TOWARDS A MECHANISM OF ACTION OF A WEAK MAGNETIC FIELD ON

BACTERIAL GROWTH

by

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Dedication

I would like to dedicate this thesis to my mother, father, and sister, all of whom have supported me in my studies.

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ABSTRACT

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The effects of weak magnetic fields (WMFs) on bacteria have attracted considerable

attention in magnetobiology. Recent studies have shown that exposure to WMFs alter

bacterial behavior at cellular and molecular scales. Classical models of

magnetobiological effects face difficulties due to a paradox in which the inherent thermal

noise in biological systems is orders of magnitude larger than the WMF interaction. The

plausibility of quantum theoretical models to describe these interactions is discussed. In

this study, the effects of static and oscillatory magnetic fields on bacteria are investigated

in vitro. *E. coli* cultures were suspended in tryptic soy broth and grown in their respective

magnetic field configurations for three consecutive generations. The optical density

(absorbance) of field-exposed and control cultures was measured as a function of time.

Emphasis is placed on understanding the WMF effects on subsequent generations of bacteria and their adaptability to such conditions. Biological effects of the oscillating magnetic fields were sustained in the second generations of *E. coli* while the effects were absent in the third generation. Our results suggest that bacteria may have a means of adapting to perturbations of a WMF on the cellular environment, depending on the field characteristics.

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CHAPTER I: INTRODUCTION

Living Organisms and the Geomagnetic Field

Deep inside the Earth, convection currents of molten iron in the outer core generate electric currents that are responsible for creating the geomagnetic field that permeates thousands of kilometers into space. On the surface, the strength of the field has small variations on the order of 10^{-4} Tesla (T) [1]. The presence of the Earth's magnetic field was and is still crucial for the evolution of life; it protects the planet from cosmic radiation and solar winds that would otherwise damage the earth's atmosphere, which as a result would expose living organisms to harmful radiation. For a period of about 3.8 billion years, life has evolved in the geomagnetic field. A natural question then arises: did the earth's magnetic field play a role in the development of life?

Recently, there have been advances in the understanding of how certain organisms interact with the geomagnetic field. A class of bacteria known as magnetotactic bacteria are known to be able to align themselves along the direction of a weak magnetic field (WMF) through a mechanism known as magnetotaxis [2]. In other organisms, the mechanism of magnetoreception is still unknown; *Drosophilia Melanogaster* are a species of fruit flies that have shown light-dependent magnetosensitivity [3], and migratory avian species such as the European Robin are believed to be able to detect variations in the local magnetic field. One of the leading hypotheses that exists to explain magnetoreception in these organisms is the radical pair mechanism, which is well-defined in the field of spin chemistry [4]. Even in humans, there is evidence that a protein in the eye could serve as a magnetoreceptor [5]. These topics are encapsulated in the field of magnetobiology.

Emergence of Magnetobiology

It is important to note that there is a key difference in the magnetic field exposure of early organisms to the exposure in the present day. That difference is the advent of electronics. smartphones, laptop computers, household appliances, and power transmission lines, which have become common sources of weak electromagnetic fields (EMFs) (see Table 1.1). Power systems in America and most European countries supply electric currents at frequencies of 50 Hertz (Hz) and 60 Hz, respectively [6]. Concerns have been raised on whether exposure to sources of static and oscillating magnetic fields could have long-term biological or biomedical impacts. Several studies have reported that strong magnetic fields are capable of inducing changes in biological systems, such as those produced by medical imaging devices [7]. Other studies have suggested that weak magnetic fields can affect ionic currents in blood circulation [8]; however, there is no experimental evidence that supports this claim. Some epidemiological studies have proposed that exposure to high-voltage power lines could be linked to cancer [9]; again, the evidence is not robust, as the amount of uncertainty and bias in these experiments tend to be significant. Currently, there is no mechanism by which non-ionizing radiation could cause cancer.

Description	Magnetic Field (μT)
Directly below 400kV power line at ground level.	40
25m from centre line of 400kV power line.	8
Directly below 132kV power line at ground level.	7
25m from centre line of 132kV power line.	0,5
Vacuum cleaner, electric drill.	2 - 20
Food mixer.	0,6 - 10
Hair dryer.	0,01 - 7
Dish washer.	0,6 - 3
Washing machine.	0,15 - 3
Fluorescent lamp.	0,15 - 0,5
Ambient field inside homes.	0,01 - 0,2

Note: Levels indicated for household appliances were measured at 30cm from the appliance.

Table 1.1: Common Magnetic Field Sources [6].

