHz. It stated that any data collected above 10kHz was no good because of the double layer interferences.

An ac impedance spectrum was taken from 65kHz, the maximum of a 1250 Schlumberger frequency response analyzer, connected to an EG&G Par 273. After some experimenting, the data collected above 10kHz appeared to be good data. There was a peak that appeared in the range of about 45kHz. This peak was associated with almost all the coupons that had substantial pits on them.

The coupons needed to be modified in such a manner as to retain the chemical properties of the coupons in use. Control was required to place a pit of a given size on the

surface. It was to be stable enough that the induced pit remained the only pit during the course of the experiment.

These requirements were to passivate 1018 mild steel in a manner that was consistent with the normal corrosion process. The passive layer needed to be an oxide coating similar to the naturally occurring corrosion products in the cathodic region. The coating needed to be mechanically and chemically sound. It had to remain intact for an extended period in a dilute sodium chloride solution without forming pits. The coating needed to be sturdy enough to allow the rough handling of processing it into a holder.

The first attempt was to heat the coupon in a flame until the color changed from a metallic silver to a blue. The thought was that the oxide coating generated would simulate the oxide naturally formed by a metal surface exposed to air. The difference would be in the thickness of the coating. The oxide film that formed was only a few angstroms thick and not totally uniform. The nature of the oxide film was such that the pit formation could not be effectively controlled.

An evaluation of what was learned from this set of experiments led to a new method. The method put an oxide coating that met the requirements of controlling pit formation on the surface of the coupon. 1018 mild steel coupons were prepared by grinding the first 1/16th of an inch

off the surface to be exposed to a test solution using 320 grit wet/dry emery paper. The reason for the initial grinding was to remove any crystal deformations that had occurred during the manufacture of the coupons. If these deformations were not removed they would cause streaks of dissimilar oxides on the coupon surface. .

The surface was then sanded in successive steps of 400,600,1500 grit wet/dry emery paper. At each step the coupons were moved in one direction on the paper and lifted to be returned to the position from which they started. This procedure was repeated 50 times, then the coupon rotated 90° and repeated 50 more times. This prevented the reintroduction of crystal deformations from sanding of the metal. The coupons were polished first with 1.0 micron alumina then with 0.3 micron alumina. The 0.3 micron alumina could not be used too extensively because pits would form in the surface of the coupon due to soft spots in the crystal structure of the metal.

The coupons were then cleansed in an ultrasonic cleaner with a series of solutions. The first was deionized water for 10 minutes to remove the majority of the grime from polishing. This was followed by three successive washes in 100% ETOH for 5 minutes each. The coupons were then inspected and wiped clean with a kimwipe soaked in 100% ETOH. The

reason for the last step was to ensure no particles or residues remained on the surface of the coupon. If there were any particles or residues left on the coupon surface, there would be a subsequent deformity in the oxide coating after heating.

Once the coupons were determined clean, they were placed in a muffle furnace for one hour at 450°C. This formed an oxide coating on the entire surface of the coupon approximately 1 micron thick. The temperature used to form this oxide coating was found to be critical. If the temperature was less than 350°C the oxide would not form in a tight coherent manner nor thick enough to prevent localized corrosion. If it was greater than 500°C the oxide coating would flake off as the coupon cooled. This was from the difference in the coefficient of expansion between the 1018 mild steel and the oxide coating.

The backs of the coupons were ground off to expose the mild steel for a good electrical contact. The coupons were cleaned in 2 successive fresh solutions of 100% ETOH. A copper wire was then attached to the back of the coupon with nickel print (a conductive paint). The coupon was then placed either in a PVC pipe plug that had been modified for this purpose or a polycarbonate tube.

The plug or tube was then filled with epoxy in such a way that only the polished surface of the coupon would be exposed to the test solution. This proved to be a lot harder than expected. After much trial and error, a method was developed to keep the surface clean. Using packing tape stretch over a glass plate with the adhesive side up, the coupons were stuck on the tape with the face to be exposed. The type of tape used makes a difference. If the adhesive is too soft, it will mix with the epoxy causing the epoxy to become soft and to not protect the edges as needed. The tube used to hold the coupon was then placed over the coupon and stuck to the tape. An acrylic epoxy was used to fill the tubes about half full. It was important to keep air bubbles from forming next to the coupon where it stuck to the tape.

A three power microscope was used to ensure that there were no holes in the epoxy and that the transition from oxide to epoxy was smooth. This was done to reduce the problems associated with edge effects. Edge effects are where a sharp edge collects electrical charge at a higher rate than the rest of the surface. The epoxy covered the nickel print on the back side to increase the durability of the electrical connection.

The Formation of The Pit.

A preexisting pit was formed on the exposed surface of the mounted coupons. This was done by coating the coupon surface with paraffin approximately 0.3mm thick. Slightly heating the coupon helps to get a thin, even coating of paraffin on the coupon surface.

A capillary was drawn to the size of the pit desired. The capillary was used to cut the paraffin where the pit was to be made. The paraffin was carefully removed from the cut region to expose the oxide underneath. Clark's solution was then used to dissolve the oxide coating on the exposed surface. The coupon was washed in deionized water in the ultrasonic cleaner for 5 minutes to neutralize the Clark's solution. All paraffin was removed with several washes of hexane in the ultrasonic cleaner. The number of washes varies with the thickness of the paraffin. The coupons were cleaned in 3 bathes of 100% ETOH. The procedure was carried out just before insertion into the reactor.

The Reactor Design.

The first reactor was designed for continuous flow over a biofilm with a gas layer above the bulk solution. There was

no attempt to control bacteria introduction or dissolved oxygen concentration. The dissolved oxygen concentration was continuously monitored in the bulk and under the biofilm. It was found to be at saturation in the bulk solution during the experiment. The types of bacteria inoculated into the reactor were *Pseudomonas Aeruginosa*, *Klebsiella pneumonia*, *desulfovibrio desulfurican*(SRB). This mixture of bacteria was used in an attempt to get the SRB's to become active in an oxygen rich environment.

There were 24 holes in the bottom of the channel to insert 2cm² coupons. A graphite rod was used as the counter electrode. The rod was coated with a polymer to keep the end of the rod the only reactive surface. The reference electrode was a silver\silver chloride reference electrode designed to fit between the working and the counter electrodes.

The biofilm was grown on the surface of the coupons exposed to the bulk solution. The mixed culture of bacteria used was designed to form a layered biofilm that would reduce the oxygen at the surface of the coupons to near zero. It was hoped that localized areas of no oxygen would allow the SRB to become active as they are anaerobic in nature. The SRB's became active in the later stages of the experiment.

The second reactor was designed to be completely filled with the bulk solution. A separate aeration tower was added

to be able to control the oxygen concentration in the bulk solution. This reactor was used to hold the controlled pit coupons with the pitted surface pointed downward. The reason for the downward orientation was to try to reduce the build up of corrosion products on the surface. This did not seem to make any difference.

The Solution.

The solution used was an artificial sea water. The exact makeup was reported in a paper by Lee and Neilsen^{59,60,61}. The solution for the hydrogen sulfide experiment was a 0.005M H₂S solution deoxygenated with argon.

Experimental Setup for The Study of Viability.

Instrumentation.

A Schlumberger 1255 frequency response analyzer was used to examine the two electrode reactor in the frequency range of 100kHz to 20MHz. Software written by the experimenters was used to collect and record the data from the Schlumberger. Quattro Pro was used to analyze the data.

Reactors.

The reactor was designed to have electrodes mounted in fixed positions on either side of the flow channel. This reactor design was a result of analysis of the data collected from earlier experiments that showed that the electrodes could be isolated from the solution. The instrument could still record the change in dielectric properties of the biofilm. This reactor design allowed the biofilm to grow between the two pairs of electrodes while preventing chemical interference at the solution/electrode interface.

Reactor design was such to have recirculating flow velocity of 0 to 2cm/sec through the chamber. The media was replenished continually to minimize the effects of changes in the solution conductivity and to insure the bacteria receive the right amount of nutrient continuously.

Biofilm Control and Makeup.

The nutrient used was Schneusner's minimal salts media with 15ppm glucose and 10ppm yeast extract as the carbon source. A 10L reservoir containing the nutrients was continually pumped into the reactor at a rate of 2mL per minute. Nutrient solution was not pumped into the system for

the first 24 hours to allow the culture to establish itself on the surface within the reactor.

The recirculation linear velocity was 1cm/sec. Recirculation rate was set to allow a thick biofilm to grow on the inside surface of the reactor chamber. The total system volume was 75ml. The bacteria used to inoculate the reactor were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Pseudomonas fluorescence*. The choice of bacteria was based on research at the Center for Biofilm Engineering, i.e., this combination was the standard that has been developed at the center for multiculture biofilms.

Biofilm Viability Tests.

The method of determining if the measured response was due to the bacteria only was to add a biocide to the reactor. The biocide used was 15ppm mercuric chloride. The choice of biocide was based on the need for a toxic compound that would kill the bacteria without disturbing the biofilm and maintain the cell integrity. The biocide was added to the nutrient reservoir to insure a complete kill of all microbes in the reactor. Background tests showed no response to the introduction of mercuric chloride to the reactor at 45ppm in an abiotic condition.

The method of testing for bacteria remaining in the reactor was to extract a glass tube mounted in the tubing used for recirculation. The biofilm was scraped off the inside of the tube and then analyzed using standard plate count methods. Two tubes were used, one removed just prior to introduction of biocide and the second after one hour.

RESULTS

A Restatement of The Hypothesis.

The purpose of this study was to determine if the frequency range above 10kHz contained any useful information. The belief was that this peak was related to the formation of an area of localized corrosion on the surface. A method of testing this hypothesis needed to be developed. The relation of the peak to the localized corrosion would permit the construction of a model that could explain the spectra.

A second area of interest was the effects of a biofilm on the impedance spectra. A method of testing just the biofilm was needed. This was to show what the biofilm did to the spectrum without the presence of corrosion products. The byproduct of this work would be a method of detecting the viability of the bacteria in the biofilm.

A Change in Philosophy.

The current method of evaluating ac impedance spectrum is to develop an equivalent circuit using the philosophies associated with electronics. The equivalent circuit must

exactly match what happens in a real electrical circuit that can be constructed. Haruyama's most recent equivalent circuit, (Figure 7, page 18), shows that the high frequency portion of the model had to be put parallel to the main circuit. This is consistent with the theory of electronics. This method of evaluating the spectra would not work for the data sets that were collected. A change in philosophy was to one of looking at the system from a chemical aspect first. The goal was to find a circuit that could model the data (with chemistry as the driving force) and not forcing the chemistry to follow the rules of electronics.

The picture of an electrode's solution/metal interface structure was documented many time in the literature. There is a double layer that contains the analyte right on the surface or within a few angstroms of the surface. This double layer interface will have a capacitance associated with it. The capacitance is the result of the ion build up in the double layer to accommodate the charge of the metal. There is a charge transfer resistance from the metal out into the double layer. This is due to the energy required to transfer an electron to an ion in the double layer and the diffusion resistance of the ions that transport the electrons into the bulk solution. This occurs at all electrode/solution interfaces. The charge transfer

resistance can be viewed as a resistor in an equivalent circuit. The equivalent circuit for the double layer is set up as a leaky capacitor, a capacitor in parallel with a resistor.

The chemical reaction consists of a capacitive and a resistive component. These were determined from the evaluation of the ac impedance spectrum data that is collected from a simple electrode system. A capacitor is required because of the build up of products within the double layer and the diffusion of the products away from the surface. The resistance component is the diffusion characteristics associated with the reactants. There is a diffusion coefficient associated with both the reactants and the products.

Randles developed a good representation of the process occurring at the electrode surface. The problem with his proposal was that he did not detect the charge transfer resistance associated with the double layer. He assumed that the capacitive and resistive components associated with the chemical reaction were in series. This was the same as saying that the charge transfer resistance and the chemical resistance were combined into one resistance. It was later determined that the chemical components were in parallel with each other. The double layer charge transfer

resistance was placed in series with the chemical components. The double layer capacitance was placed in parallel with the other components, since the chemical components were a part of the double layer, and the charge transfer resistance is the resistance across the double layer.

The Basic Unit Equivalent Circuit.

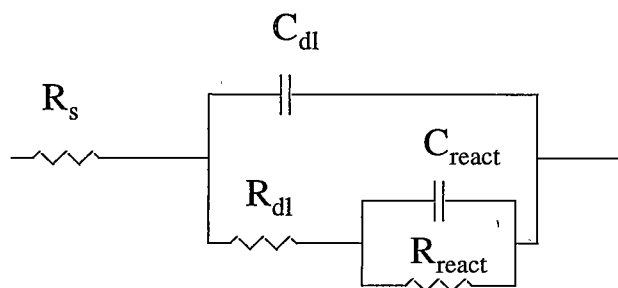


Figure 8. Equivalent circuit for the basic unit of the model.

