DIELECTRIC PROPERTIES OF AZOTOBACTER VINELANDII
IN A MICROWAVE FIELD

THESIS

Presented to the Graduate Council of the North Texas State University in Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

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A resonant frequency cavity was used to determine the dielectric properties of various preparations of Azotobacter vinelandii ATCC 12837. It was found that the bacteria investigated did interact with microwave radiation in the absence of free water. The data presented here indicate that bacteria demonstrate frequency specific dielectric properties. The techniques employed in these experiments may also be used to determine microwave spectra of other species of bacteria in different physiological stages.
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CHAPTER I

INTRODUCTION

Modern technology has made possible the production of numerous devices which utilize microwave radiation. The fact that microwave production is simple and economical has created what could be called a "microwave fad" in the home as well as in industry. Due to the increasing number of microwave generating devices, there is a growing concern about possible deleterious effects of microwaves on biological systems. The mechanisms of interaction between microwaves and biological systems are, at this time, only partially understood.

In general, electromagnetic (E) radiations can be defined as wavelike entities of energy particles, or quanta of energy, which propagate through space by means of simultaneous oscillations of magnetic and electric fields which are perpendicular to each other. These electromagnetic waves propagate at a velocity of \(3 \times 10^8\) meters per second in free space and in a direction perpendicular to both the electric and magnetic fields. The oscillations of the electric and magnetic fields have different periodicities (frequencies of oscillation) which in turn produce different wavelengths of electromagnetic radiation.
It is now understood that all the various wavelengths and corresponding frequencies form a continuous spectrum of electromagnetic radiation. While the limits of the spectrum have not yet been determined, the known portion of the spectrum is composed of wavelengths from less than $10^{-3}$ Å to more than $10^{14}$ Å. This spectrum includes ionizing and non-ionizing radiation. Ionizing radiations contain extremely high energy levels, are particulate in nature, and induce the formation of ions in the medium through which they pass. Electromagnetic radiation of wavelengths less than 160 Å is considered ionizing. The types of ionizing radiations are cosmic rays, gamma rays, x-rays, and the shorter wavelength end of the ultraviolet range.

Non-ionizing radiation is more wavelike in nature and propagates at a lower energy level than ionizing radiation. Electromagnetic radiation of wavelengths greater than 160 Å is considered to be non-ionizing. The types of non-ionizing radiation are ultraviolet, visible, infrared, microwave, and radiowave.

Historical Development and Definition of Microwaves

The long electromagnetic waves, characteristic of radiofrequency, were first discovered by the German physicist Heinrich Hertz in 1888. Hertz demonstrated that these waves could be propagated through space between points of different electrical potentials (6).
Subsequent technological advances proved that these electromagnetic waves could be propagated in any unbounded isotropic medium such as free space and also through the air. This discovery ultimately led to the use of long electromagnetic waves in radio communications.

The use of radiowaves in radio communications and broadcasting promoted military interest, and during World War II a particular region of the radiofrequency band was found to be useful for navigation and in a new application, for the detection of certain targets in space. This new development was called radar (radio detecting and ranging), hence the term radarwave. For reasons of military security, the government attempted to maintain secret the characteristics of these radarwaves, and information was not made public until the end of the war in 1945 (12). Even before the end of the war, however, secret information had been released and electromagnetic waves in the radar range or microwave range were found to have great diversity of application.

In the electromagnetic spectrum, the microwave region lies between the infrared and radiofrequencies. While it is understood that no clear lines of demarcation can be drawn which separate the various regions of the electromagnetic spectrum, microwaves are generally considered to be of wavelengths from 1 meter to 1 millimeter and of
frequencies between 300 MHz \((\text{MHz} = 10^6 \text{ cycles per second})\) and 300 GHz \((\text{GHz} = 10^9 \text{ cycles per second})\). The microwave region of the electromagnetic spectrum has been further subdivided into more specific wavelength and corresponding frequency bands, each of which represents a characteristic frequency of common usage. These subdivisions are given in Table I.

Applications of Microwave Radiation

Microwaves can be manipulated in unique and novel ways which make them useful for many purposes. They can be reflected, refracted, and focused. These characteristics enable them to be used for radio and television communications. Generally, frequencies in the very high range are used for television, FM broadcasting, air traffic control, and radio navigation. Frequencies in the ultra high range are used in television, broadcasting, citizens band radio communications, microwave point-to-point communications, telemetry, tropo scatter studies, meteorological radar, and microwave ovens. Super high frequencies are utilized for satellite communication, airborne weather radar, altimeters, shipborne navigational radar, and microwave point-to-point communication. The microwave band nearest the infrared region is called the extra high frequency band. Microwave radiation of this region is used in radio astronomy, cloud detection radar, and analysis.
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of the atmospheric gases of celestial bodies (5). The ability of microwaves to penetrate deeply into tissues facilitates their use in medical diathermy (21) and also for food processing (7, 11). Recently, microwave radiation in the ultra high frequency region has been under investigation for use in agriculture as a method of weed control (25).

