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

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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.

THE CORROSION OF MILD STEEL:
A NEW INTERPRETATION OF AC IMPEDANCE SPECTRA OF
CORRODING MILD STEEL

By

Michael Lee Morrison

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Michael Lee Morrison

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TABLE OF CONTENTS

TABLE OF CONTENTS-Continued

TABLE OF CONTENTS-Continued

LIST OF TABLES

Table   Page

LIST OF FIGURES

LIST OF FIGURES-Continued

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 that 10kHz are evaluated.

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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

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

for resistance,

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

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

$$W = \frac{1}{Y_o \sqrt{j\omega}} \qquad \text{Equation 3}$$

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_O 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 potential[1]. 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 today[2]. 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_{i1} 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.
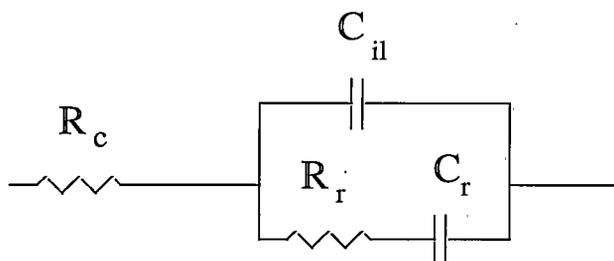
Figure 1. Randles' equivalent circuit for the impedance interpretation of a simple electrode interface.

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,

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

and for resistance in equation 5,

$$R_r = \frac{RT}{n^2 F^2 A C} \left(\sqrt{\frac{2}{\omega D}} + \frac{I}{k} \right)$$
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 frequencies[7]. 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 literature[8] . 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 1981[3], 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_Ω 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.
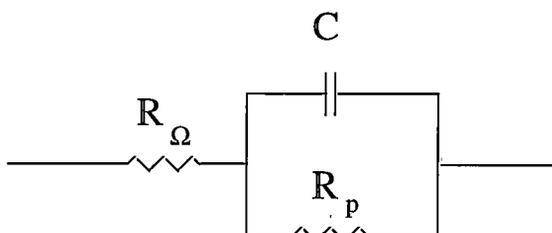
Figure 2. Mansfeld's first equivalent circuit

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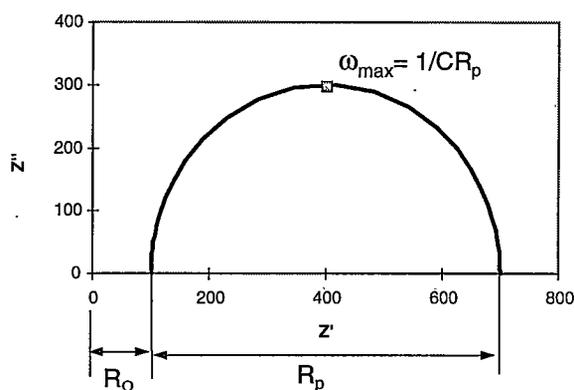
Figure 3. Nyquist plot of Mansfeld's initial equivalent circuit model.

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

$$R_p = 2|Z|\tan\delta_{\text{max}}$$ 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.
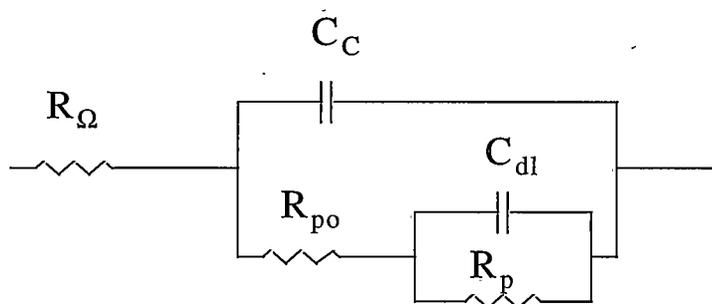
Figure 4. Mansfeld's final equivalent circuit.