The basic model for a simple electrode in contact with a solution would be the equivalent circuit diagrammed in Figure 8. The resistance associated with the solution is R_s . The capacitance associated with the double layer is C_{dl} . The charge transfer resistance across the metal/solution interface is R_{dl} . The reaction equivalent capacitance that

is from the diffusion characteristics of the reactants and products is C_{react} . The charge transfer resistance associated with the transfer of an electron to the reactants is R_{react} .

This is the basic circuit from which many of the models reported in the papers were derived. The model was changed to meet with the differing view of the chemical process on the coupon's surface. The double layer capacitance, C_{dl} , is reported as the coating capacitance in the other models. C_{creat} and R_{react} are the double layer factors in the other models. This basic model will be used to give a new interpretation of what is happening at the metal/solution interface of a corroding mild steel surface.

The equation for this simple case is given in Equation 8. The variables given in equation are the same as the component names in Figure 8.

$$Z = \frac{1}{j\omega C_{dl} + \frac{1}{R_{dl} + \frac{1}{j\omega C_{\text{creat}} + \frac{1}{R_{\text{react}}}}}} \quad \text{Equation 8}$$

This was the equation used as the basis for all of the modeling in this report. This was a change of philosophy on the makeup of the solution/metal interface.

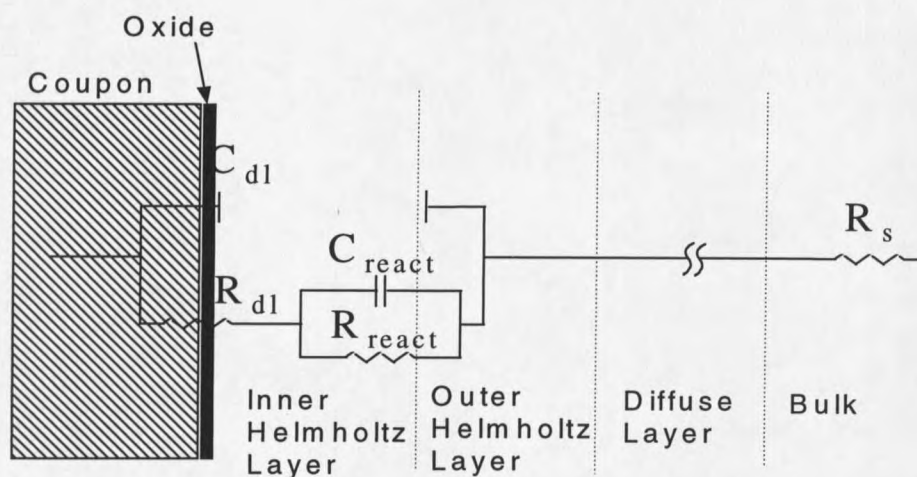


Figure 9. Overlay of the equivalent circuit onto a representation of a blank coupon surface.

Overlaying the equivalent circuit over a diagram of the surface interface of the metal gives more meaning to what is proposed. Figure 9 is the overlay diagram. The basic circuit does mimic what would be expected if it were an actual circuit.

The Evaluation of a Blank Coupon.

The data in Figure 10 is of a mild steel coupon that was treated for an hour in a muffle furnace. The equivalent circuit used to fit this data was the basic circuit as defined in Equation 8. The fit was good with the parameters given in the graph. The fit in the high frequency region was not great between the experimental and theoretical data. This was because of a minor inductance that affected the spectra at frequencies greater than 10kHz. Tests indicate

that this was inherent of the instrument and the nature of mild steel. The model does not account for this inductance. The correlation between the experimental and theoretical curves was greater than 0.999.

The ac impedance spectrum from the blank is indicative of a single double layer and chemical reaction occurring on the coupon's surface as defined in Figure 8. If this was equivalent to a mild steel coupon that has only a passive layer over the surface, the model would indicate that the chemistry on the entire surface of the coupon was the same. This would then lead to the conclusion that there was no corrosion of the metal by the solution. Since no observable corrosion of the coupon occurred while in the test solutions, the conclusion was considered sound.

Explanation of the Major Figures.

Figure 10 and the subsequent figures containing impedance data have a lot of information in them. The three graphs are the three major methods of viewing ac impedance spectra. The phase angle plot is the shift of phase between the current signal and the applied potential. A capacitor causes a shift of the phase angle 90° behind the applied phase. A resistor has no effect on the applied phase and

therefore has a phase shift of 0° . An inductor causes a shift in the phase to be ahead of the applied sine wave.

The phase angle was plotted as the negative of the measured phase shift to make the graph conform to the standard method of reporting this type of data. The information that can be read from this type of graph was the relative capacitance and resistance. The position of the peak corresponds to the capacitance, (the lower in frequency the higher the capacitance). The height of the peak is related to the resistance, (the lower the height the smaller the resistance).

The second representation is the Bode plot. This is the log of the impedance of the system versus the log of the frequency. This plot basically gives the total resistance of the system under study at a given frequency. The value that you can read from the high frequency end of the graph is the solution resistance. The resistance at the shoulder corresponds with the resistance associated with the parallel portion of the equivalent circuit. The method of calculating the impedance is given in Equation 9.

$$Z = \sqrt{Z'^2 + Z''^2}$$

Equation 9

The third graph is a Nyquist plot. It is the real impedance versus the imaginary impedance. This should be a perfect semicircle if the system under study is a resistor in parallel with a capacitor. The Nyquist plot is similar to a Cole-Cole plot. The Cole-Cole plot is a graph of the real portion of the dielectric plotted against the imaginary part of the dielectric.

The equation used to make the ac impedance spectrum and the values used for the graph are given. This information was given in the figure to consolidate all of the information from one experiment in one place. The values that are given are consistent with the value reported in the literature. The capacitance for the double layer should be around $60\text{microfarads/cm}^2$. The capacitance is low in comparison to later spectra, as was predicted. The values in the figures are not corrected to account for area.

The last thing included in the figure is a representation of the equivalent circuit used as a basis for the model. The three main components in this circuit are the solution resistance, R_s , the metal/solution interface circuit, and a Warburg's impedance element. These components are in series to imitate our view of the structure of the surface of the metal. Solution resistance is definitely in series to the rest of the components. The

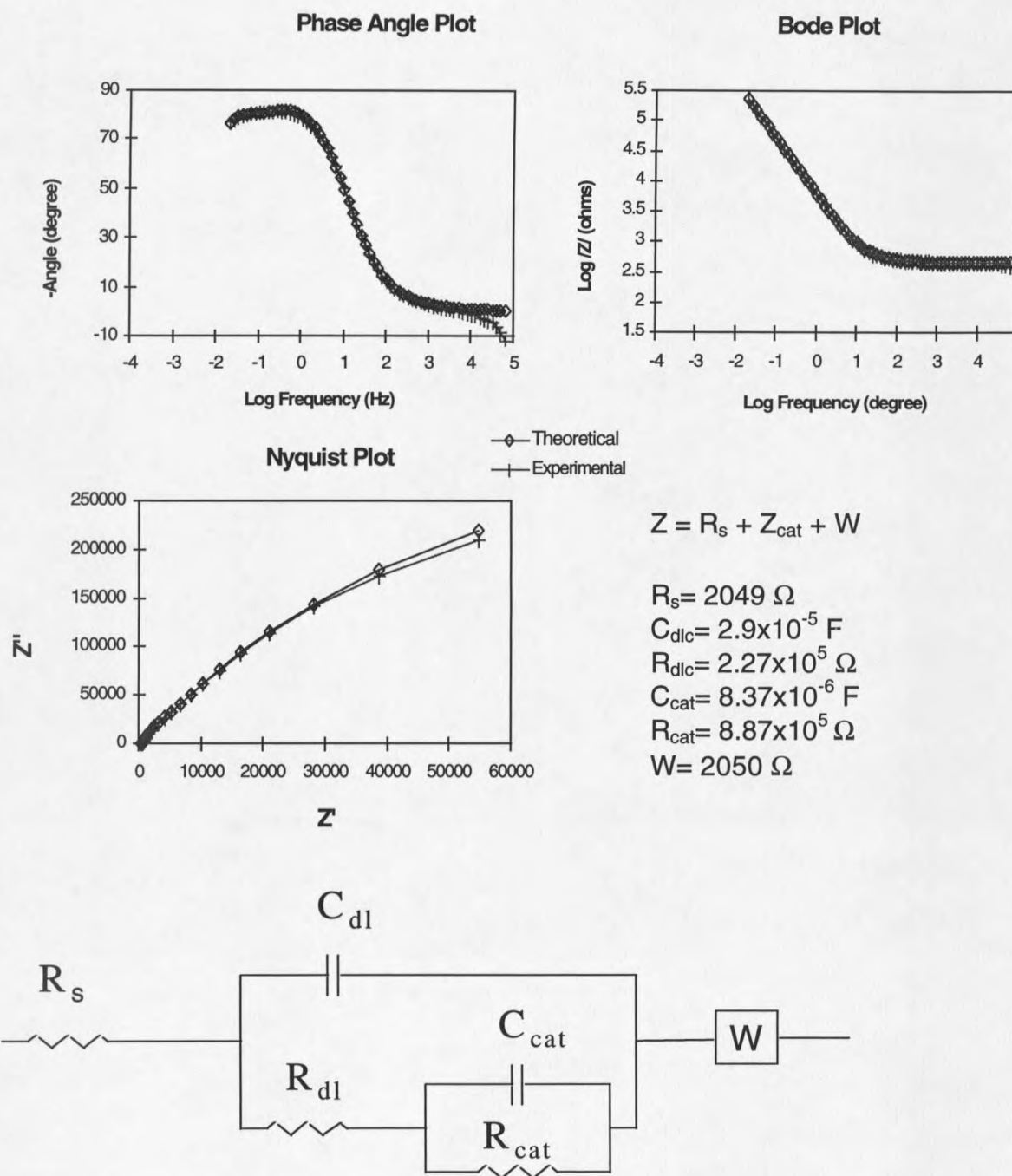


Figure 10. The model for an oxide coated coupon with no pits in the coating. The Bode, phase angle and Nyquist plots are given with both the experimental data and the theoretical data. The equation used and the equivalent circuit is given.

Warburg's impedance was put in series to simulate the assumption that only cathodic chemical reactions are occurring at the surface of the coupon. The assumption was that the diffusion of the reactants and the products would be the same across the entire surface of the coupon. The Warburg's impedance then was in series with the surface of the coupon based on this assumption.

The Relevance of The High Frequency Peak.

The purpose of developing a procedure to control the pit on the coupon's exposed surface was to determine if the information at frequencies greater than 10kHz resulted from the pit. Figure 11 is of a series of coupons that had a single pit of the size indicated on the surface. A blank coupon was added to ensure that the signal does not appear if there is not a pit present. The graph indicates that the first part of the hypothesis is correct. There is a correlation of the high frequency peak to the presence of a pit.

Data was collected on a coupon with a 3mm pit in the coating, but it reacted as if the pit was only 0.5mm in diameter. When the coupon was removed, there were two small pits within the exposed surface each of about 0.2mm in

diameter. There was a limit to the pit size for this type of coupon. This limit was due to the ratio of the anodic and cathodic regions. If the anodic region is made large enough with comparison to the cathodic region, then the chemistry will attain an equilibrium with a smaller anodic region.

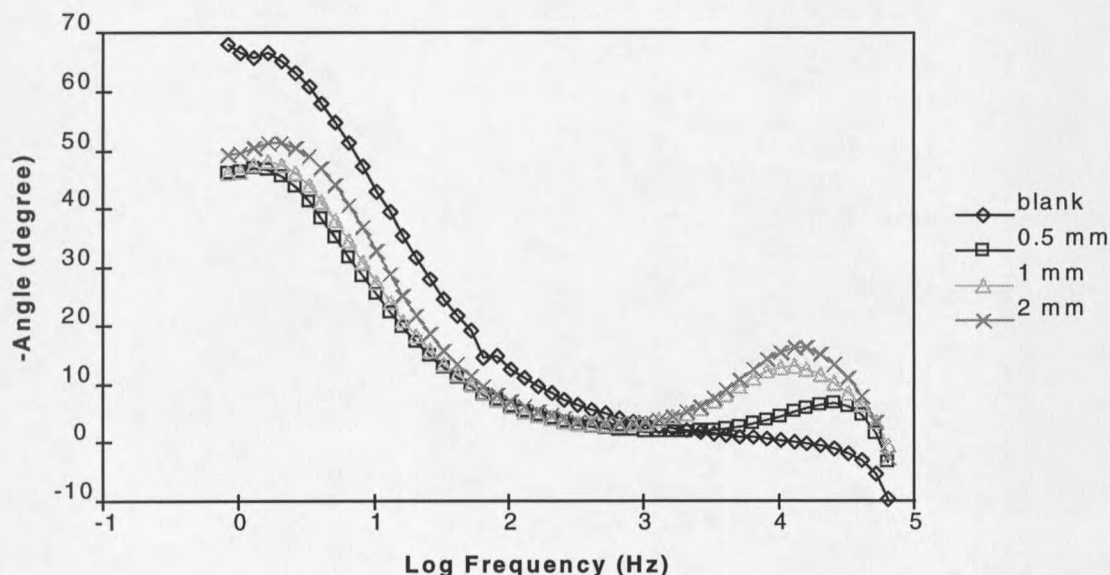


Figure 11. Series of known diameter pits on the surface of oxide coated coupons.