As of late, a fair amount of interest has been taken in magnetobiology, the study of the effects of weak static and extremely low frequency (ELF) magnetic fields on biological systems. The relevant range of frequencies dealt with is put into perspective in Figure 1.1. The two major aspects of static and ELF-WMFs are their non-ionizing nature and inability to cause significant thermal effects in biological tissue. It has been observed in a subset of magnetobiological studies (discussed in chapter III) that bacterial growth is affected by WMFs. These results remain inconclusive, and the mechanism of action is still unknown. In this regime, biological effects give rise to a paradox, as the energy due to a WMF is much less than the thermal energy in a biochemical transformation. The problem then reduces to understanding how such a weak magnetic signal causes any significant change amid the stochastic noise inherent in biological systems.
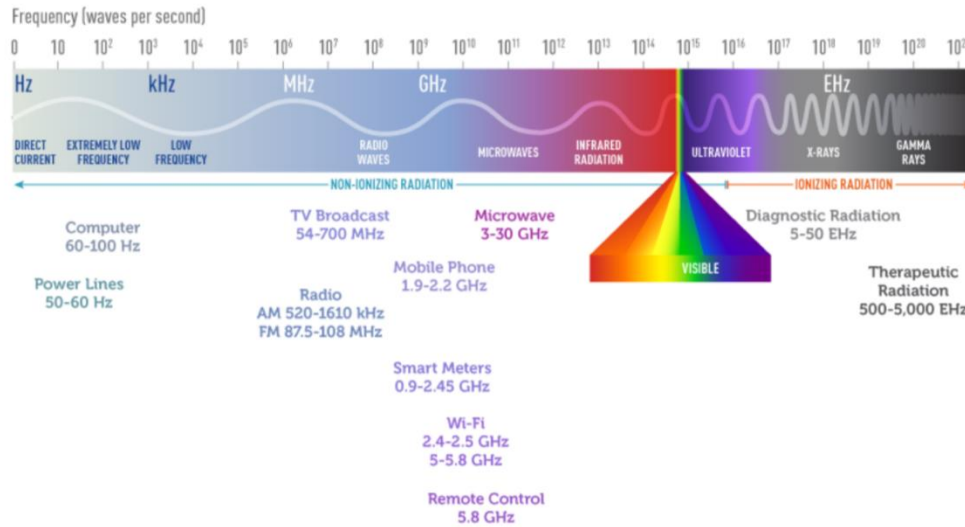
Figure 1.1 Electromagnetic Range of Frequencies.
https://www.cancer.gov/about-cancer/causes-prevention/risk/radiation/electromagnetic-fields-fact-sheet

Why Study Bacteria?

Bacteria were among the first forms of life on Earth. Today, more than 35,000 species are known to exist [10]. They are found in all sorts of habitats, from soil to radioactive waste [11], and even the human digestive tract [12]. Some bacteria are important in regulating the nutrient cycle of the atmosphere, while others are known to cause infectious diseases; some find application in biotechnology in microbial fuel cells [13] and the food industry [14]. The structure and organization of bacterial cells are relatively simple compared to that of more complex multicellular life forms such as plants and animals. Furthermore, their fast rate of reproduction conveniently allows for the understanding of how they grow and respond to external stimuli, making them the ideal candidates for studying the biological effects of magnetic fields.

Several preliminary studies have been conducted on different species of bacteria in a variety of magnetic field configurations in the biophysics group at the University of

Houston Clear-Lake. Among these studies are master's theses that have explored the role of electromagnetism [15] [16], weak magnetic fields [17] [18], and the interaction of weak magnetic fields and antibiotics on bacterial growth [19]. Additionally, a multitude of conference presentations have detailed the preliminary findings of these studies [20] [21] [22] [23] [24]. A few papers have also been submitted and accepted for publication in peer-reviewed journals [25] [26] [27]. These studies altogether form the crux of this thesis.

The contents of this thesis strive to illuminate the present condition of the experimental and theoretical facets of magnetobiology while instilling a motivation to push the field further towards elucidating a mechanism of action of WMFs on bacteria. In Chapter II, an introduction to the organization and growth of a bacterial cell will be given. Chapter III contains an overview of the physical aspects of magnetic fields and the relevant body of literature of their effects on bacteria. The details of a growth experiment conducted on *E. coli* in static and time-varying WMFs is presented in Chapter IV, with the results and discussion following in Chapter V.

CHAPTER 2: BACTERIA

Prokaryotic cells are better characterized by the features that they lack when compared to the more complex eukaryotic cell that possess a nucleus and membrane-bound organelles. We restrict ourselves to the discussion of bacteria, as their relatively simple structure (see Figure 2.1) and rapid rate of reproduction make them the ideal candidates for which we can study the biological effects of magnetic fields. In this chapter, an introduction will be given to the basic structure of bacteria and conditions for their growth.
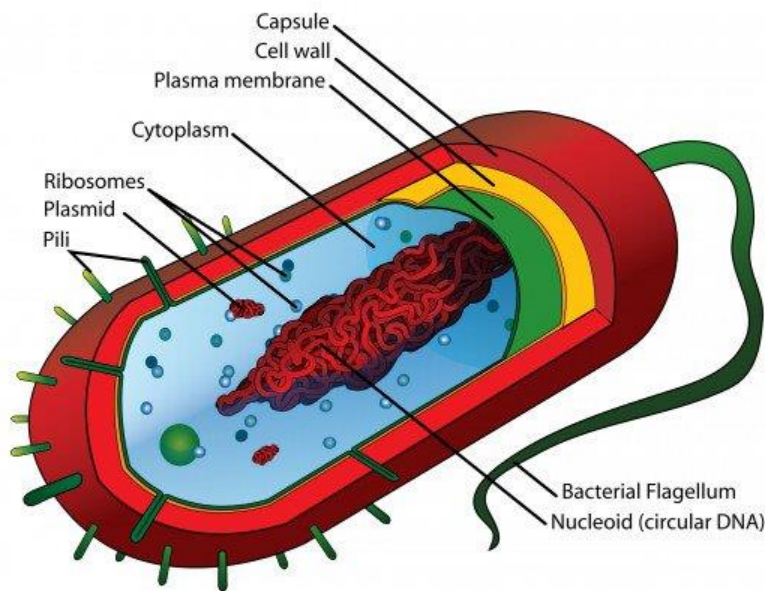
Figure 2.1 Structure of a Bacterial Cell.
https://owlcation.com/stem/What-Are-Cells-Made-Of-Prokaryotic-Cell-Structure-Part-3-of-3