Sources of Microwave Radiation

Cleary (6) points out that, although man intentionally produces microwave radiation for his own purposes, microwaves are analogous to ionizing radiation in that there is a natural background (noise) level of microwave radiation occurring from sources such as solar radiation, lightning, cosmic radiation from galactic sources and specific stars, rain precipitation, and black body radiation from the earth. Also contributing to this background noise level are man-made oscillators such as spark plugs of internal combustion engines and household appliances which "gap" and create radiation pulses. The microwaves produced naturally and unintentionally, however, seem to be of little importance to man except that they may cause noise interference in radio and television communications.

The common production sources of microwave radiation are designed to be electrically and/or mechanically tuned to produce electromagnetic radiation at desired
frequencies and energy densities for specific application. There is now a variety of microwave generating devices, or microwave tubes. Examples of these are the magnetron, the klystron, and the amplitron.

**Measurement of Microwave Radiation**

Detection of microwave radiation is accomplished by means of a reflectometer which may vary in composition and design depending on its specific use. Many reflectometers are designed to detect power densities by converting heat energy to electrical energy. Still others are designed to act in a manner analogous to a television antenna which detects and differentiates microwave signals or changes in an applied microwave signal. The later type was used in conducting this investigation (Figure 1, Chapter II).

**Biological Effects of Microwave Radiation**

The expansion of microwave technology in the last thirty years has proved very beneficial to man. It has also created a need for a thorough understanding of the nature of interaction between microwaves and biological systems. As a consequence, extensive research has been conducted to examine the biological effects of microwave radiation. A compendium of representative reports of this research is presented here.
Effects of Microwaves on Animals

It has been clearly demonstrated that chronic exposure to high intensity microwave fields induces violent, lethal effects on laboratory animals (8, 15). Whole body radiation and partial body radiation cause various physiological aberrations. Some of these reported abnormalities are hematologic variations (8, 19), changes in lymphoid spleen cells (27), testicular damage (13), and cataractogenesis (12, 20, 24). These experiments employed frequency ranges from 2.0 to 3.0 GHz and power densities greater than 10 milliwatts (mW) per cm². Several investigators have reported central nervous system effects at lower power densities. It was recently reported by the news media that the U. S. Embassy in Russia was bombarded with low intensity microwave radiation. The microwaves were allegedly intended to interfere with competency and efficiency of embassy personnel (18), but information on the effects of such uses of microwaves has not yet been presented in suitable scientific form. There is great diversity in statements concerning the effects of microwaves on man and other organisms. Cleary, in his review article (5), stated:

The results of recent studies of the effects of microwave radiation on the mammalian CNS, as manifested by changes in nerve structure, or behavior, may be summarized by stating that low intensity fields appear, in some instances, to induce detectable changes. (p. 149)
Effects of Microwaves on Insects

In 1930, McKinely and Charles (16) reported that radiofrequency radiation, now called microwave radiation, was lethal to parasitic wasps. In 1968 Hamid et al. (10) proposed treatment of storage grains to control grain insects. Further investigation indicated that infrared heating was preferable to microwave radiation in grain insect control (14).

Effects of Microwaves on Microorganisms

The lethal effect of microwave radiation on fungi, bacteria, and viruses has been a focal point of the majority of investigations reported in the more recent literature (1-4, 9, 23, 25, 28). The general consensus is that the lethality is due to a thermal effect only. It has been demonstrated that the destruction of soil microorganisms by microwave radiation at 2.45 GHz at power intensities up to 40,000 joules per cm² is directly proportional to the water content of the cells, and furthermore, it appears that in situ the soil bacteria are extremely resistant to microwave radiation (25, 29).

It has also been reported that at frequencies from 65 to 75 GHz (power levels not given) bacteria absorb energy, and cell reproduction is stimulated. These findings suggest that there may be non-thermal, frequency-specific alterations due to microwave exposure (26).
Mechanisms of Interaction Between Microwaves and Microorganisms

There is disagreement among scientific investigators concerning mechanisms by which microwaves interact with biological systems in general. The disparity revolves around the question of whether or not there exist non-thermal effects of microwave radiation on biological systems.

**Thermal Mechanism**

It has been well established that the destruction of bacterial cells, due to microwave radiation, is predominantly attributable to an increase in temperature (29). This increase in temperature results from interaction between the permanent dipole moment of the water molecule and the electric field component of the applied microwave field. The torque on the dipole of the water molecule, when placed in an alternating electric field, induces the molecule to orient itself parallel to the electric field. Work is required for orientation, and due to frictional resistance of the medium, heat is evolved (6).

**Non-Thermal Mechanism**

Although several reports (5, 17) indicate a direct non-thermal interaction between a cell or its constituents and a microwave field, the mechanism of interaction remains obscure.
The Purpose of This Experiment

It has been well-documented that whenever an object, whether biological or inert, is perturbed by microwave radiation, the microwaves are reflected, refracted, or transmitted (conducted) through the object. It has further been established that when the medium through which the microwaves propagate is biological, the interaction between the microwave radiation and the medium is due to the electrical properties of the biological material (22). Therefore, if we assume that there are possible non-thermal effects of microwaves on bacterial cells, these effects would certainly be related to the electrical properties of the bacterial cells.