This was the result of the equilibrium shifting from having the anodic chemistry the limiting factor to that of the cathodic chemistry.

The data showed that a pit caused a deviation in the spectra above 10kHz if the anodic region had adequate size. The limitations of the instrumentation made it necessary to put a single anodic surface on the coupon. This was

required to bring the shift in the impedance low enough to be detected and to control the area of the anodic region. By controlling the anodic region's size and location, it was shown that the occurrence of the peak at frequencies greater than 10kHz was related to the anodic region. Now the spectra can be divided into two regions, a cathodic and an anodic region. A model of this process could lead to a method of directly determining the corrosion rate that was accurate. The method would be non destructive to the sample coupon, and allows the direct interpretation of the pit formation and propagation. Such a method would be very useful to industry for the control of corrosion. This new method would use the area associated with the anodic region's capacitance to determine the extent of pitting.

Model for Pitted Surface.

The model that was developed for the interpretation of this phenomenon is a shift from the path that others have taken. The assumption was that the chemistries on the surface were connected, but not in a way that is consistent with electronic theory. The assumption was that the anodic and cathodic regions could be treated as two separate electrodes.

This would mean that the ac impedance was a sum of two or more separate processes. A good reproduction of the experimental ac impedance spectra was calculated using the basic equivalent circuit for each region and summing them together. This was the case. The direction the theory was going was promising.

Figure 12 is the result of a calculation based on this assumption with the corresponding experimental data. The high frequency and low frequency data were calculated simultaneously. The total equation used for this model was

$$Z = R_s + \frac{1}{j\omega C_{dlc} + \frac{1}{R_{dlc} + \frac{1}{j\omega C_{cat} + \frac{1}{R_{cat} + W}}}} + \frac{1}{j\omega C_{dla} + \frac{1}{R_{dla} + \frac{1}{j\omega C_{an} + \frac{1}{R_{ant}}}}} \quad \text{Equation 10.}$$

The factors in the equation are the same as that given in the equivalent circuit diagram of Figure 12. Data from the single pit experiment. The pit size was 2mm in diameter. The subscript a and c were added to distinguish the anodic and cathodic processes double layer phenomenon. Subscript

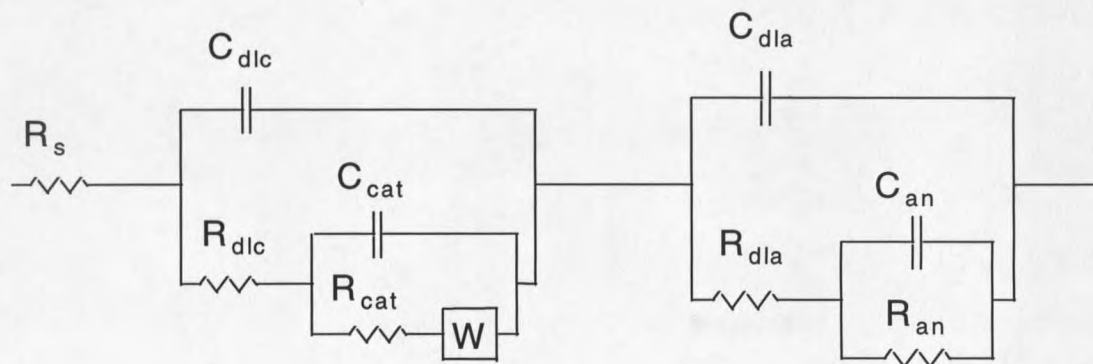
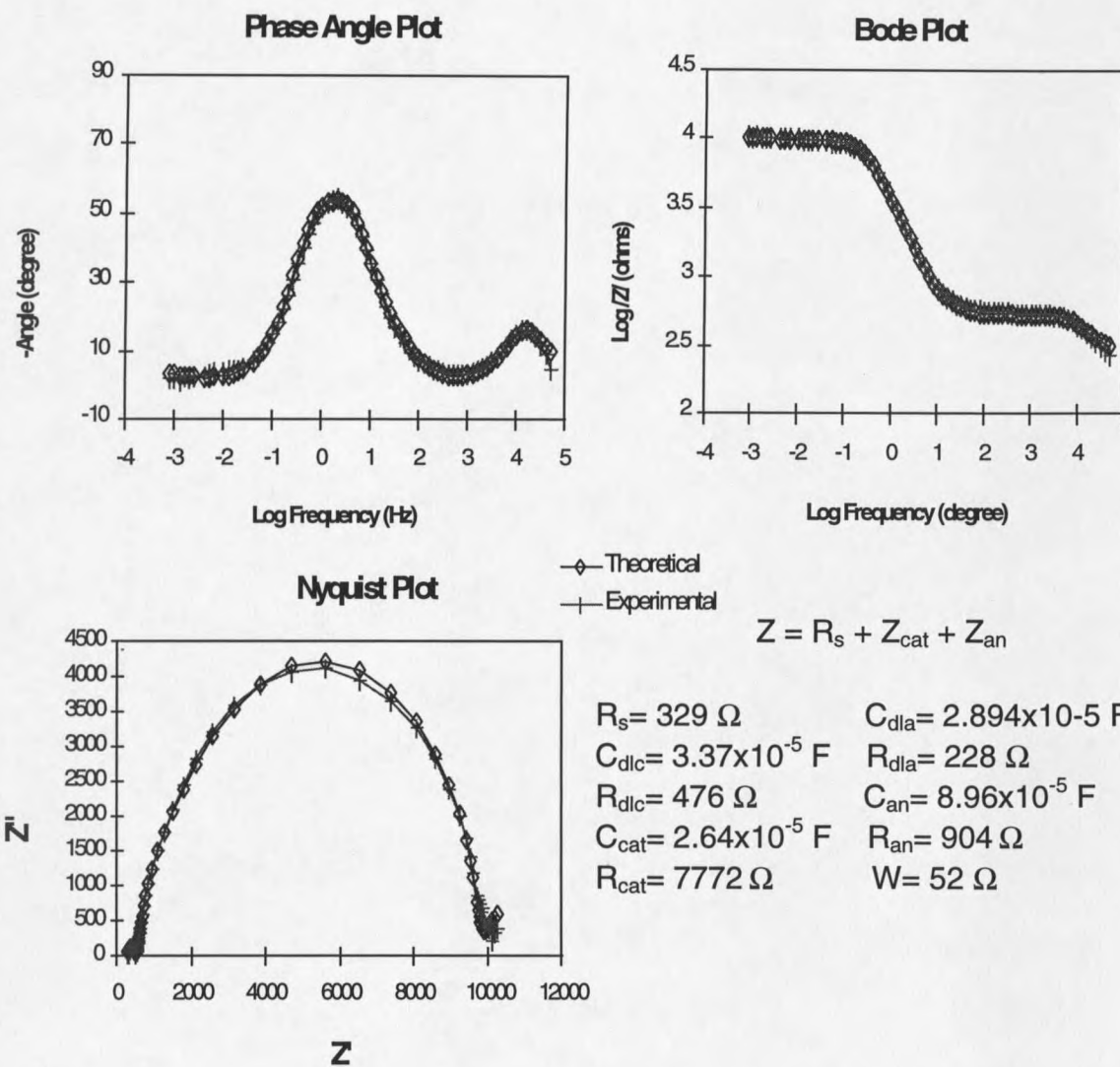


Figure 12. Data from the single pit experiment. The pit size was 2mm in diameter.

cat and an are used to identify the cathodic and anodic parameters.

The resulting correlation between the theoretical and experimental data was 0.999. What this meant was that the assumptions made about the views of the system were justified. The assumptions were not consistent with pure electronic theory. It was necessary to have to look at what is happening at the surface in regard to the chemistry first, and then deduce how it would affect the ac impedance spectrum. The assumption that there are two separate regions on the surface of the coupon, the anodic and cathodic regions, models the system.

The chemistry occurring in the anodic region is the dissolution of iron into the solution. A concentrated solution chemistry containing a larger number of anions than the bulk solution is found in the immediate area of the anodic region. This is because of the large number of cations going into solution from the metal. The reaction of the iron ions with water causes the pH within the pit to drop to a range of about 5.5, consistent with the pH obtained from the reaction of Fe(II) with water. This was consistent with the pH measured near the coupon's surface with a microelectrode.

The chemistry at the cathodic region is most probably the reduction of oxygen and its byproducts for an aerobic system. The literature is full of many differing opinions as to the exact mechanism of the chemistry occurring at the cathode. The consensus seems to conclude that a series of intermediates is present as the reaction proceeds. The series of steps could include O_2 to a super oxide, then to a peroxide, then to a hydroxide. The pH in the solution directly above the cathodic region was measured at 9.5. This was consistent to what $Fe(OH)_2$ dissolved in water would have been. This was the values that were measured at the surface with a microelectrode.

The separating of the two regions and treating each as a separate electrode was based on the rates of reaction. The most probable reaction occurring at the anode is $Fe(II) \rightleftharpoons Fe$. This reaction would occur very fast. The only influence this reaction would have on the spectra would be at high frequencies. If the frequency was slower than the reaction rate, the only thing measured would be the diffusion of the products out into the bulk.

At high frequencies the chemistry occurring at the cathode would not have much of an effect. There would not be enough of a perturbation in the active species to cause a change in the spectrum. The reaction rate for the cathodic

species is much slower than the anodic species. The double layer associated with the anodic region would have no real effect at the lower frequencies because it would saturate quickly.

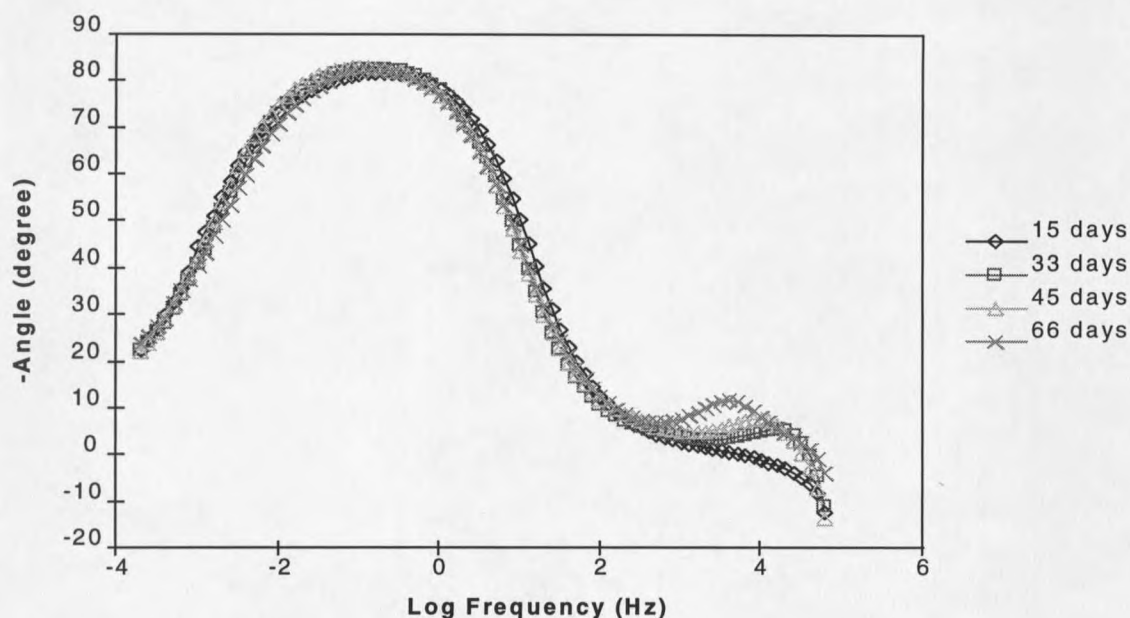


Figure 13. Series of ac impedance spectrum of a blank oxide coated coupon that failed with the formation of a single pit on the surface. This set of data illustrates the change in the high frequency peak as the pit grows.

Figure 13 was a series of spectra for a blank coupon that had a localized corrosion area form in the coating. Three weeks into the run a pit formed in the oxide coating. The data sets show how the phase angle spectrum changed over time as the localized corrosion grew. In the high frequency end of the spectra, a peak forms over time. The formation

and movement of the high frequency peak were the same as that observed in impedance spectra taken earlier with the biofilm experiments. This was also the same type of peak observed with the controlled pit experiments. This was further evidence that the information at frequencies greater than 10kHz could be useful.

The Effects of an Artificial Film.

The model needed to be extended to a point to see if it would work for a system that contains a biofilm. Experiments were done to expand the understanding of how a biofilm affects the corrosion of mild steel. AC impedance was tested to see if it could be useful for this study. The impedance spectrum was only one of many different techniques used on the coupons under study.