Cellular Organization

Cells can be broken down into four organic constituents: nucleic acids, proteins carbohydrates and lipids. These macromolecules form the basis of the machinery that contribute to cellular growth and function (see Figure 2.2).

Deoxyribonucleic acid (DNA) is an essential molecule for living organisms. It is composed of biopolymers called nucleic acids which are built out of nucleotides—monomers that contain a five-carbon sugar, nitrogenous base, and at least one phosphate group. The nitrogenous bases (adenine, thymine, guanine, and cytosine) are selectively paired by hydrogen bonds and eventually wound into a double helix structure (see Figure 2.3). Nucleotides also form the basis of adenosine triphosphate, the energy currency of cells. Proteins are synthesized from the genetic information encoded in the DNA and are responsible for many functions in an organism. They are built out of sequences of amino acids. Carbohydrates belong to a chemical group called saccharides consisting of carbon, hydrogen, and oxygen. They serve as a major source of energy for living organisms and are also components of ribonucleic acid (RNA) and DNA. Lipids play an important role in energy storage as well and are the main structural components of cell membranes.
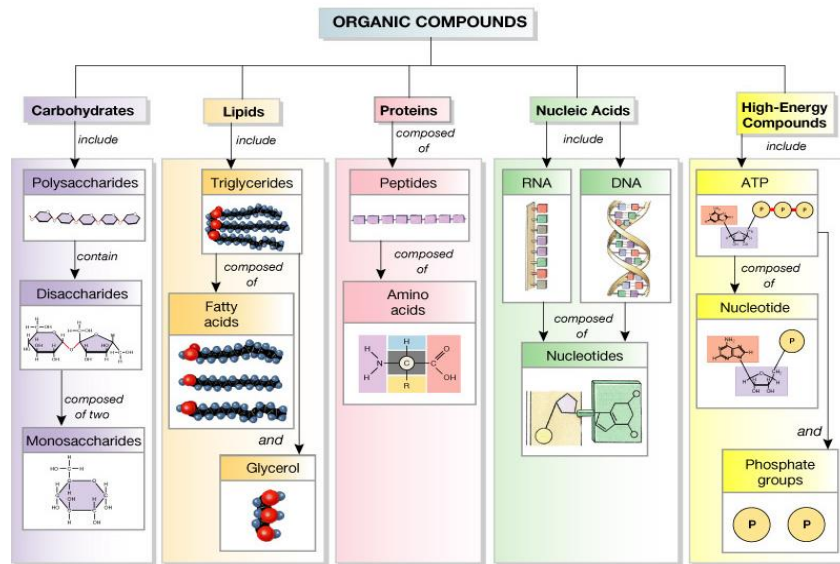
Figure 2.2 Organic Constituents of Living Organisms.
http://www.newcriticals.com/what-is-life-part-ii-biomolecules-and-the-genetic-code/print
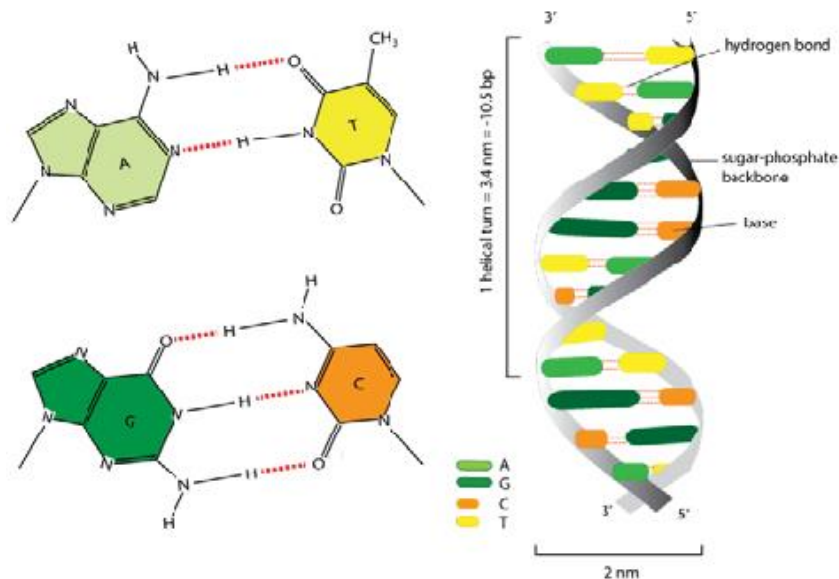
Figure 2.3 Double-helix Structure of DNA.
https://www.researchgate.net/figure/Complementary-base-pairs-left-and-secondary-structure-of-a-DNA-molecule-right_fig11_259848448