This research is intended to demonstrate a method of determining the dielectric properties of bacterial cells. The objective of this experiment is to bring into focus possible mechanisms of interaction between microwaves and bacterial cells other than those interactions involving free water.
CHAPTER BIBLIOGRAPHY


CHAPTER II

MATERIALS AND METHODS

Culture

Azotobacter vinelandii ATCC 12837 obtained from the North Texas State University stock culture collection was utilized in all the experiments reported here.

Dried Cell Preparations

Pure cultures were obtained from stock cultures on Burke's nitrogen-free agar medium (2) incubated at room temperature (20-26°C). After four days incubation, individual colonies were selected and transferred to 250 ml portions of Burke's nitrogen-free liquid medium and incubated on a reciprocal shaker at room temperature for four days. The cells were harvested by centrifugation at 3,000 x G for fifteen minutes, resuspended in sterile distilled water, and collected by centrifuging again at 3,000 x G for fifteen minutes. This washing process was repeated three times. The cell paste was divided into three portions. One portion was spread over the surface of sterile glass petri dishes and dried in a drying oven at 80°C for twelve hours.
The second portion of the wet cell paste was also spread on a sterile glass petri dish and dried in a vacuum desiccator over drierite (CaSO$_4$) for seventy-two hours.

The third portion was suspended in a minimal amount of sterile distilled water. These cells were then lyophilized using a Virtis lyophilization apparatus (Gardiher, New York). Standard lyophilization procedures were utilized except that no litmus milk or other stabilizing material was added.

The dried cell preparations were transferred to clean sterile mortars and pulverized. The powdered cells were packed into clean, pre-weighed sections of polyethylene tubing approximately 2.5 cm in length with an inner diameter of 0.336 cm. The wall thickness of the tube was determined to be 0.072 cm. Polyethylene was selected due to its inability to absorb microwave radiation.

After being packed with cells, the ends of the tube sections were sealed using surgical forceps and heat. Once sealed, the tubes were weighed again, and the mass of the cell samples determined. Each dried cell sample contained approximately $6 \times 10^{10}$ cells.

Using standard serial dilution and spread plate techniques, the viability of the dried cell samples was determined and found to be less than 0.2 per cent.
Vegetative Cell Preparations

Pure cultures of cells were obtained by the technique already described. One loopful of growth was transferred to fresh plates of the same medium and streaked for confluent growth. The cells were incubated at room temperature until the characteristic green pigment of *Az. vinelandii* became apparent. Harvesting of the cells was accomplished by carefully scraping the colonies from the surface of the agar using a razor blade. The cells were transferred into sections of polyethylene tubing as described below. Each sample tube contained approximately $5 \times 10^9$ cells. No more than two hours elapsed from the time of harvesting to the time of irradiation.

Wet Lyophilized Cell Preparation

A cell paste was prepared by adding water to lyophilized cells so that the mass of the paste was 70 per cent water and 30 per cent dry cells. This paste was packed into polyethylene tubing using a pasteur pipette. Each sample contained approximately $5 \times 10^9$ cells.

Controls

Soil and water samples were used as controls in this investigation. The soil samples were dry sandy loam pulverized in clean mortars and packed into polyethylene tubes. The water samples were distilled, sterilized and transferred
into the same type tube sections using a pasteur pipette. Wet soils were also prepared. These consisted of 70 per cent water and 30 per cent soil.

Equipment

All weight determinations in these experiments were made using a Metler (Sargent, Des Plaines, Illinois) analytical balance accurate to 0.0002 gram. The electronic equipment utilized in conducting this investigation is shown in block diagram form in Figure 1. The functions of the individual components of the apparatus are described below.

Power Supply

Power was supplied from a standard laboratory source which provided suitable voltage and frequency to heat the filaments, power the electrodes of the reflex Klystron and drive the system.

Reflex Klystron

The klystron employed was an L1633 (Raytheon, Dallas, Texas) microwave oscillator which was driven by a rapidly varying (Sawtooth) voltage signal derived from the time base of the cathode ray oscilloscope.
Figure 1. Block diagram of experimental apparatus used in this investigation.
Modulator

The modulator (Hewlett Packard, Palo Alto, California) provided a smooth sinusoidal "chopper signal" of 31 KHz (KHz = 10^3 cycles per second) to modulate the sawtooth signal at a suitable frequency for amplification by the tuned amplifier.

Frequency Multiplier

The frequency multiplier employed was a TB 40BBU Hewlett Packard (Palo Alto, California) oscillator which provided the reference or marker signal of such a nature that it could be harmonically mixed to beat with the output signal from the reflex klystron.

Auxiliary Signal Generator

The function of the auxiliary signal generator (Tektronix, Portland, Oregon) was to provide a means of tuning the output from the frequency multiplier on a vernier scale so that frequency measurements could be made.