In the first attempt to use the new coupons, the pit was covered with a 2% agar gel with the same concentration of salt as the bulk solution. The theory was that the agar would simulate the presence of a biofilm.

The agar was placed over just the pit to try to imitate the conditions under the biofilm. Research had shown that the biofilm in nature was not a homogeneous mass of bacteria and extra cellular polymer (ECP). This was consistent with

the model of the biofilm being heterogeneous. The way the biofilm was heterogeneous was that the bacteria formed clusters in the ECP. Channels formed around the clusters to allow nutrients to reach a cluster from most directions. The channels and clusters allowed for the formation of concentration gradients that induced galvanic corrosion of the metal.

To simulate this, the cathodic surface was left uncovered to insure the agar did not impede the diffusion of oxygen to the surface. The agar was over the exposed surface to simulate the presence of a cluster. The cluster impedes the diffusion of the corrosion products and oxygen causing a tubercle to form over the pit at a higher rate than in an abiotic system. This was the theory at the time these experiments were conducted. The agar did give an ac impedance spectrum that was similar to a biofilm's ac impedance spectrum. The ac impedance spectrum was flattened out in a manner similar to what was observed with coupons exposed to a biofilm.

The importance of the clusters within the biofilm is that a galvanic cell can be formed. The concentration of oxygen at the cathodic region is greater than at the anodic region. This concentration gradient allows for necessary conditions for galvanic corrosion to occur. If there was no

gradient, then the electrochemical cell would not be a complete circuit and no localized corrosion can occur.

Modification of The Model to Fit a Simulated Biofilm.

The ac impedance spectrum and data for a coupon with a 2mm pit covered in agar is in Figure 14. The ac impedance spectrum has been smoothed over and flattened. The model for the uncoated coupon would not fit this data. Two more components were added to account for the changes. The first was a parallel RC circuit to simulate the capacitive effect of the agar. The agar would have a concentration gradient within it causing it to act as if it were a leaky capacitor described earlier.

The second component was a hyperbolic-tangent function that was developed to account for the change in the diffusion of the active species. The function is given in Equation 11.

$$Z_o = \frac{T a n h \left[\frac{B}{\sqrt{j \omega}} \right]}{Y_o \sqrt{j \omega}} \quad \text{Equation 11}$$

The impedance that accounted for changes in diffusion was labeled Z_o . B contains diffusion constants and dimensions of the coating, and Y_o contains the diffusion constant and material constants. The Z_o element was inserted to account

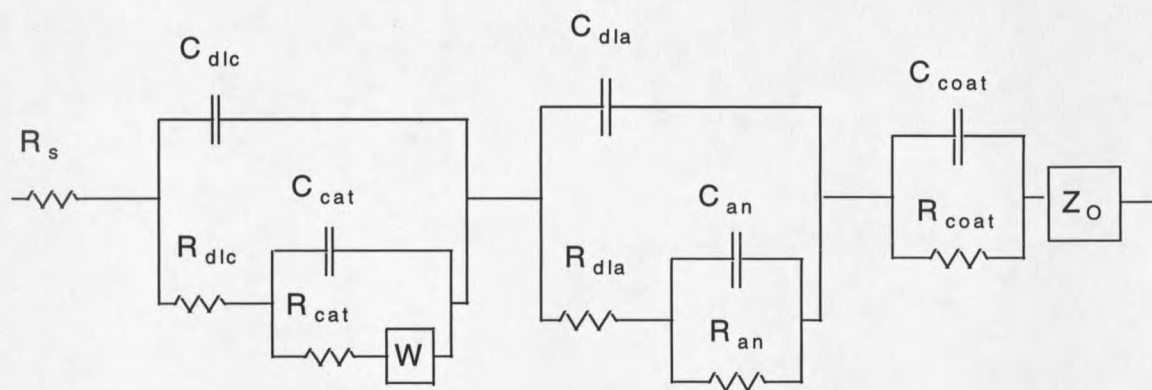
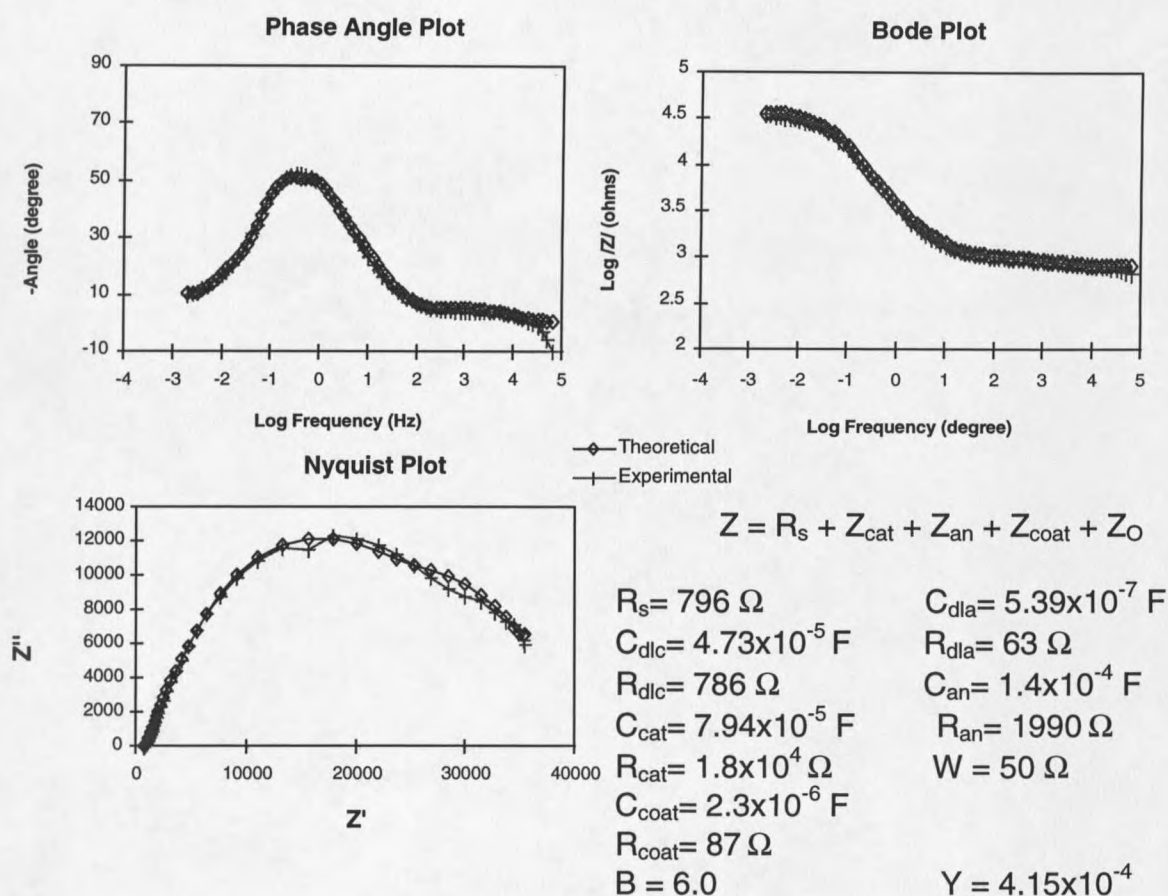


Figure 14. Data set for a coupon with an artificial biofilm before corrosion products were present.

for the finite length diffusion imposed by a boundary that fixes the concentration or activity for the diffusing species. This was the factor that caused the impedance spectrum to become flattened.

The addition of this element was based on the methods used by the program Equivalent Circuit written by Bernard A. Boukamp. This program was the starting point for all the models shown in this paper. The interpretation of the value of B was that if it was a large value then the information could be taken as a Warburg's impedance. If it was a small value then the information could be treated as a parallel RC circuit. The values reported for B are not large enough nor small enough to be considered a RC circuit or a Warburg's impedance.

The hyperbolic-tangent function deals with the finite diffusion associated with a boundary that imposes a fixed concentration of the diffusing species. The boundary is permeable for the diffusing specie, but impedes their transport to the surface of the electrode. The example that would work to demonstrate this concept is the diffusion of oxygen through a membrane in the Clark's dissolved oxygen probe.

The addition of these components was based on the assumption that the EPC would affect both the anodic and

cathodic regions. The agar would impede the diffusion of oxygen at the point where the agar overlaps onto the oxide/cathodic region.

An Artificial Film with Corrosion Products.

The data presented in Figure 15 is of an agar coated pitted coupon after two weeks. The agar was filled with corrosion products to the point of forming a tubercle. This added another component to the model as a parallel RC circuit. The added RC circuit was necessary to include the effects of the corrosion products on the ac impedance spectrum. Fifty percent of the agar had dissolved into the bulk solution.

The corrosion products exhibit the equivalent circuit of a parallel RC circuit because of the dielectric effect. The iron compounds in the tubercle can be polarized. The induced dipole of the iron compounds would align themselves in such a manner as to oppose the electric field. The flipping of these dipoles causes a phase shift and a resistance to the electric field. The result was an enhancement of overall capacitance and resistance to the system at the resonance frequencies of the iron compounds.

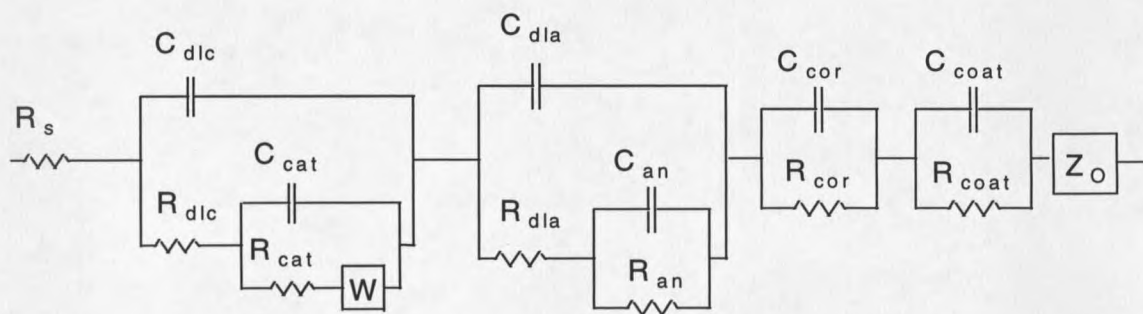
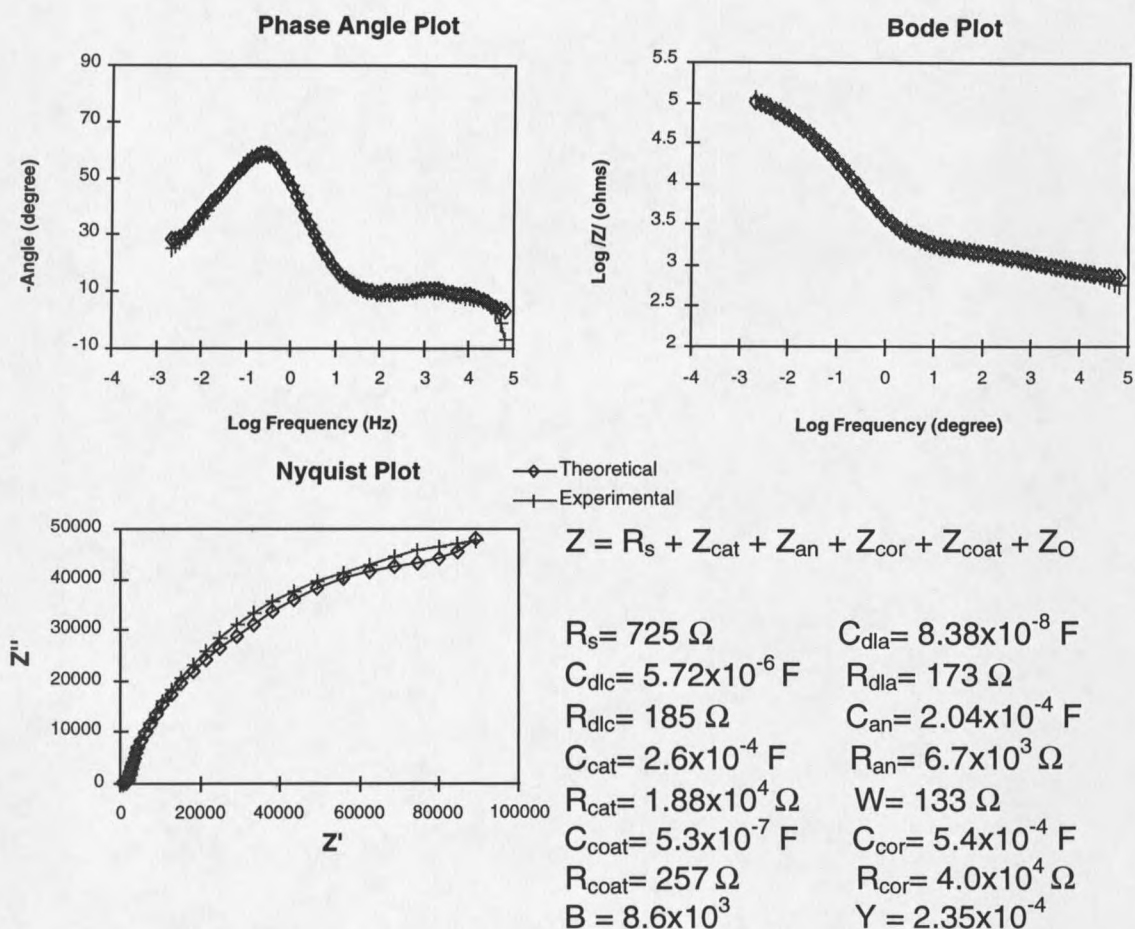


Figure 15. The fitted curves with the equation and equivalent circuit of the model for an artificial biofilm with corrosion products in the agar.