Internal Structure

The internal contents of a cell are housed by the cytoplasm, a water-based solution composed of sugars, amino acids, and salts. Inside a region called the nucleoid lies the DNA, the molecule that contains the genetic instructions for cellular heredity. In bacteria, the DNA is of a circular form and wrapped around a single chromosome. There is another source of genetic information in the cytoplasm which comes from satellite DNA structures called plasmids. These small, circular double stranded DNA molecules are typically transferred from cell to cell via bacterial conjugation and the genes they express provide situational advantages to cells—such as antibiotic resistance and the ability to break down different types of nutrients—depending on the state of the cellular environment.

Structures that exist outside of the nucleoid region include ribosomes, enzymes, and other protein-bound organelles. Ribosomes are structures that link together sequences of amino acids from messenger RNA (mRNA) to synthesize various proteins. The functions of these proteins include DNA replication, nutrient transport, cellular signaling, and catalyzing biochemical reactions. Enzymes are biological catalysts consisting of protein complexes that participate in breaking down nutrients into useable chemical forms as well as synthesizing new macromolecules. Cells also use protein-bound organelles to provide microcompartments that localize metabolic reactions.

The Cell Envelope

Throughout its environment, a cell may encounter a variety of mechanical and chemical influences, some of which may have negative effects on its growth. Two important structures exist to separate the inside of the cell from the outside: the cell (cytoplasmic) membrane and cell wall.

Cell Membrane

The cell membrane consists of a bilayer of phospholipid molecules and embedded proteins. Each of the phospholipids have 2 hydrophobic fatty acid tails attached to a hydrophilic phosphate head (see Figure 2.4). The membrane structure and dynamics are currently described in terms of the fluid mosaic model (see Figure 2.5) as a two-dimensional fluid that suppresses the lateral diffusion of certain membrane components while allowing for the diffusion of others [28]. Only water, dissolved gases such as carbon dioxide and oxygen, and lipid-soluble molecules may freely diffuse across the bilayer. Proteins also able to diffuse through the membrane, their main tasks being to regulate intracellular signal transduction, ion and molecular transport, and intercellular communication.
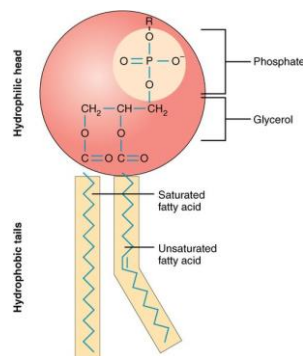
Figure 2.4 Phospholipid Molecule.
https://en.wikipedia.org/wiki/Phospholipid
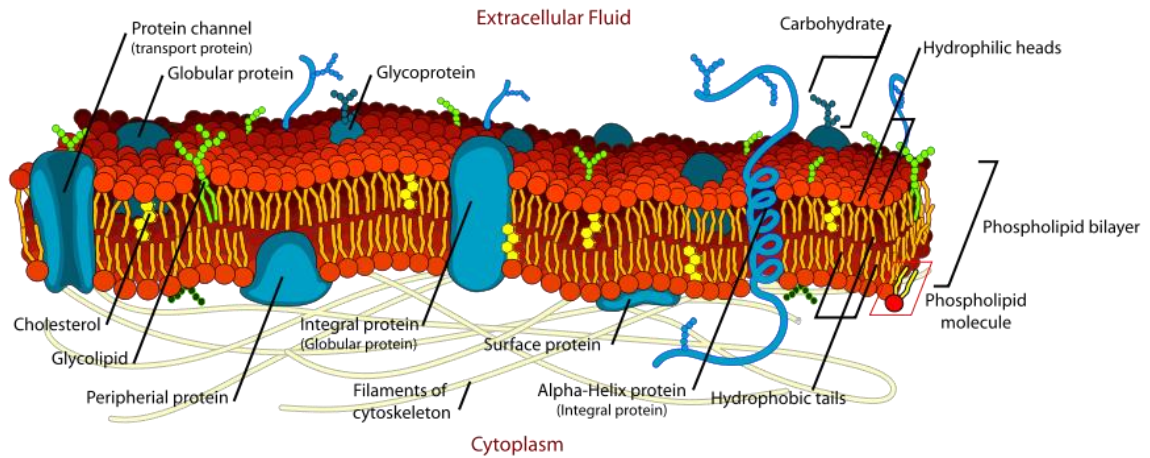
Figure 2.5 Fluid Mosaic Model.
https://en.wikipedia.org/wiki/Fluid_mosaic_model#/media/File:Cell_membrane_detailed
_diagram_en.svg

Energy plays a significant role in the diffusion of substances through the cell membrane. In general, there are three ways substances are transported throughout a cell. The first is through passive diffusion, by which a system of molecules will tend towards equilibrium by minimizing the chemical potential energy. The second way is through facilitated diffusion, where a membrane protein binds itself to a specific insoluble molecule or ion to transport it from an area of high to low solute or charge concentration. The flow of ions around the cellular environment can signal protein channels to open or close by inducing changes in the membrane electrochemical potential. The third mechanism is active transport, a process in which membrane proteins transport materials against the concentration gradient by using the energy from ATP. In addition to transport, the cell membrane also has the task of regulating biochemical signaling. An example of a process in which this occurs is quorum sensing [29], where cells respond to certain chemical molecules that are secreted by other bacteria. Protein receptors on a cell

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membrane can recognize changes in the local density of these signaling molecules upon binding and as a result will modulate gene expression to compensate for changes in the cellular environment. Quorum sensing enables unicellular entities such as bacteria to behave as a collective, multicellular entity to complete large-scale tasks such as forming biofilms.