Radio Receiver

The Navy model R-441A/Srr-13 radio receiver (Radio Corporation of America, Camden, New Jersey) with a frequency range of 2.0 to 30 MHz received the output signal from the frequency multiplier-mixer network, and transmitted this signal to the oscilloscope.
Coupler

The coupler (Tektronix, Portland, Oregon) utilized had a diode device which served to mix the signals from the frequency multiplier, auxiliary generator, and klystron and provide a usable signal to the radio receiver.

Reflectometer

The reflectometer used was a device which detected and differentiated signals from the resonant cavity.

Tuned Amplifier

The tuned amplifier designed by the North Texas State University Physics Department electronics shop (D. Maxon) served to maintain the entire system at a very low power density (25 mW) to prevent thermal damage to the sample within the cavity. It also served the function of amplifying the output signal of the reflectometer so that it could be observed on the oscilloscope.

Oscilloscope

The oscilloscope employed was an LA 545 Tektronix (Portland, Oregon) dual trace oscilloscope which allowed observation of the marker signal and the resonance signal simultaneously.

Resonant Cavity

This cavity was the heart of the entire system. It was a cylindrical device designed to give optimum resonance
of microwave signals at wavelengths of less than 10 cm. The samples were placed into this device, and the activity of its resonance was the source of all the data.

**Micrometer Drive**

The micrometer drive was a screw type micrometer (Starrett, Athol, Massachusetts), which allowed samples to be inserted at precise depths into the cavity.

**Measurements**

The microwave signal output from the klystron was electronically and mechanically tuned to the desired frequency by means of a sawtooth voltage signal and a mechanical tuning screw. The tuned output signal from the klystron propagated through the waveguide to the cylindrical resonance cavity, which at a specific frequency absorbed energy from the applied signal. As this energy was absorbed, there ensued resonance within the cavity, thereby impressing an absorption signal (i.e., resonance signal) in the reflectometer. The resonance signal was amplified after differentiation and transmitted to the oscilloscope, where it was observed and referenced to the frequency marker signal. The marker signal was generated by the signal generator in conjunction with the frequency multiplier-mixer network and transmitted to the oscilloscope via a radio receiver. The two signals, absorption due to the cavity and the
marker signal, were displayed simultaneously on the dual trace oscilloscope.

The frequency measurements were made by tuning the radio receiver at a specific frequency so that the marker signal was coincidental with the center of the resonance signal. When a sample was inserted into the resonant cavity, the characteristic resonance frequency and quality factor of the cavity were changed (i.e., frequency shift and width of profile change). The marker signal was then tuned to coincide with the new center position of the resonance by tuning the auxiliary signal generator. The distance in MHz from the original resonance frequency to the new resonance frequency represented the change in frequency ($\Delta f$) due to the dielectric qualities of the sample and its position within the cavity.

The samples were incrementally lowered into the cavity by means of a micrometer drive, and frequency measurements were made at every 0.0625 cm of insertion into the cavity to a depth of 1.0 cm. The samples were then extracted to a depth of 0.9375 cm and measurements made every 0.0625 cm on extraction. This provided a frequency measurement every 0.0625 cm from depths 0 to 1.0 cm.

Attempts were made to measure changes in width of signal ($\Delta Q$) by the same procedure. The microwave source used, however, was not adequately stable to measure very small changes
in frequency; therefore, significant results were not obtained. It is projected that our future experiments will provide these data on width changes. However, more suitable frequency locking techniques must be employed before such small changes as were encountered can be assumed to be significant.

**Density Determinations**

In order to compare changes in frequency in a relative manner, the density of each sample was determined by the classical equation shown immediately below:

\[
D = \frac{M}{V}
\]

where:

- \(D\) = density
- \(M\) = mass
- \(V\) = volume

**Dielectric Properties**

The changes of resonant frequency versus depth of penetration of the samples into the cavity could be expressed by the equation

\[
\Delta f = A + BX
\]

where \(\Delta f\) = change in resonant frequency
- \(A\) = \(Y\) intercept, 0 (under ideal experimental conditions \(A = 0\))
- \(B\) = coefficient of linear regression (slope)
- \(X\) = change in depth of penetration into cavity (\(\Delta l\))
It was assumed that

\[ \Delta f_{H_2O} = B_{H_2O} \times, \]

\[ \frac{\Delta f}{X} = B_{H_2O} = c\varepsilon_{H_2O} \]

where:  
- \( c \) = a constant
- \( \varepsilon \) = dielectric constant

In order to compare \( \varepsilon \) of each individual sample (\( \varepsilon_x \)) to the \( \varepsilon_{H_2O} \), the \( B \) of each sample (\( B_x \)) was corrected to relative density by the equation

\[ \frac{B_x}{B_{H_2O}} = B_x \text{ corrected} = B_{xd} \]

\( B_{H_2O} \), therefore, became "standard", so that the dielectric properties of bacterial cells could be compared to the dielectric properties of water and expressed by the ratio:

\[ \frac{\varepsilon_x}{\varepsilon_{H_2O}} = \frac{B_{xd}}{B_{H_2O}} \]