There was a build up of material on the surface of the coupon that looked similar to corrosion products. This buildup covered the entire coupon at the time this data set was taken. Since the corrosion products covered the entire coupon, the RC circuit for the corrosion products was placed in series with the other components. The spectrum that was created was a summation of the many differing processes occurring between the electrodes.

The Justification for The Changes.

The comparison of the theoretical and experimental data shows a close fit. A model that fit a corroding coupon with a known pit and an artificial biofilm has been presented. This model was considered to be a reasonable representation of the systems studied since it incorporated the previous model and still fits so well.

An explanation for each of the components conforms to the overall view of the processes occurring at the surface. The introduction of two new factors to modify the ac impedance spectra coincides with the conditions used to develop the theory. There is a RC time constant associated with the agar due to the ions within it. The change in the diffusion characteristics of the active species is due to

the hindrance in the diffuse layer by the agar. The agar concentrates some species at the surface under it.

Effects of a Mixed Culture Biofilm Under Aerobic Conditions.

The Biofilm without Corrosion Products.

The purpose of this work was to see if it could be applied to a biofilm covering mild steel coupons. The next two sets of data are the application of the model for an artificial biofilm to systems that contain biofilms. The data was from coupons treated with the flame method. The data sets are presented in Figure 16 and Figure 17.

The coupons were mounted in the first reactor described in the experimental chapter. The early biofilm coupon was in the reactor for 2 weeks. This was long enough to grow a biofilm about 50 μm in thickness. The biofilm was not uniform on the coupon's surface, but covered the surface completely.

The corrosion products that had formed up to this time were removed by the recirculation of the bulk solution. The model that should be able to fit this data set was the model for the artificial biofilm without corrosion products. The

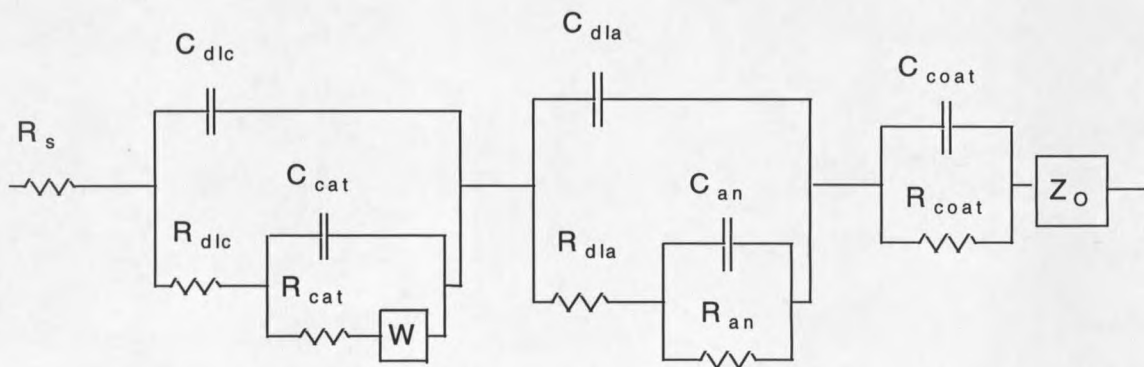
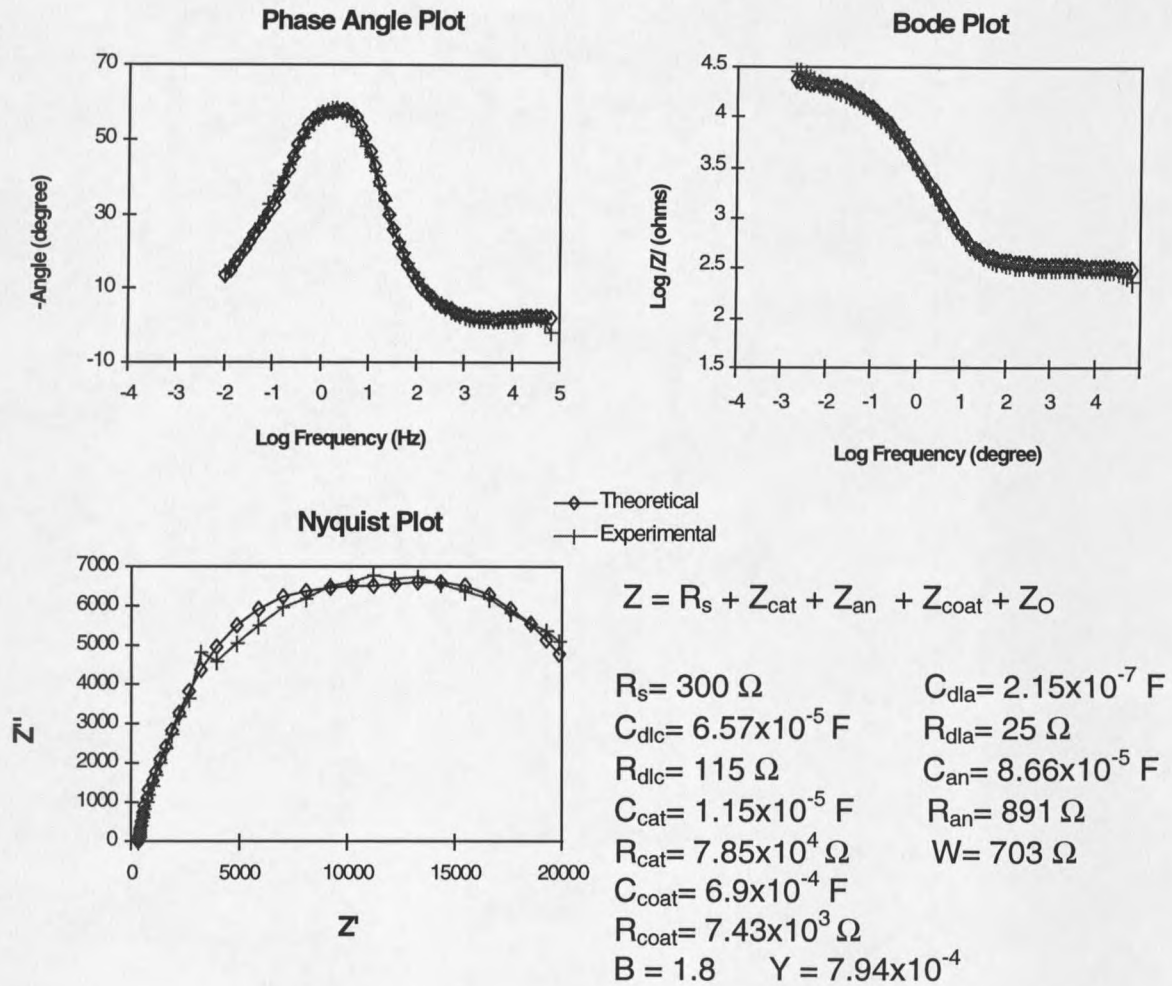


Figure 16. Fitted data set for a biofilm coated coupon in the early stages. The biofilm was about 50 microns thick.

model for the artificial biofilm without corrosion products was fitted to the experimental data as shown in Figure 16.

The model fit the experimental data without the corrosion products present with a correlation of 0.999. The fitted data points closely match those of the experimental data. This was a major step in the development of this theory. No other model reported to date could account for the effects of a biofilm on the ac impedance spectra.

The Model Applied to a Biofilm with Corrosion.

The model for an artificial biofilm with corrosion products was applied to the biofilm when it had grown thick and was full of corrosion products. The data is given in Figure 17. The model for an artificial biofilm with corrosion products fits to a correlation of 0.994. The data gave a good correlation because of the even distribution of data points above and below the experimental data set. The main reason that it was not a close fit was the presence of the bacteria.

The bacteria come in many shapes and sizes. The fact that they are polarizable makes them able to affect the spectrum. Due to this variety of sizes and shapes they affect most frequencies in the test range. The factor to account for this phenomenon has not been found. The fit is

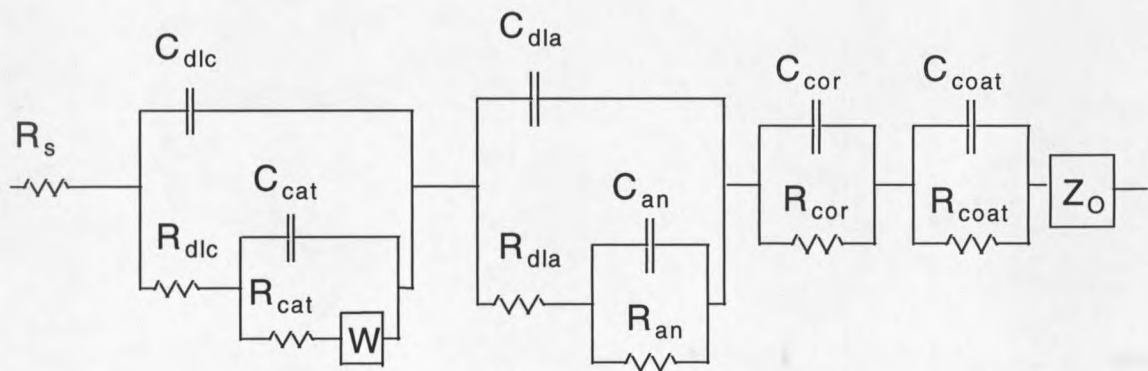
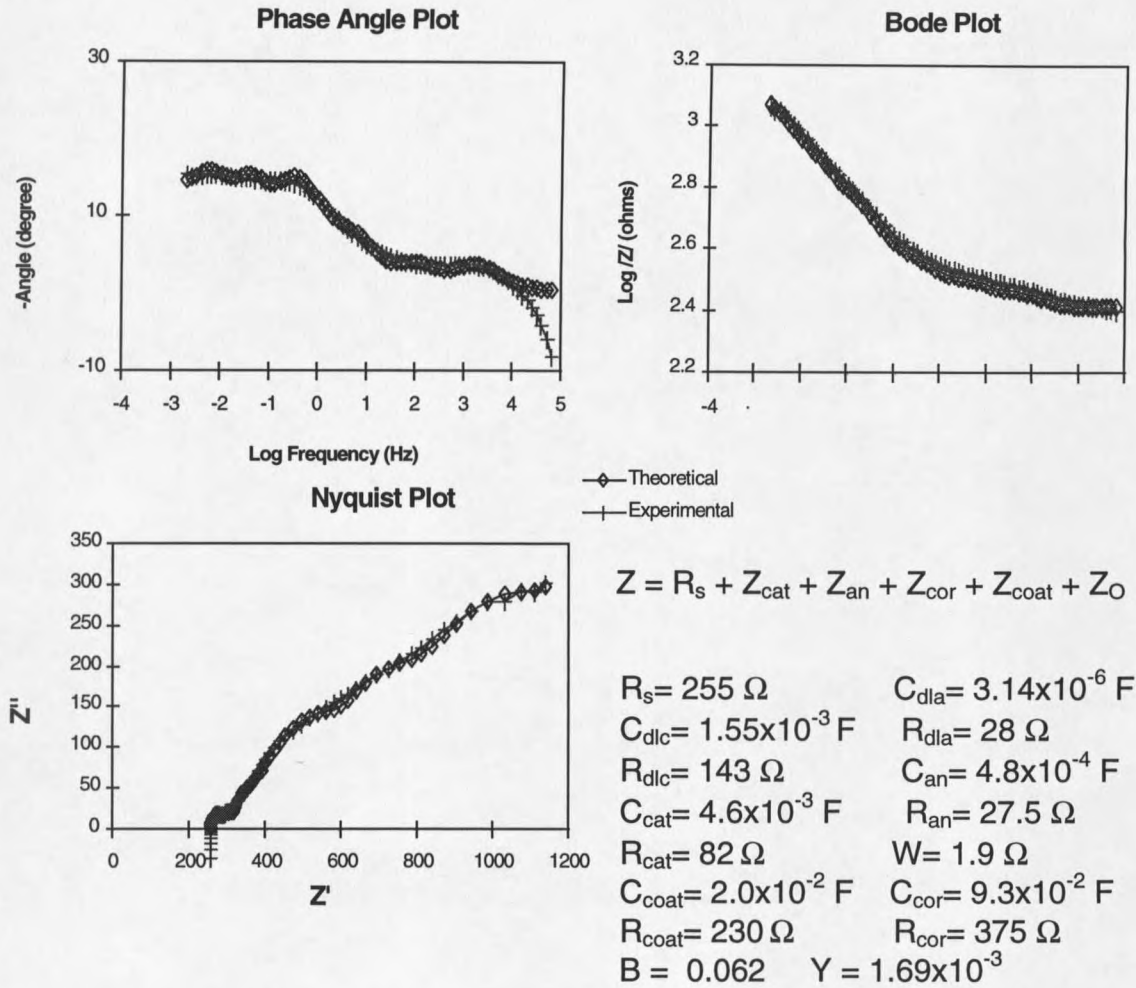


Figure 17. Fitted curves for the biofilm covered coupon. Biofilm thickness was about 2cm.

good enough to give a good idea as to what is happening in the system.