Cell Wall

The bacterial cell wall is composed of a porous substance known as peptidoglycan, a polymer consisting of sugars and amino acids. It is a means of protection from various environmental factors that can cause cell lysis or death. Bacterial cell walls are common targets of antibiotics that inhibit peptidoglycan synthesis. If cell wall synthesis is blocked, then no new cells will be able to form, resulting in the death of a population.

Cell walls also play an important role in determining the shape of a cell and allow bacteria to be differentiated through Gram-classification. By using a staining technique, certain types of bacteria can be identified by their cell wall structure. Gram-positive bacteria have cell walls ranging from 20 nm to 80 nm in thickness whereas Gram-negative cell walls are comparably thinner on the order of 1 nm to 3 nm. In the staining procedure, a crystal violet dye is used to colorize the peptidoglycan layer. A decolorizing agent is then used to wash out the crystal violet color. The gram-positive bacteria will tend to retain the crystal violet after a counter-stain of safranin is added. Gram-negative bacteria will lose the crystal violet, and instead are stained pink due to the safranin.

External Structure

      The interactions of bacteria with their extracellular environment are facilitated through external protein structures. Species of bacteria that can actively move throughout their environments are designated as motile, while species that passively move through their environment are called non-motile. Motile bacteria possess flagella, which are whip-like structures that can be found in a variety of arrangements protruding from the cell wall. E. coli is one of the more well-known species of motile bacteria. Their motility is based on the mechanism of chemotaxis in which their movement is biased towards chemical attractants and away from repellents [30]. Non-motile bacteria such as *S. aureus* typically diffuse through liquid environments or secrete surfactants on solid surfaces [31]. Some bacteria also have fimbriae and pili, which are used to attach to surfaces or transfer genetic information in between cells.

Growth and Nutrition

The growth and reproduction of bacteria are closely related to each other. A cell begins to gain mass as it takes in nutrients and eventually reaches a point when it must divide. The critical time or size at which a cell divides can vary from cell to cell and is still an active area of research [32] Furthermore, the average growth rate of a bacterial population depends on the physical and chemical state of the environment. On the population level, quantitative models of growth are used to understand how bacteria respond to external factors. These models are often verified through quantitative laboratory methods.

Conditions of Growth

Binary Fission

Bacteria reproduce asexually through the process of binary fission. Cell division begins when the DNA uncoils from its circular form. Once the DNA has been duplicated, both copies migrate to their respective ends of the cell before the cell wall splits. Two daughter cells are formed after the cell wall regenerates, with each one containing ribosomes, plasmids, and the copies of DNA. An illustration of the process is shown in (see Figure 2.6). The timescale through which binary fission depending on environmental factors and varies across bacterial species.
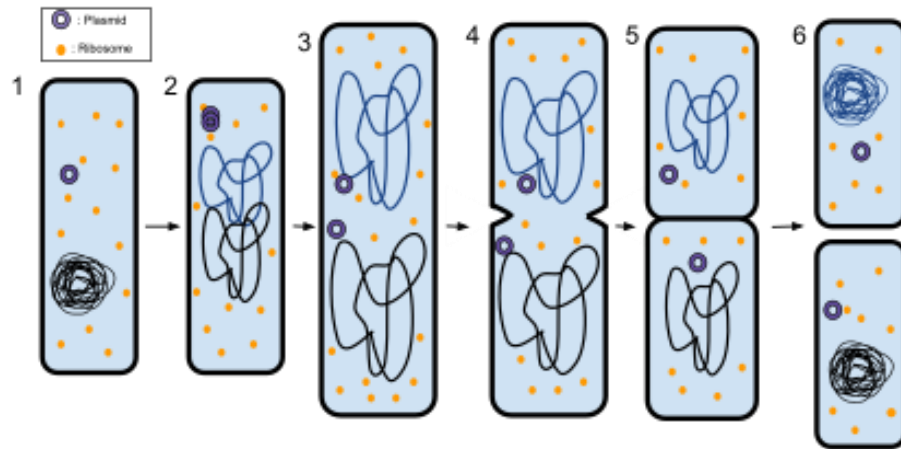
Figure 2.6 Binary Fission Process.
https://en.wikipedia.org/wiki/Fission_(biology)

Environmental Factors

For bacterial cells to grow and divide, nutrients must be collected and broken down into useable forms. A few of the common essential compounds utilized by bacteria are sugars, carbohydrates, and amino acids. In general, bacteria will not use all the available nutrients, as certain enzymes are designed for high specificity towards the essential ions or molecules required by the species. Nutrients are selected depending on the metabolic enzymes the cell possesses.