This proportionality was used in conjunction with a previously determined value of \( \varepsilon_{H_2O} \) (1), so that a comparison of these data could be made with other values.
of $\varepsilon$ which were available. The theoretical values of $\varepsilon$ were derived by the equation:

$$\varepsilon_x = \frac{B_{xd}}{B_{H_2O}} (\varepsilon_{H_2O})$$
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CHAPTER III

RESULTS

Figures 2 - 8 are representative examples of the patterns which the various sample materials demonstrated when frequency shift was plotted versus depth of penetration into the resonant cavity. The zero displacement of the Y-intercept of the calculated slope resulted from mechanical inability to see into the cavity. The displacement indicates only that the sample material did not enter the cavity at exactly zero. This experimental error is of no consequence in the data, because the slope of the line representing frequency shift/depth into cavity is not dependent on the Y intercept. For practical purposes, the Y intercept should be as near zero as possible.

Each point on the graphs shown in Figures 2 - 8 represents a frequency shift in MHz of the resonant cavity due to the sample at a specific depth ($\lambda$) of penetration into the cavity. The points were individually calculated using the equation given below.

\[ c = f_o \]

\[ \Delta f = f_i - f_o \]
where: \( c \) = center of resonance
\( f_0 \) = frequency measurement at zero penetration depth
\( f_i \) = individual frequency measurements at specific depths of penetration.

The two mode frequencies utilized in these experiments were 2.35 GHz and 7.6 GHz. The resonance frequencies ranged from 20 MHz to 45 MHz as the cavity was perturbed. The frequency resonance observed on the oscilloscope screen represented an interval of 20 MHz on the mode. Examples of frequency shift calculations, indicated by stars in Figure 2, are given immediately below.

\[ \Delta f \text{ due to water, where } \lambda = 1.0 \text{ cm} \]
\[ \Delta f = 39.6 \text{ MHz} - 39.1 \text{ MHz} \]
\[ \Delta f = 0.5 \text{ MHz} \]

\[ \Delta f \text{ due to lyophilized cells, where } \lambda = 0.5 \text{ cm} \]
\[ \Delta f = 30.3 \text{ MHz} - 30.1 \text{ MHz} \]
\[ \Delta f = 0.2 \text{ MHz} \]

\[ \Delta f \text{ due to vegetative cells, where } \lambda = 0.75 \text{ cm} \]
\[ \Delta f = 29.7 \text{ MHz} - 29.2 \text{ MHz} \]
\[ \Delta f = 0.5 \text{ MHz} \]

The bold lines shown in Figures 2 - 8 represent the "least squares" of the frequency shift determinations.

At least three measurements were made using each sample of dry bacteria. Measurements using water and dry soil were
Figure 2. Representative examples of resonant frequency shifts of resonant cavity due to water, lyophilized cells and vegetative cells at 2.35 GHz as depth of penetration into cavity is varied.
Water

Lyophilized cells

Vegetative cells
Figure 3. Representative examples of resonant frequency shift of resonant cavity due to water at 7.6 GHz as depth of penetration into cavity is varied.
Figure 4. Representative examples of resonant frequency shifts of the resonant cavity due to vacuum dried, heat dried and lyophilized cells at 7.6 GHz of penetration into cavity is varied.
Figure 5. Representative examples of resonant frequency shifts of resonant cavity due to vegetative cells at 7.6 GHz as depth of penetration into cavity is varied.
Figure 6. Representative examples of resonant frequency shifts of resonant cavity due to soil and wet soil at 7.6 GHz as depth of penetration into cavity is varied.
Figure 7. Representative examples of resonant frequency shifts of resonant cavity due to wet lyophilized cells at 7.6 GHz as depth of penetration into the cavity is varied.
Figure 8. Representative examples of resonant frequency shifts of resonant cavity due to empty carrier at 2.35 GHz, empty carrier at 7.6 GHz and dry soil at 2.35 GHz as depth of penetration into carrier is varied.
Soil at 2.35GHz

Empty carrier at 2.35GHz

Empty carrier at 7.6GHz
made at the beginning of each series of measurements. The wet soil sample was measured only once, and the wet bacteria sample only twice. Figure 8 shows that the dry soil at a mode frequency of 2.35 GHz, and the empty carrier at both 2.35 GHz and 7.6 GHz did not induce frequency shifts of significant values to "fit" the linear regression equation.

The linear regression coefficient ($B_x$) was determined for each sample, except dry soil at 2.35 GHz and the empty carrier at mode frequencies of 2.35 GHz and 7.6 GHz. The value of each $B_x$ determination was corrected for density ($B_{xd}$). Those data obtained at a frequency of 2.35 GHz are given in Table II.