The modeling of the system from an abiotic coupon with localized corrosion to one covered with a biofilm was not expected. The biofilm was not considered stable enough to allow for this type of calculation. The fact that it can work under these differing conditions lends credibility to the model and the theory.

Interpretation of The Data.

The fit of the model was good for all the different systems examined thus far. The next thing to address is if the data and models actually give numbers that relate to the real world. The data for the fit of the pitted coupon, the coated coupon and the coated coupon with corrosion products is in Table 1.

The data in Table 1 indicates that the double layer that was associated with the high frequency peak does follow what was expected. The area associated with this capacitance was the same for both coupons. Based on the area of the anodic region was $3.14 \times 10^{-2} \text{cm}^2$, the capacitances for these two coupons are identical. This means that two different coupons under different conditions had the same chemistry present in their anodic regions. The resistance is

different due to the presence of the agar. This hinders the diffusion of Fe(II) out into the bulk solution.

	C_{an} (F/cm ²)	R_{an} (Ω /cm ²)	C_{dla} (F/cm ²)	R_{dla} (Ω /cm ²)	E_{cor} (mV)
pit	2.85E-03	2.88E+04	2.07E-06	7.23E+03	-
coated	4.78E-03	6.66E+04	2.04E-06	3.98E+02	4.35E+02
pit					-
coated	6.66E-03	2.13E+05	2.67E-06	5.48E+03	5.87E+02
pit					-
corroded					4.73E+02

Table 1. Table of the normalized data for the controlled pit experiments.

The capacitive value for the anodic process shows an increase from the simple pitted to the pitted with corrosion. The change was due to a difference in area. The corroded coupon's capacitance was corrected on the assumption that its anodic region was the same size as for the previous two coupons. The higher capacitance per unit area for the corroded coupon indicates that the area was not $3.14 \times 10^{-2} \text{cm}^2$. If it was assumed that the capacitance per unit area was constant for all mild steel coupons then the area on the corroded coupon should be $4.5 \times 10^{-2} \text{cm}^2$.

The examination of Equation 4 and 5, the relation of the capacitance to the area, diffusion coefficient, and the concentration are directly proportional for the capacitance

and inversely proportional for the resistance for the chemical aspects of the circuit. The variables examined here are C_{an} and R_{an} . These relationships indicate that the increase in corrosion rate at the anode increases the concentration at the anode and its area. The capacitance changes in the same direction.

It was stated that the diffusion coefficient would change as a coating developed. The relation of the diffusion coefficient to the resistance value is inversely proportional to the square of the resistance. The change of the diffusion coefficient with respect to capacitance is proportional to the square of the capacitance. The result is that it takes a much larger change in the diffusion coefficient to affect the calculated capacitance and resistance. This leads to resistance being inversely proportional to area times concentration, and capacitance proportional to area times concentration.

Test of The Model with a Sulfide Coated Coupon.

Data was collected on mild steel coupons exposed to a solution of hydrogen sulfide to test the response of the ac impedance spectra to these conditions. The coupon used was a cylinder 1cm in diameter and 2cm in length. The result of

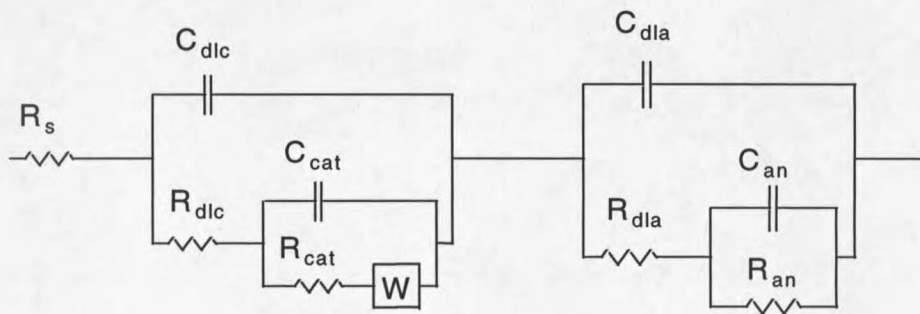
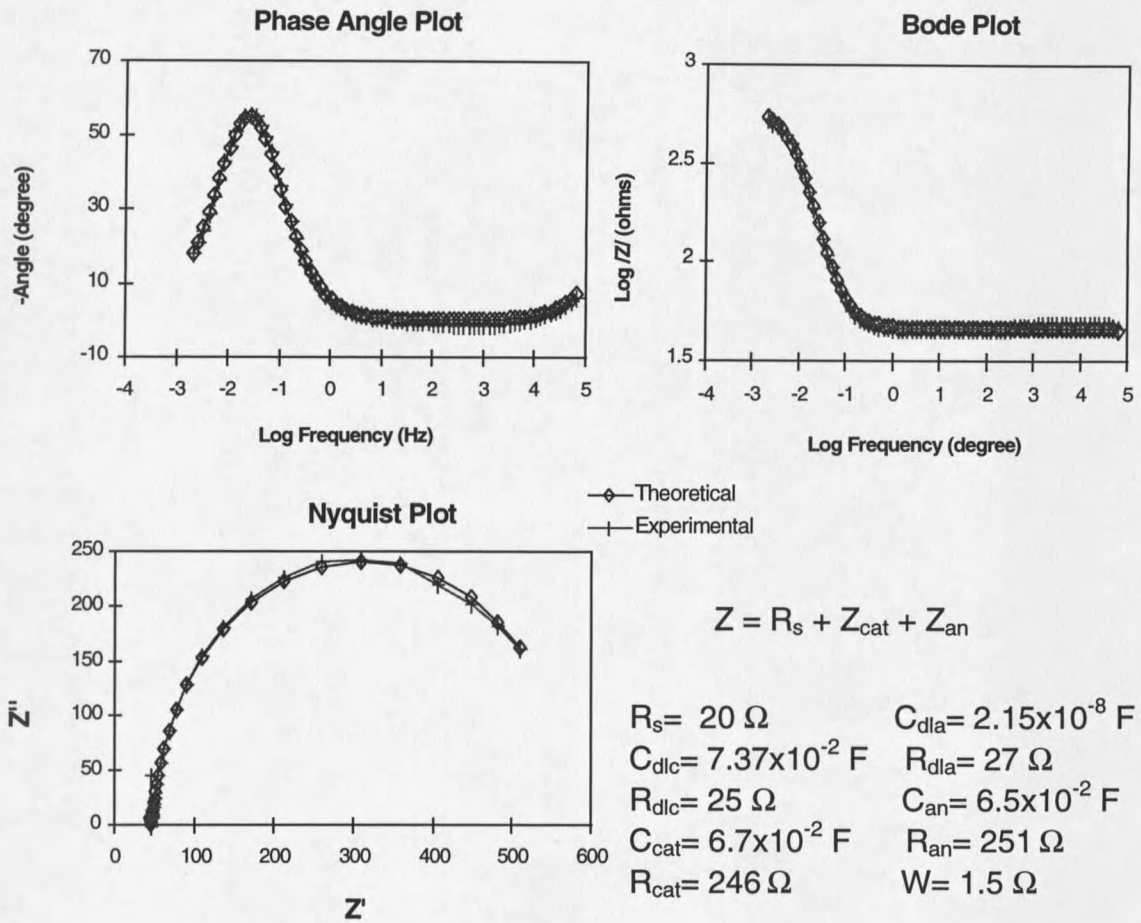


Figure 18. Fitted curves for a coupon exposed to hydrogen sulfide without the presence of oxygen.

trying to fit the model for a pitted coupon is given in Figure 18.

The model does fit the experimental data for these conditions. This expands on the usefulness of this model. The ability of the model to give a fit to a system that it was not originally designed for was promising. The capacitance is high for the chemistry variables because of the change in the coating of the metal. This was the same range of capacitance observed with coupons exposed to SRB byproducts long enough for a change in the surface coating.

Test of The Model on Biofilm Covered Stainless Steel Coupon.

The stainless steel data was collected by Wayne Dickenson, Ph.D. student jointly with the Chemistry Department and the Center for Biofilm Engineering here at MSU. The data was taken from a 316 stainless steel coupon 1.6cm² in diameter. The coupon was mounted in the PVC plug adapted for this purpose as described in the experimental chapter. The coupon was being used for the study of ennoblement of the metal by the presence of a biofilm. The biofilm was grown using the water taken from a creek. There were no nutrients added.

Ennoblement is when the open circuit potential becomes more positive than can be explained using simple

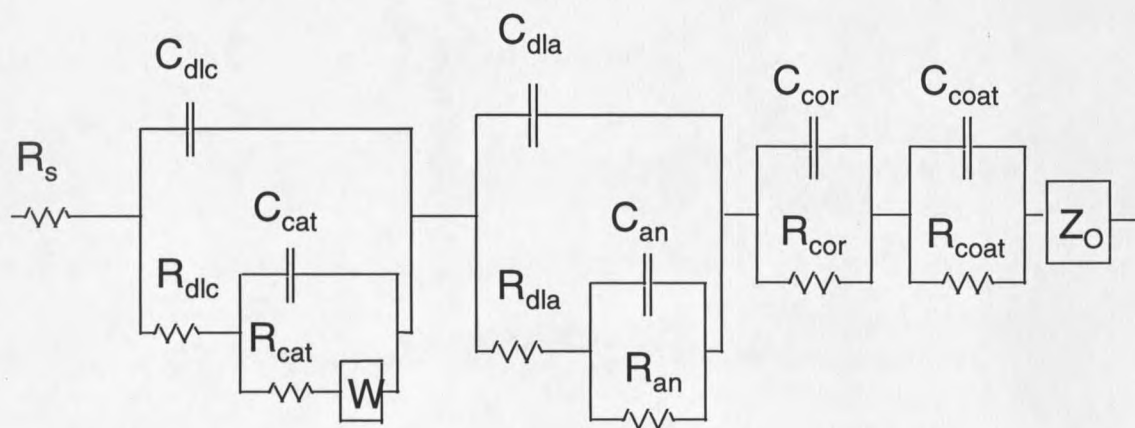
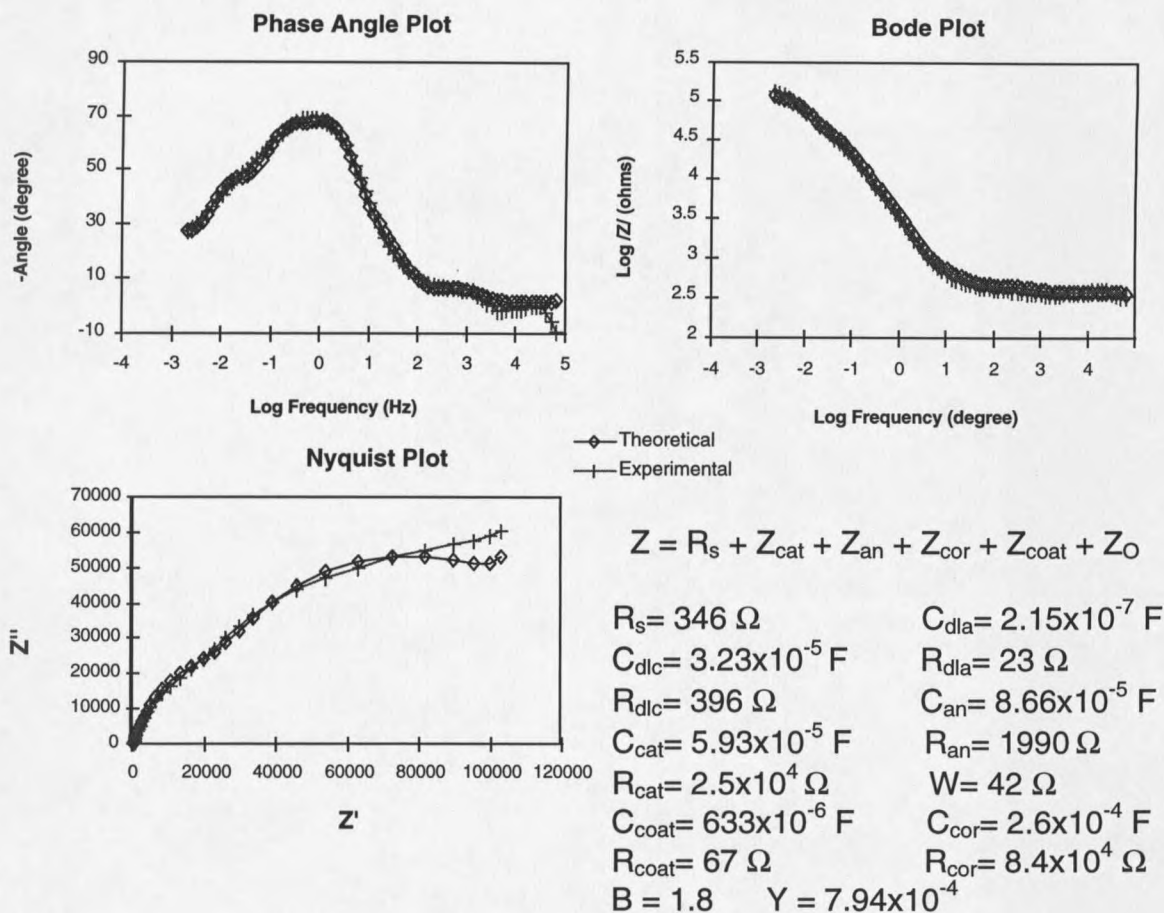


Figure 19. AC impedance data from a stainless steel coupon. The coupon was part of an experiment to determine the reason for bacterial induced ennoblement. Data set courtesy of Wayne Dickenson.

electrochemistry. The bacteria in the biofilm enhance the passivation of the coupon by increasing the open circuit potential. An example would be that a coupon without ennoblement had an open circuit potential of -150 mV, where a coupon under the same conditions but ennobled had an open circuit potential of +100 mV.