Roughly 70% of a bacterial cell's mass is comprised of water. It serves as a medium that transports nutrients and waste and helps to regulate the osmotic pressure built up by changes in solute concentrations. The amount of water needed for survival varies across different species of bacteria.

A source of energy is vital to the overall growth of a bacterial cell. Some species of bacteria harvest their energy from light while other species of bacteria harvest most of their energy through chemical processing of compounds collected from their environment. In cellular respiration, the biochemical energy stored in nutrients is

converted into ATP and waste products through a series of catabolic and metabolic reactions [33]. The availability of oxygen is important for species that undergo aerobic respiration, while species that grow without oxygen use anaerobic respiration; bacteria that can use both are called facultative anaerobes. Cellular respiration generates ATP, which is the energy currency used in various cellular processes.

Temperature and pH also have a significant impact on cell proliferation. Most bacteria require a near neutral pH value for ideal cellular growth and reproduction; however, there are some species that are known to survive in more acidic or basic conditions [34]. The temperature of the cellular environment plays a vital role in the upkeep of cellular growth mechanisms. Bacteria generally have a temperature range outside of which growth will not occur, depending on the species. *E. coli* divides on average every 20 minutes at 37 °C [35][36], which is the temperature where its maximum growth rate occurs. As the temperature is increased or decreased from the optimum temperature, the growth rate decreases marginally.

Population Growth Model

Bacterial growth is intimately related to the metabolic and regulatory processes that govern cell division. However, information can be extracted from a quantitative description of population growth without understanding the underlying mechanisms of single cell growth.

The simplest model of population growth assumes that no growth limiting factors are present. Let $N(t)$ denote the number of bacteria at a time t. The change in the number of cells in a time interval dt will be proportional to the number of cells, given by

$$\frac{dN}{dt} = \alpha N(t), \qquad\qquad (2.1)$$

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where α is the specific growth rate. Separating variables and solving for $N(t)$ yields the solution

$$N(t) = N_0 e^{\alpha t}, \tag{2.2}$$

where N_0 is the number of cells at time $t = 0$. Setting $N(t_d) = 2N_0$ in equation 2.2 gives the mean generation time t_d, which is the time at which the number of cells in a population has doubled:

$$\alpha = \frac{\ln(2)}{t_d}. \tag{2.3}$$

Hence, if it is assumed that there are no limiting growth factors, the population of cells would grow exponentially with time. In the laboratory, the conditions of growth can only be controlled to a reasonable extent. As it turns out, the exponential growth model needs to be modified to account for factors that limit growth.

Quantitative Methods

In a laboratory, bacteria are typically grown in solid or liquid media. Agar plates are examples of solid media in which certain compounds can be added to influence growth. Bacteria that are placed on plates form individual colonies which can be counted upon adequate dilution of a liquid culture. In liquid cultures, the growth of bacteria can be measured by visible light spectrophotometry.

Colony counting is a common technique used to estimate the number of bacterial cells in a known volume of a sample. By making successive dilutions of a culture and then plating them on agar, one can count the number of colonies formed. Assuming that each colony came from one viable bacterium, it is straightforward to calculate the colony forming units (CFUs) per unit volume and hence the number of cells in the original sample by taking into account the dilution factors.

Another common laboratory method makes use of a visible light spectrophotometer, which measures the turbidity or optical density (OD) of bacteria suspended in liquids. The basic layout of such a device is illustrated in Figure 2.7. Initially, light passing through a wavelength selector (monochromator or diffraction grating) is incident on a sample inside a cuvette. As the light travels through the cuvette, it can be scattered or absorbed depending on the properties of the sample. The light that emerges from the cuvette reaches a detector that converts the signal to a reading of transmittance T, which is the ratio of the transmitted to incident light intensity. More commonly used is the absorbance A, which is related to the transmittance by the equation:

$$A = -\log_{10} T. \qquad (2.4)$$

The absorbance is related to the concentration C of a sample by

$$A = \int_0^l \varepsilon C(z) \, dz, \qquad (2.5)$$

where l is the pathlength that the light has traveled through the sample, z is the distance along the direction of the beam, and ε is the molar attenuation coefficient. If there is uniform attenuation of light, then equation 2.5 becomes

$$A = \varepsilon l C \qquad (2.6)$$

which is the Beer-Lambert equation. Equation 2.6 can be used to estimate the concentration of an analyte; in the case of a bacterial suspension, this is the number of cells or biomass per unit volume.
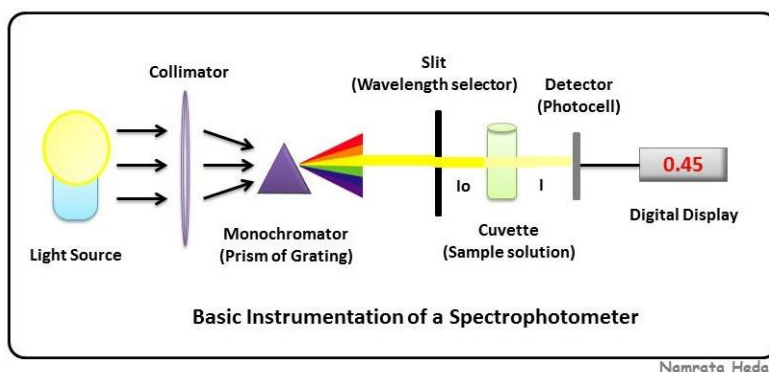
Figure 2.7: Absorbance Spectrophotometer.
http://namrataheda.blogspot.com/2013/06/spectrophotometry-part-1.html