The value of $B_x$ of a sample material of very low density was increased significantly when considered in terms of its density. The $B_{xd}$ values of the lyophilized cell samples are the most graphic example of the importance of density correction. The $B_{xd}$ values of the lyophilized cell samples showed a rather wide range of variation; however, in every case the $B_{xd}$ value of the lyophilized cell samples were at least two times greater than the $B_{xd}$ value of water. The vegetative cell samples showed only slightly higher $B_{xd}$ values than the water samples.

The zero displacement ($A$) of the Y intercept showed that the experimental technique was acceptable because the values of $A$ were very close to zero. The linear
### TABLE II
LINEAR REGRESSION DATA AT 2.35 GHz

<table>
<thead>
<tr>
<th>Sample Material</th>
<th>Density</th>
<th>$B_x^*$</th>
<th>A**</th>
<th>$B_{xd}^*$</th>
<th>$\tilde{B}_{xd}^*$</th>
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<tr>
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<td>7.9</td>
<td>6.25</td>
<td>7.9</td>
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</tr>
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<td>10.5</td>
<td>10.9</td>
<td>...</td>
</tr>
<tr>
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<td>10.6</td>
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<tr>
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<td>11.4</td>
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<td>Lyophilized Cells</td>
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<td>7.9</td>
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<td>13.4</td>
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<td>12.3</td>
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*Values expressed at $10^{-4}$.  
**Values expressed at $10^{-2}$.  

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<th>Sample Material</th>
<th>Density</th>
<th>$B_{x}^{*}$</th>
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<th>$B_{xd}^{*}$</th>
<th>$\tilde{B}_{xd}^{*}$</th>
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<td>0.92</td>
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<td>3.18</td>
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<tr>
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<td>29.0</td>
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<td>63.5</td>
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<td>37.0</td>
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<td>5.0</td>
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<tr>
<td></td>
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<td>25.7</td>
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<td>45.0</td>
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</tr>
<tr>
<td></td>
<td>0.55</td>
<td>14.2</td>
<td>5.0</td>
<td>25.8</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>0.58</td>
<td>14.8</td>
<td>5.0</td>
<td>25.5</td>
<td>34.5</td>
</tr>
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<td>35.7</td>
<td>7.75</td>
<td>33.0</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>1.08</td>
<td>41.2</td>
<td>7.75</td>
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<td>...</td>
</tr>
<tr>
<td></td>
<td>1.08</td>
<td>40.0</td>
<td>1.25</td>
<td>37.0</td>
<td>36.0</td>
</tr>
</tbody>
</table>

*Values expressed x $10^{-4}$.

**Values expressed x $10^{-2}$.
regression data obtained at 7.6 GHz are given in Table III. At 7.6 GHz the water B_x values were reproducible to 10 per cent, but only two measurements were recorded. The dry soil was reproducible at about 25 per cent. The B_x values of lyophilized cells were reproducible to about 25 per cent. The wet cell samples produced almost identical data, but again, only two samples were measured.

The data on vacuum dried cells were reproducible to about 20 per cent, but those on heat dried cells were not. There was a 40 per cent variance among the four samples with respect to B_x values. The data from the vegetative cell samples were within 15 per cent reproducibility.

In order to make these data more meaningful, the average B_xd value of each sample was determined (B_xd̅) and the Y intercept (A) was set to equal zero, so that

\[ \Delta f = B_{xd} \Delta \ell \]

for each measurement series. The dielectric properties could then be compared to the dielectric properties of pure water. Examples of these calculations are given below, and are graphically illustrated in Figures 9 - 11.

When calculating the experimental regression coefficients by the method of "least squares" to fit a linear equation, metric units for \( \Delta \ell \) were not used. In performing these experiments, the samples were lowered in increments
of 25 arbitrary units on the micrometer scale. Four hundred units on the micrometer scale equal 1.0 cm. These values were converted to metric units for convention in reporting the data.

\[ \Delta f \text{ due to water at } 2.35 \text{ GHz} \text{ (Figure 9)} \]
where \( \lambda = 1.0 \text{ cm} \)

\[ \tilde{E}_{xd} = 9.8 \times 10^{-4} \text{ (from Table II)} \]
\[ \Delta f = (9.8 \times 10^{-4})(400) \]
\[ \Delta f = 0.392 \text{ MHz} \]

\[ \Delta f \text{ due to vegetative cells at } 7.6 \text{ GHz} \text{ (Figure 10)} \]
where \( \lambda = 1.0 \text{ cm} \)

\[ \tilde{E}_{xd} = 36.0 \times 10^{-4} \text{ (from Table III)} \]
\[ \Delta f = (36.0 \times 10^{-4})(400) \]
\[ \Delta f = 1.44 \text{ MHz} \]

\[ \Delta f \text{ due to water at } 7.6 \text{ GHz} \text{ (Figure 11)} \]
where \( \lambda = 0.75 \text{ cm} \)

\[ \tilde{E}_{xd} = 42.4 \times 10^{-4} \text{ (from Table III)} \]
\[ \Delta f = (42.4 \times 10^{-4})(300) \]
\[ \Delta f = 1.27 \text{ MHz} \]

All lines given in Figures 9 - 11 were determined in the same manner.