The data was taken from a coupon that did not ennoble and exhibited the characteristics of a coupon with crevice corrosion. The result of the fitting of the model for a biofilm with corrosion is in Figure 19. The correlation for this data was 0.999.

Comparison of The Data Between All of The Coupons.

The data given in Table 2 is for the values corrected to the same area. This was done to normalize all of the data to compare how it was related. There are many relationships that have yet to be explored, but not for this report. The ones that are of interest are the relation for the cathodic chemical capacitance, the cathodic double layer capacitance, and the corrosion capacitance.

The cathodic double layer capacitance increased as the amount of material was deposited on it. The material was

	blank	pit	coated pit	coated pit corroded	early biofilm	late biofilm	H ₂ S	Stainless Steel
Ccat	1.07E-05	3.36E-05	9.86E-05	3.32E-04	5.70E-06	2.30E-03	6.82E-03	2.97E-05
Rcat	1.13E+06	9.90E+03	2.29E+04	2.39E+04	3.92E+03	4.10E+01	2.51E+01	1.25E+04
Cdlc	3.69E-05	4.29E-05	6.84E-05	7.30E-06	3.29E-05	7.80E-04	7.54E-03	1.62E-05
Rdlc	2.89E+05	6.05E+02	8.98E+02	2.36E+02	5.75E+01	7.15E+01	2.55E+00	1.98E+02
Can		2.85E-03	4.78E-03	6.66E-03				
Ran		2.88E+04	6.66E+04	2.13E+05				
Cdla		2.07E-06	2.04E-06	2.67E-06				
Rdla		7.23E+03	3.98E+02	5.48E+03				
Ccoat			1.66E-06	6.75E-07	3.45E-04	1.00E-02		1.65E-06
Rcoat			1.13E+02	3.27E+02	3.72E+03	1.15E+02		3.35E+01
Ccor				6.88E-04		4.65E-02		1.30E-04
Rcor				5.10E+05		1.88E+02		4.15E+04
Yo			5.29E-04	2.99E-04	1.01E-03	2.55E-03		1.18E-02
B			7.64E+00	1.10E+04	2.29E+00	7.90E-02		8.79E-03
W	2.61E+03	6.62E+01	4.20E+02	1.69E+02	8.97E+02	2.36E+00	2.47E+00	5.31E+02
Rs	5.49E+02	4.18E+02	1.01E+03	9.25E+02	3.82E+02	3.25E+02	2.42E+01	4.39E+02

Table 2. Normalized data from all of the experiments discussed in the text.

biofilm, corrosion products and/or agar. The change in this value could be a change in the make up of the double layer contents. As the coating changes in nature then the contents of the double layer would change accordingly.

The cathodic chemical capacitance was relatively constant between the various systems. The only set of systems that differed was the biofilm. This was probably due to the ECP trapping a higher amount of compounds that would react at the set potential.

The corrosion capacitance again is similar between the mild steel and the stainless steel. The corrosion capacitance for the biofilm was higher than the other two due to the amount of corrosion and the amount of corrosion trapped by the biofilm.

Look at The Effects of Biofilm at Very High Frequencies.

Overview of The Work Done.

Cell counts can be measured directly with the use of impedance. Living cells can be separated from dead cells of the same species if the right frequency is used. The use of impedance as a tool to monitor the viability of a biofilm was

explored by Hugo Schmidt, Stewart Hutton of the physics department, and myself. Zbigniu Lewandowski initiated the idea and saw over the project. A study of the feasibility to utilize ac impedance as a tool to monitor the growth and changes in a biofilm were done at the Center for Biofilm Engineering.

A frequency, from the analysis of the ac impedance spectrum, that was associated with the bacteria was chosen for monitoring. Once the frequency was determined and base capacitance, the change in the impedance was monitored. The impedance is split into two parts, a real and an imaginary impedance. The real impedance is a parameter that is related to the conductivity between the plates in the reactor. The imaginary impedance is a parameter that is related to the capacitance between the plates in the reactor.

The imaginary impedance is the parameter that was assumed to be affected by the biofilm the most. A change in the capacitance at a given frequency should be mostly due to a change in the number of dipoles affected by the ac signal. The characteristics of the material will enhance the total capacitance of the capacitor by the effect of freely rotating dipoles. These dipoles align themselves to counter the potential across the capacitor, thus reducing the net potential measured between the plates for the charge applied.

The structure of a microbe is a single cell containing a cytoplasmic membrane surrounding a conductive solution. There is an electrochemical potential across the membrane generating a capacitance as well. It is believed that at a given frequency of the ac signal that corresponds to this cytoplasmic membrane capacitance, this signal can cause a dipole to form in the microbes. This induced dipole will affect the imaginary impedance in a way that is different from a system with no biofilm. The change in the imaginary impedance could be correlated back to the number of microbes present. If the membrane is damaged, then no dipole can be induced and there will be no difference between a reactor with sterilized EPC and a biofilm in which all the microbes were killed.

The experiments carried out indicate that the four electrode cell was not necessary. A two electrode reactor would work. The electrodes are not exposed to the solution therefore preventing any interference from the chemistry occurring at the surface. For this study, the two electrode reactor was used.

The FRAs used were not designed to operate in the frequency range of interest. The Schlumberger 1255 was designed to measure from 0.000001Hz to 20MHz. This was the correct range, but not with the required potentiostat. The

potentiostat would allow the 1255 to only function up to 100kHz. The frequency range of interest was between 100kHz and 20MHz.

The HP 4140A had a frequency range from 1MHz to 10GHz. The problem with it was that it could not function well below 20MHz. The data was erratic and the calibration could not be done to the same level as at frequencies above the 20mhz range. The reactor used in this experiment was a rectangle quartz tube. The dimension of the tube was 3mm x 9mm. The measured values were right near the limits of the instrument. Work was continuing in this area in the Physics Department.

SUMMARY

Determination of The Meaning of The Data Above 10kHz.

The results of experiments to determine the nature of the data collected above 10kHz confirmed the interrelation with the formation of a region of localized corrosion. A method was developed to control where and how large the region of localized corrosion could be. This allowed the testing of changes due only to the known size of the region. The increasing of the size of the localized corrosion was concurrent with the increase in the perturbation in the high frequency region.

A model was developed to create a theoretical spectrum to match the experimental data for a blank coupon and one with a localized corrosion present. The models gave a reasonable representation of the experimental data. The correlation was not less than 0.994 for all fitted data. This was determined to be a reasonable fit.

The model for the blank coupon assumed there were only the chemical processes associated with the cathodic region of a corroding coupon. This was based on the belief that there was very little localized corrosion on the surface of the coupon. The model for this coupon was the double layer

capacitance in parallel with the charge transfer resistance and the chemical components. The charge transfer resistance was in series with the chemical components. The chemical resistance and the capacitance were determined to be in parallel.

The model for a coupon with a single localized corrosion area assumed that the processes occurring at the anodic region and the cathodic region could be treated as separate electrodes. The spectrum could be calculated for each region and then summed together to approximate the experimental data. The result was an exceptionally good fit of the curve.

The Controlled Localized Corrosion Results for Artificial Biofilm.

The results of the experiments with the controlled pit size and location with the artificial biofilm showed that the data collected above 10kHz was due to the localized corrosion. The change in the chemical capacitance and resistance conformed to the predicted outcome. The artificial biofilm causes the concentration of reactive redox couples to increase. This causes the capacitance to increase and the resistance to decrease.

The model was adjusted to account for the coating by including an RC circuit for the diffusion gradient within the coating. The coating capacitance was in the range of what was expected.

A second term added was the finite diffusion impedance. This term was added to try to model the changes in the diffuse layer. The diffuse layer had the diffusion coefficient changed because of the agar. The chemistry at the surface was not affected by the coating, but was affected by this change in the diffuse layer. The change in the spectra was a spreading and smoothing.

The total model for the presence of an artificial biofilm gave reasonable values for the capacitive and resistive elements.

The Model with a Biofilm Present.

The model for the artificial biofilm should fit the experimental data for a real biofilm if it was valid. The fit with the early stage biofilm was excellent. The model for an artificial biofilm without corrosion products fit to a correlation of 0.999. The values required to fit the data were as predicted. The capacitance increased and the resistance decreased.

The model for the late stage biofilm was not as good a fit. This was determined from a literature search to be the result of the bacteria's effect on the spectrum. The bacteria can have a dipole induced within their structure by the applied ac fields. This dipole will cause an increase in the capacitance. The effect would not be for a specific frequency as desired. It would affect most frequencies in the lower range. This is due to the variations in size and shape of the bacteria. The species of a given size and shape would only affect its resonance frequency. Since there are many different sizes and shapes, each with its own resonance frequency, the whole spectrum was affected.

The fit was still reasonable. The model could not account for the effects of the biofilm bacteria, but gave a representative analysis of the conditions present at the coupon surface.

Tests with Other Systems.

The models were tested on two different systems to determine if the model would hold up if conditions changed. The data for the hydrogen sulfide experiment indicated that it could. The data from the experiment reproduced the experimental data accurately. The parameters required for

the fit were much higher than for the previous experiments. This was due to the nature of the sulfide coating and the concentration of the reactant.

The stainless steel coupon with a corroded surface and covered by a biofilm was modeled using the biofilm with corrosion products. The fit for this experimental data was to a correlation of 0.999. The model gave parameter values that were similar to that of the mild steel. The similarity was predicted since both systems have the same general chemistry occurring at the surface.

The Evaluation of The Use of AC Impedance to Monitor The Viability of Bacteria.

The experiments that were performed to evaluate the feasibility of using ac impedance to monitor the viability of bacteria were informative. The limitations of the instrumentation available allowed only a glimpse to what was possible. New instruments designed for this type of work are required. The possibility of getting both corrosion data and biofilm viability simultaneously is still only a theory.

APENDIX

PROGRAM FOR SCHLUMBERGER 1255 FRA

APENDIX

This appendix is the source code for the independent operation of the Schlumberger 1255 FRA. The program was written to collect data and store that data in a compressed file. The computer used to collect the data was a Zenith 190 that did not have a hard drive installed. The data sets that were collected were in excess of 3M bytes. The floppy drives could not hold that much data at one time uncompressed.

The compression program used was LHA.EXE. This program is a shareware program available on the internet. Another source is game programmers. They use LHA.EXE to compress their programs for sale. The compression ratio for the text files that were generated by the program was about 5:1. That meant that a 3M byte set of data was only 600k bytes compressed.

The program was written with Turbo C++ from Borland. This was the programming language that was the most familiar. There was some code taken from the source code sent with the GPIB II/IIa interface card. This code was the error checking routines in the presented source code. I thank National instruments for the help in getting the bugs figured out for this program. They were very helpful in supplying both technical support and documentation.