The wavelength of light used in spectrophotometry is also important, as it determines how much light is absorbed or scattered. It is chosen based on the physical and chemical characteristics of the sample being observed. For growth rate experiments, a single wavelength is used, and the absorbance due to the nutrient broth is accounted for by zeroing the instrument. By plotting the values of absorbance over time, a growth curve is obtained, from which the doubling time of bacteria may be deduced (see equation 2.3). In order to quantify the amount of substance in a sample, the spectrophotometer is calibrated to produce a standard curve with known concentrations and their corresponding values of absorbance. For bacteria, this is typically done by plotting the absorbance as a function of the number of CFUs per milliliter.

Although both methods find their use in microbiology labs, it is important to understand their limitations. Counting CFUs usually requires between 30-300 colonies to ensure a statistically sound analysis of growth. Enumeration of colonies by eye can be cumbersome, although image analysis software and other laboratory methods do exist for this purpose. Furthermore, the assumption that each colony arises from an individual cell only sets a lower bound on the number of bacteria present, as some colonies have the possibility of merging with others throughout the growth process. Measuring cell growth using spectrophotometry has its advantages in being less labor intensive; however, this method falls short in its inability to distinguish between live and dead cells. The detector only sees the amount of light that makes it through the sample, but it cannot differentiate between scattering and absorption by the object that is attenuating the light. Also, at high concentrations, the relationship between absorbance and cell number as given in equation 2.6 becomes nonlinear due to chemical and instrumental factors [37]; in this regime, dilutions of the sample must be made and factored in to obtain a value of OD that accurately reflects the number of cells.

Microbial Growth Curve

Growth curves can be obtained by plotting the absorbance of a population as a function of time. Bacteria that are cultured in a laboratory setting are subjected to limiting growth factors. For this reason, there are four distinct phases that arise in a typical growth curve (see Figure 2.8). Initially, newly inoculated bacteria enter a lag phase where they must synthesize enzymes for the processing of specific metabolites in their environment. Cells in this phase will increase in size, but there will be no cell division. Once the cells become acclimated to the nutrient medium, they enter the logarithmic phase and begin to divide exponentially through binary fission. The rate of proliferation will depend on the environmental conditions previously discussed as well as the physiological capability of the individual species. Since the growth in this phase is exponential, the specific growth rate can be extrapolated from the slope of a semi-logarithmic plot of the absorbance vs. time. Equation 2.3 then can be used to calculate the mean generation time. In a closed environment such as a test tube or culture flask, the amount of essential nutrients will be depleted as a bacterial culture continues to grow. Additionally, metabolic waste products will accumulate in the growth medium to levels that can inhibit further growth. As a result, the curve will enter a stationary phase in which the viable cell number remains approximately constant due to competition between the cell growth and death rates. Finally, in the death phase, the cell number falls exponentially with time.
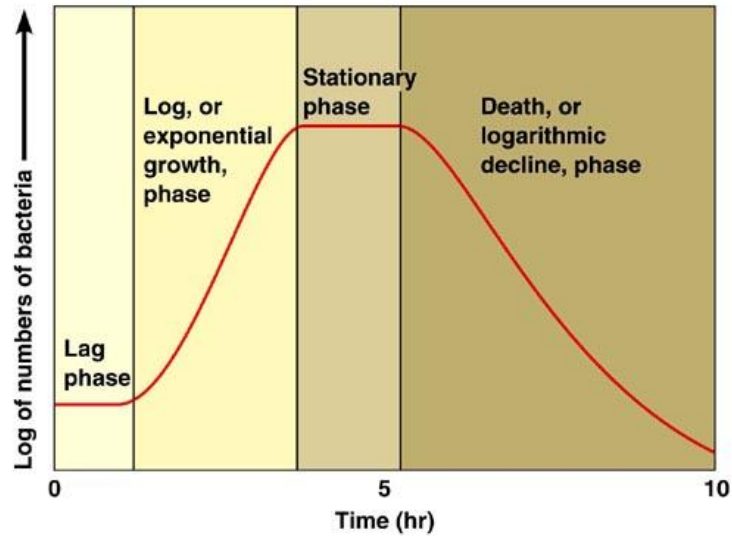
Figure 2.8 Microbial Growth Curve.
http://accounts.smccd.edu/case/biol230/growth/

The shape of the bacterial growth curve as shown in figure 2.7 is sigmoidal, and follows the logistic population growth model [38]:

$$\frac{dN}{dt} = \alpha N \left(1 - \frac{N}{K}\right) \tag{2.7}$$

where K is the carrying capacity of the biological species. In equation 2.7, the αN term is the same as the one on the right-hand side of equation 2.1. The second term accounts for the interspecies competition due to the limited amount of resources in the environment. In a more general case, K can be dependent on time or even on the population at an earlier point in time [39].