Figure 9 shows that at 2.35 GHz, the relative frequency shift due to lyophilized bacterial cells is much
Figure 9. Comparative resonant frequency shifts of resonant cavity at 2.35 GHz due to water, vegetative cells and lyophilized cells, as depth of penetration into cavity is varied.
I S (.0- 0.75 1.0 Al (cm) Lyophilized cells Vegetative cells Water
Figure 10. Comparative resonant frequency shifts of resonant cavity at 7.6 GHz, due to water, lyophilized cells, wet lyophilized cells, and vegetative cells.
Resonant Frequency Shift (MHz)

Δl (cm)

--- Lyophilized cells (wet)
--- Lyophilized cells (dry)
--- Water
--- Vegetative cells
Figure 11. Comparative resonant frequency shifts of resonant cavity at 7.6 GHz due to water, soil, wet soil, heat dried and vacuum dried cells, as depth of penetration into cavity is varied.
greater than the frequency shift due to water. The vegetative cells showed very nearly the same dielectric characteristics as water. The difference observed in the latter comparison was too small to be considered significant.

Figure 10 indicates that, at 7.6 GHz, the frequency shift due to water is much less than the frequency shift due to lyophilized cells. It is also evident that the combination of water and lyophilized cells causes a higher frequency shift than either dry lyophilized cells or water. The frequency shift due to vegetative cells was significantly less than water. Dead cells of the same water content caused frequency shifts approximately two times greater than the frequency shifts induced by the vegetative cells.

It can be readily observed in Figure 11 that at 7.6 GHz bacterial cells dried by vacuum at room temperature exhibit only slightly different dielectric properties than the heat dried cells. Both heat dried and vacuum dried cells caused a greater frequency shift than inert dry soil, and slightly greater frequency shift than the wet soil sample. The frequency shift due to water is significantly greater than frequency shifts due to heat dried cells, vacuum dried cells, dry soil, and wet soil.

Table IV gives a summary of the data obtained from this experiment. It shows a relative comparison of the
<table>
<thead>
<tr>
<th>Sample Material</th>
<th>$\tilde{B}_{xd}$</th>
<th>$\tilde{B}<em>{xd}/\tilde{B}</em>{H_2O}$</th>
<th>$\varepsilon_x$</th>
<th>$\tilde{B}_{xd}$</th>
<th>$\tilde{B}<em>{xd}/\tilde{B}</em>{H_2O}$</th>
<th>$\varepsilon_x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Soil</td>
<td>....</td>
<td>....</td>
<td>23.9</td>
<td>0.56</td>
<td>26.5</td>
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</tr>
<tr>
<td>Wet Soil</td>
<td>....</td>
<td>....</td>
<td>29.0</td>
<td>0.68</td>
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<tr>
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<td>95.2</td>
<td>55.5</td>
<td>1.30</td>
<td>62.4</td>
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<td>....</td>
<td>31.3</td>
<td>0.71</td>
<td>34.1</td>
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<tr>
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<td>....</td>
<td>....</td>
<td>34.5</td>
<td>0.80</td>
<td>38.4</td>
<td></td>
</tr>
<tr>
<td>Vegetative Cells</td>
<td>12.3</td>
<td>1.1</td>
<td>37.4</td>
<td>36.0</td>
<td>0.84</td>
<td>40.32</td>
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<td>....</td>
<td>63.5</td>
<td>1.49</td>
<td>71.52</td>
<td></td>
</tr>
</tbody>
</table>

*Values expressed x 10^{-4}.

**$\varepsilon_{H_2O}$ at 2.35 GHz = 34 (1).

***$\varepsilon_{H_2O}$ at 7.6 GHz = 48 (1).
theoretical dielectric properties of the cells of Az. vinelandii. The $\tilde{\varepsilon}_{xd}$ values from Tables II and III were used to determine the values of $\varepsilon$ relative to water. The $\varepsilon$ values of the samples were proportionally equal to the $\Delta f/\Delta\lambda$ relationships because constant values of $\varepsilon_{H_2O}$ were used in the $\varepsilon$ determinations.

At 2.35 GHz it was observed that the $\varepsilon$ value of lyophilized cells was almost three times as great as the $\varepsilon$ of water, while the vegetative cells demonstrated only slightly greater $\varepsilon$ values than water at that frequency.

At 7.6 GHz, it appears that dry soil and wet soil have very nearly the same values of $\varepsilon$, and their values were more than half of the $\varepsilon$ value of water.

The $\varepsilon$ value of the lyophilized cells at 7.6 GHz was about 30 per cent greater than the $\varepsilon$ value of water. The heat dried and vacuum dried cells had $\varepsilon$ values approximately three-fourths the value of $\varepsilon$ for water, and slightly less than the vegetative cells. Curiously, the $\varepsilon$ value of the wet dead cells was about 50 per cent greater than the $\varepsilon$ value of water, and about 15 per cent greater than the $\varepsilon$ value of dry lyophilized cells.
CHAPTER BIBLIOGRAPHY

CHAPTER IV

DISCUSSION

The data obtained from this experiment indicate that bacterial cells may in fact interact with microwaves in some manner other than in heat exchange. It should be understood that without data on quality changes within the cavity, no unequivocal statement supporting nonthermal effects can be made. These data, however, should not be regarded as superfluous. The relationship between dielectric properties of biological systems and their response to electromagnetic radiation has been a perplexing subject for many years. The problem seems to be profoundly intricate. It was assumed in these experiments that frequency changes were due to absorption of energy from the microwave signal.