PROGRAM

```

#include <stdlib.h>
#include "decl.h"
#include <conio.h>
#include <dos.h>
#include <string.h>

void dvmerr(char *msg, char code); /* device error function */
void gpiberr(char *msg);          /* gpib error function */
void convdats(void);
void file_out(void);
long float getjuldats(void);
void elapse_tim(void);
void compress(void);
void stop(void);

/* Program to run 1255 FRA as a stand alone instrument.
   Written by Michael Morrison and Casey Parker June
   1994*/

    char rd[512],*FRQ[15]; /* variables for data buffer*/
    int  dvm,gpib,i,j,k,k1,l,m,errorc[15]; /* device
number and counter variables */
    char spr;              /* serial poll response byte */
    float freq[15],a[15],b[15]; /* variables for data sets
*/
    char header,tdump; /* variables for cleanup of data sets
*/
    FILE *out_fr,*out_fi;char *filimag,*filr; /* file
labels */
    char frq[15],zr[12],zim[12],zerr[2];      /* used to
split string from instrument */
    long float eltime,basetime;                /*for time data */
    int flag1,flag2,intrvtm;
    long float tm,dt,month,year,day,hr,min,sec;

main()
{
    char temp[25],temp1[25],*t1,*t2,*t3,*filnam;
    int tin;

    /* set variables to zero */
    eltime=0;basetime=0;hr=0;

    system("cls");

```

```

    printf("For the operation of a 1255 FRA\n");
    printf("\n");

/* To initialize the gpib board */

    if ((gpib = ibfind ("GPIB0")) < 0) {
        gpiberr("ibfind Error");
        exit(1);
    }

    ibsic(gpib);
    if(ibsta & ERR)
    {
        gpiberr("Send IFC Error");
        exit(1);
    }

    ibsre(gpib,1);
    if(ibsta & ERR)
    {
        gpiberr("System Control Error");
        exit(1);
    }

/* Assign an unique identifier to the frequency analyzer at
primary address 6. */

    if ((dvm = ibfind ("FRA")) < 0) {
        gpiberr("ibfind Error");
        exit(1);
    }
    printf("The address for the FRA is %d.\n\n",dvm);

/* clear the device.
*/

    if (ibclr (dvm) & ERR) {
        gpiberr("ibclr Error");
        exit(1);
    }

    delay(1000);

```

```

/* Write initializing instruction to the FRA. */

    printf("Initializing the instrument.\n");

    ibwrt (dvm,"TT1",3L);
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }
    delay(1500);
    clrscr();

/* Set up FRA to take data set. */

    printf("Enter data file name (max 6 char):");
    gets(filnam);
    printf("\nEnter number of runs: ");
    scanf("%d",l);
    printf("\nEnter time interval between data sets in
    whole minutes: ");
    scanf("%d",intrvtm);

    ibwrt (dvm,"VA 1",4L); /* set generator amplitude */
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }

    ibwrt (dvm,"CV0",3L); /* set coordinate display */
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }

    ibwrt (dvm,"OP 2,1",6L); /* set data out put */
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }

    ibwrt (dvm,"AU1",3L); /* set analyser to aout */
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }

```

```
ibwrt (dvm,"BP0",3L); /* set error beep off */
if (ibsta & ERR) {
    gpiberr("ibwrt Error");
    exit(1);
}

ibwrt (dvm,"IS 1E-1",7L); /* set analyser
integration time */
if (ibsta & ERR) {
    gpiberr("ibwrt Error");
    exit(1);
}

ibwrt (dvm,"DC 1,0",6L); /* set V1 coupling to dc */
if (ibsta & ERR) {
    gpiberr("ibwrt Error");
    exit(1);
}

ibwrt (dvm,"DC 2,0",6L); /* set V2 coupling to dc */
if (ibsta & ERR) {
    gpiberr("ibwrt Error");
    exit(1);
}

ibwrt (dvm,"OU 1,1",6L); /* set V1 to floating */
if (ibsta & ERR) {
    gpiberr("ibwrt Error");
    exit(1);
}

ibwrt (dvm,"OU 2,1",6L); /* set V2 to floating */
if (ibsta & ERR) {
    gpiberr("ibwrt Error");
    exit(1);
}
```

```
/* set frequencies to be used in array */
```

```
FRQ[0]="FR2.0000E7";
FRQ[1]="FR1.1240E7";
FRQ[2]="FR8.3300E6";
FRQ[3]="FR7.5500E6";
FRQ[4]="FR6.0000E6";
FRQ[5]="FR5.1240E6";
FRQ[6]="FR4.3300E6";
FRQ[7]="FR2.5500E6";
FRQ[8]="FR1.0000E6";
FRQ[9]="FR9.1240E5";
FRQ[10]="FR7.3300E5";
FRQ[11]="FR6.5500E5";
FRQ[12]="FR4.0000E5";
FRQ[13]="FR3.1240E5";
FRQ[14]="FR1.0000E5";
```

```
flag1=0;
flag2=0;
basetime=getjuldat();
delay(2000);
```

```
for(j=0;j<1;j++)
{
    clrscr();
    t3=j; /*Makes a new file name for each data set.*/
    t1="zr.";
    strcpy(temp,filnam);
    strcat(temp,t1);
    strcat(temp,t3);
    filr=temp;

    t1="zi.";
    strcpy(temp1,filnam);
    strcat(temp1,t1);
    strcat(temp1,t3);
    filimag=temp1;

    delay(1000);
    elapse_tim();
    hr=eltime*24;
```

```

sec=eltime*86400;
min=eltime*1440+1;

```

```

printf("Elapsed time: %f hr.\n",hr);
printf("Elapsed time: %f sec.\n\n",sec);
flag2=1;
for(i=0;i<15;i++)
{

```

```

    ibwrt (dvm,FRQ[i],10L); /* set analyser frequency */
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }

```

```

    ibwrt (dvm,"SI",2L); /* do a single measurement */
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }

```

```

    ibwrt (dvm,"DO",2L); /* output last result */
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }

```

```

/* get data */
if (ibrd (dvm,rd,40L) & ERR) {
    gpiberr("ibrd Error");
    exit(1);
}

```

```

rd[ibcnt] = '\0';

```

```

convdats();
printf("Freq= %f",freq[i]);
printf(" Zr= %f",a[i]);
printf(" Zi= %f",b[i]);
printf(" error= %d\n",error[i]);

}

```

```

file_out();
compress();

```

```

        printf(" Run number %d.",j);

/* Generate a twirling icon to show that the program is
running */

    _setcursortype(_NOCURSOR);

    gotoxy(1,25);
    printf("Delay in progress.");

    for(k=0;k<intrvtm;++k) /* to display a changing
        character on the screen for operation purposes */
    {
        for(k1=0;k1<150;++k1)
        {

            gotoxy(40,25);
            printf("|");
            delay(100);
            gotoxy(40,25);
            printf("/");
            delay(100);
            gotoxy(40,25);
            printf("-");
            delay(100);
            gotoxy(40,25);
            printf("\\");
            delay(100);
        }
    }

    _setcursortype(_NORMALCURSOR);
    printf("\nDelay completed. \n");

}

/* Write a break instruction to the FRA.*/

    ibwrt (dvm,"BK",2L);
    if (ibsta & ERR) {
        gpiberr("ibwrt Error");
        exit(1);
    }
    return 0;
}

```

```
/* this section was from the National Instruments
distribution disk for programing the GPIB -PCII/IIA. */
```

```
void gpiberr(char *msg) {
```

```
/*This routine would notify you that an IB call failed*/
```

```
    printf ("%s\n", msg);
    printf ( "ibsta=&H%x  <", ibsta);
    if (ibsta & ERR ) printf ( " ERR");
    if (ibsta & TIMO) printf ( " TIMO");
    if (ibsta & END ) printf ( " END");
    if (ibsta & SRQI) printf ( " SRQI");
    if (ibsta & RQS ) printf ( " RQS");
    if (ibsta & CMPL) printf ( " CMPL");
    if (ibsta & LOK ) printf ( " LOK");
    if (ibsta & REM ) printf ( " REM");
    if (ibsta & CIC ) printf ( " CIC");
    if (ibsta & ATN ) printf ( " ATN");
    if (ibsta & TACS) printf ( " TACS");
    if (ibsta & LACS) printf ( " LACS");
    if (ibsta & DTAS) printf ( " DTAS");
    if (ibsta & DCAS) printf ( " DCAS");
    printf ( " >\n");
    printf ("iberr= %d", iberr);
    if (iberr == EDVR) printf ( " EDVR <DOS Error>\n");
    if (iberr == ECIC) printf ( " ECIC <Not CIC>\n");
    if (iberr == ENOL) printf ( " ENOL <No Listener>\n");
    if (iberr == EADR) printf ( " EADR <Address error>\n");
    if (iberr == EARG) printf ( " EARG <Invalid argument>\n");
    if (iberr == ESAC) printf ( " ESAC <Not Sys Ctrlr>\n");
    if (iberr == EABO) printf ( " EABO <Op. aborted>\n");
    if (iberr == ENEB) printf ( " ENEB <No GPIB board>\n");
    if (iberr == EOIP) printf ( " EOIP <Async I/O in prg>\n");
    if (iberr == ECAP) printf ( " ECAP <No capability>\n");
    if (iberr == EFSO) printf ( " EFSQ <File sys. error>\n");
    if (iberr == EBUS) printf ( " EBUS <Command error>\n");
    if (iberr == ESTB) printf ( " ESTB <Status byte lost>\n");
    if (iberr == ESRQ) printf ( " ESRQ <SRQ stuck on>\n");
    if (iberr == ETAB) printf ( " ETAB <Table Overflow>\n");
    printf ("ibcnt= %d\n", ibcnt1);
```

```

printf ("\n");

/* Call the ibonl function to disable device DVM.*/

    ibonl (dvm,0);
}

void dvmerr(char *msg,  char spr) {

/* This routine would notify you that the DVM returned an
   invalid serial poll response byte */

    printf ("%s\n", msg);
    printf("Status Byte = %x\n", spr);

/* Call the ibonl function to disable device DVM. */

    ibonl (dvm,0);
}
/* End the Nation Instruments program section.62 */

/* simple routine to stop the program at any point, for
diagnostics */

void stop(void)
{
    char x;

    x=getchar();
    x=x+x;
    return 0;
}
/* routine to convert data string recieved from FRA to
seperate numeric values */

void convdats(void)
{
    strncpy(frq,rd+0,14);
    frq[14]='\0';
    freq[i]=atof(frq);
    strncpy(zr,rd+15,11);
    zr[11]='\0';
    a[i]=atof(zr);
    strncpy(zim,rd+27,11);
    zim[11]='\0';
    b[i]=atof(zim);
}

```

```

    strncpy(zerr,rd+39,1);
    zerr[2]='\0';
    errorc[i]=atoi(zerr);
}

```

```

/* output file for Quattro spreadsheet */

```

```

void file_out(void)
{

```

```

    int c,d;
    out_fr =fopen(filr,"a+t"); /* real file */

    /* to insert freq at top of spreadsheet */

    if(flag1==0)
    {
        for(c=0;c<15;c++)
        {
            fprintf(out_fr,"%f",freq[c]);
        }
        fprintf(out_fr,"\n\n");
    }

```

```

/* for the output of the real data */

```

```

    for(c=1;c<2;++c)
    {
        fprintf(out_fr,"%f",hr);

        for(d=0;d<15;++d)
        {
            fprintf(out_fr,"%f",a[d]);
        }

        fprintf(out_fr,"\n");
    }

```

```

    fclose(out_fr);

```

```

/* for the out put of the imaginary data */

```

```

    out_fi =fopen(filimag,"a+t"); /* real file */

    if(flag1==0)

```

```

{
    for(c=0;c<15;c++)
    {
        fprintf(out_fi,"%f",freq[c]);
    }
    fprintf(out_fi,"\n\n");
}
flag1=1;

for(c=1;c<2;++c)
{
    for(d=0;d<15;++d)
    {
        fprintf(out_fi,"%f",b[d]);
    }
    fprintf(out_fi,"\n");
    fclose(out_fi);
}

/* routine to get the date and time and convert to julian to
remove errors of day/ date changes */

long float getjuldat(void)
{
    struct time t;
    struct date d;

    tm=0;dt=0;month=0;year=0;day=0;hr=0;min=0;sec=0;
    gettime(&t);
    hr=t.ti_hour;
    min=t.ti_min;
    sec=t.ti_sec;
    tm=hr/24;
    tm=tm + (min/1440);
    tm=tm + (sec/86400);

    getdate(&d);

    switch (d.da_mon) {
        case 1: month=0; break;
        case 2: month=31; break;
        case 3: month=59; break;
        case 4: month=90; break;
    }
}

```

```

        case 5: month=120; break;
        case 6: month=151; break;
        case 7: month=181; break;
        case 8: month=212; break;
        case 9: month=243; break;
        case 10: month=273; break;
        case 11: month=304; break;
        case 12: month=334; break;
        default: break;
    }

```

```

    year=d.da_year;
    day=d.da_day;
    dt=(year-1900)*1000;
    dt=dt + month;
    dt=dt + day;

```

```

    dt=dt + tm;

```

```

    return (dt);
}

```

```

/* routine to determine elapsed time from beginning of
experiment */

```

```

void elapse_tim(void)

```

```

{
    long float newtime;
    newtime=0;
    newtime=getjuldat();
    printf("newtime= %f\n",newtime);
    eltime=newtime - basetime;
}

```

```

void compress(void);

```

```

{
    char *filec,temp3[8];

    t3=".lzh";
    strcpy(temp3,filnam);
    strcat(temp3,t3);
    filec=temp3;

    system("lha a %s",filec);
}

```

```

End of program.

```

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REFERENCES CITED

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