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 frequencies[11]. 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.
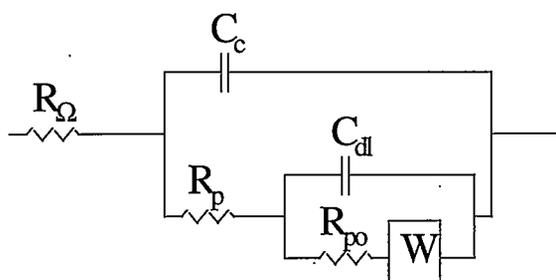
Figure 5. Mansfeld's final model corrected for the low frequency response of the data.

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.
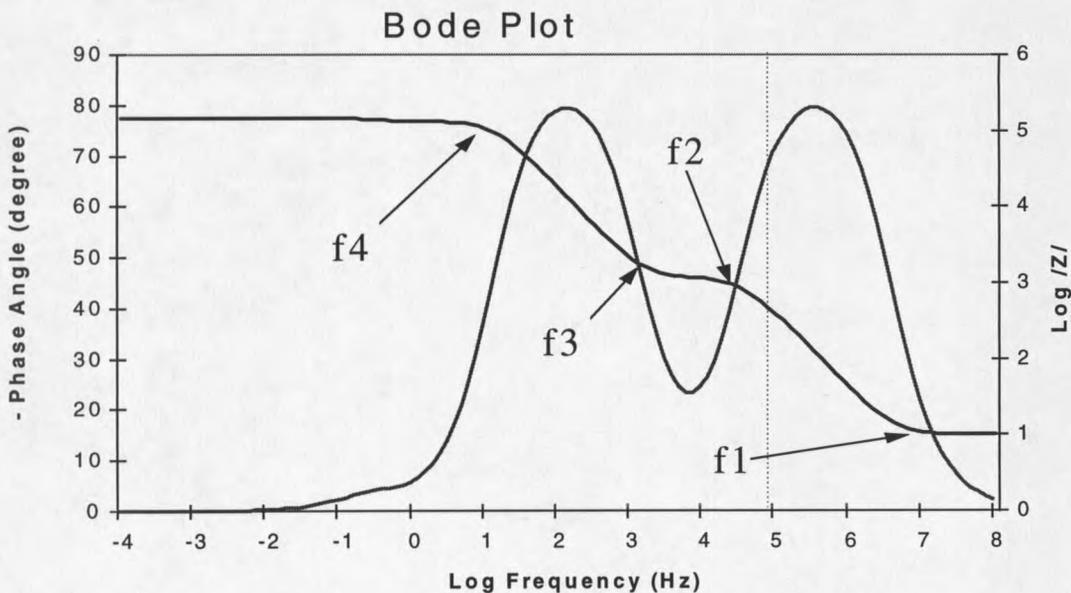
Bode Plot

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 f2. 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 f2 and f4 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.
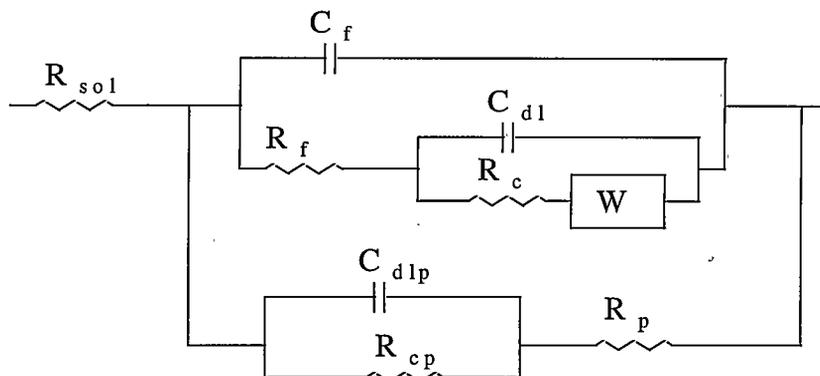
Figure 7. Haruyama's break point equivalent circuit.

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 f2. 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 Haruyama[17]. 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 technique[18]. 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 polyurethane[19]. 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 theory[20]. 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 article[21]. 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 respirate. 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 medium[27] . The second method is to directly stain the cells with a cytochemical or immunological stain[28] . 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 sample[33]. 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 cells[37] .

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 used non-uniform ac fields to separate living bacterial cells from dead cells in 1968[55]. 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 desolved oxygen concentration. The disolved 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, Kleibsiella 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 $2cm^2$ 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 H_2S 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