The observation that viable cells have very nearly the same dielectric properties as water at 2.35 GHz was not surprising. The literature predicted this. At 7.65 GHz, the proportional decrease in $\Delta f$ by the viable cells could be explained as a dampening effect on the freedom of motion of water molecules due to viscosity of the cell cytoplasm (1). This reasoning could also account for the lower than expected frequency shift exhibited by the wet soil sample.
The relatively high frequency shift exhibited by the lyophilized cells indicated that the cells or their constituent molecules were interacting with the microwave field in some manner independent of water molecules. The amount of water was assumed to be less than one per cent, and therefore could not be considered a major contributor to the phenomenon. The possibility of interaction of microwaves with macromolecules such as proteins, amino acids, and nucleic acids was therefore considered. This consideration is not consistent with data presented by Schwan (2), who states that proteins absorb microwaves in a frequency range of 300 to 2,000 MHz. Schwan, however, was reporting on hydrated (80 - 90 per cent water) animal tissues and their liquid extracts. Dry bacterial cells would understandably have much higher protein concentrations and much lower water content than animal tissues.

By the addition of water to the lyophilized cells, one could have predicted the increase in frequency shift. The increase was not, however, proportionally equal to the additive effect expected. This could reasonably be explained by the dampening effect already described. The observation that the dead cells with 70 per cent water displayed a much higher $\Delta f$ than the vegetative cells indicates that the integrity of the living system may offer some resistance to microwave radiation.
Considering the Δf of the heat dried and vacuum dried cells, it would be reasonable to assume that due to the harshness of the drying procedure the macromolecules of the cells were destroyed.

The unusually high dielectric constant of the soil sample at 7.6 GHz was probably due to inadequate drying. It should be noted that the technique used in this experiment assumed predetermined values for the dielectric constants for water, and these may not have been accurate.

The technique of measuring perturbation of a resonant cavity by a small sample of material has, in the last few years, grown in popularity among physicists, but only recently has this technique been used in biological sciences. By use of this technique, it is believed that future investigations will provide an absorption spectrum for various microorganisms.

Previous experiments performed by the author using a similar technique revealed the possibility of evolution of ammonia (NH₃) by various species of azotobacter. This possibility was considered on observing a linear decrease in Δf at 7.6 GHz over a period of thirty days after lyophilizing the cells in the same manner as was described in this experiment. (The cells were not coated with a stabilizing substance.) The absorption of microwave energy by NH₃ characteristically occurs from 12 GHz to 36 GHz (7). Dampening of the freedom of motion of the NH₃ molecule when it is bound may change the absorption frequency
spectrum by a factor of two or perhaps three. Enzyme-bound NH₃ would probably have a much lower frequency spectrum than free NH₃.

It was observed that Δf of wet cell paste immediately after harvesting was too great to be measured by the technique employed. After lyophilization, the Δf of the cells was measured every three to five days. The drawback of this technique was the rectangular resonant cavity which did not possess the quality characteristics of the cylindrical cavity used in the experiments described here. The rectangular cavity did not employ a microwave drive. All measurements were therefore taken at a single position within the cavity.

Conclusions

Under the conditions of this experiment, bacterial cells were shown to interact with microwave radiation in some manner other than that which can be explained by the presence of free water. It cannot be assumed, however, that the interaction would be lethal to the bacteria. These experiments demonstrated a reproducible technique for the determination of dielectric properties of bacterial cells. Vegetative Az. vinelandii cells possess dielectric properties very similar to the dielectric properties of pure water. Dry bacterial cells, however, possess markedly different dielectric properties than either vegetative cells or water.
CHAPTER BIBLIOGRAPHY


APPENDIX

Burke's nitrogen-free medium:

\[
\begin{align*}
K_2HPO_4 & \quad 0.64 \text{ gm} \\
KH_2PO_4 & \quad 0.16 \text{ gm} \\
NaCl & \quad 0.20 \text{ gm} \\
MgSO_4 \cdot 7H_2O & \quad 0.20 \text{ gm} \\
CaSO_4 \cdot 2H_2O & \quad 0.05 \text{ gm} \\
Na_2MoO_4 \cdot 2H_2O & \quad 0.001 \text{ gm} \\
FeSO_4 & \quad 0.003 \text{ gm} \\
\text{Glucose} & \quad 0.20 \text{ gm} \\
H_2O & \quad 1.01
\end{align*}
\]
BIBLIOGRAPHY


Osborne, S. L. and J. N. Frederick. 1948. Microwave radiations. JAMA 137:1036-1040.