CHAPTER 3: BIOLOGICAL EFFECTS OF MAGNETIC FIELDS

Physical Aspects of Magnetism

Magnetic Field Characterization

Electromagnetic phenomena are defined in terms of the electromagnetic force, which is the fundamental interaction between electrically charged particles. The space surrounding a source of charged particles is permeated by a vector quantity called the electric field. It is a vector field that gives information on the force per unit charge that would be felt by a test charge. Charged particles are accelerated by an electric field, which gives rise to an electric current. These physical quantities are studied in electrodynamics—a classical theory that describes how electricity and magnetism are manifestations of the same phenomenon; electrical currents generate magnetic fields, while changing magnetic fields generate electric fields. The macroscopic description of electromagnetic interactions in matter is contained in Maxwell's equations [40]:

$$\vec{\nabla} \cdot \vec{D} = \rho_f \tag{3.1a}$$

$$\vec{\nabla} \cdot \vec{B} = 0 \tag{3.1b}$$

$$\vec{\nabla} \times \vec{E} = -\frac{\partial \vec{B}}{\partial t} \tag{3.1c}$$

$$\vec{\nabla} \times \vec{H} = \vec{J}_f + \frac{\partial \vec{D}}{\partial t}. \tag{3.1d}$$

The sources of electric and magnetic fields are the free charge density ρ_f and free current density $\vec{J}_f$, respectively; $\vec{E}$ is the electric field, $\vec{B}$ is the magnetic induction $\vec{D}$ is the electric displacement, and $\vec{H}$ is the auxiliary magnetic field. The latter two terms are

distinguished from the former when considering the effects of electromagnetism in matter; otherwise, they are equivalent in free space.

The magnetic field is a vector quantity that is produced by moving charge distributions. Consider the simple case of a particle with charge q and mass m moving at a velocity v through an electromagnetic field. Its motion is described by the Lorentz force equation:

$$\vec{F} = q\left(\vec{E} + \vec{v} \times \vec{B}\right) \tag{3.2}$$

where $\vec{E}$ and $\vec{B}$ are the electric and magnetic fields, respectively. In the absence of an electric field, the net force acting on the particle is orthogonal to the direction of the charge velocity. It follows from equation 3.2 that the work done by a uniform magnetic field is zero. If $\vec{B}$ is a homogeneous field (independent of position or time), the particle will undergo uniform circular motion with a radius

$$R = \frac{mv}{qB} . \tag{3.3}$$

Setting $v = 2\pi R f$, it follows that

$$f = \frac{qB}{2\pi m} , \tag{3.3b}$$

where f is the Cyclotron frequency.

Field Strength

Charges in motion constitute electrical currents, which are measured in units of amperes—or coulombs per second. Analogous to the way that an electric field can be calculated from a stationary charge distribution using Coulomb's Law, the magnetic field strength can be calculated from an electrical current using the Biot-Savart Law [40]. In a conducting wire, a unidirectional current produces magnetic field lines that circulates in the plane orthogonal to the direction of the current. In the case of a bar magnet, the field lines emanate from the north pole and eventually pass through space and enter through the south pole. The density of field lines corresponds to the strength of the magnetic field produced. The direction of the field lines is given by the right-hand rule and can be visualized through the deflection of a compass needle or with iron fillings.

Magnetic Flux

When a magnetic field is present in a medium (including free space) a magnetic flux exists. The flux Φ_m is a measure of the net amount of field lines passing through a surface, more properly defined by the surface integral over the normal component of the magnetic field passing through an infinitesimal area element dA:

$$\Phi_m = \int_{\partial s} \vec{B} \cdot d\vec{A},\tag{3.4}$$

where ∂s is the boundary of the surface.

Magnetic Induction

At this point, it is important to distinguish between the magnetic field strength H and the magnetic induction B. The value of H is a measure of the magnetic force in free space, i.e. all the force due to the magnetic field that generates it. Its units are amperes per meter.

When an external magnetic field passes through a material, the response of the material produces an induction field B, which is related to H by

$$\vec{B} = \mu_m \vec{H},$$
(3.5)

where μ_m is the permeability—a quantity which measures the tendency of a material to form a magnetic field within itself or magnetize. In other words, the quantity H arises due to the free current that generates it, while B arises due to the bound currents induced in the material response. The unit of magnetic induction is weber/meter^2 or tesla (T). In a vacuum, μ_m is replaced by $\mu_0 = 4\pi \times 10^{-7} \frac{V \cdot s}{A \cdot m}$, which is the permeability in free space. Equation 3.3 does not hold when dealing with ferromagnetic and ferrimagnetic materials, as the relationship between B and H becomes non-linear [41].

The concept of electromagnetic induction enters the picture with Lenz's law, which states that when the magnetic flux through a conductor changes, an electromotive force ξ is induced such that the direction of current flow creates a magnetic field to counteract the change. The description of this phenomenon follows from Faraday's Law of Induction [40]:

$$\xi = -\frac{\partial \Phi_m}{\partial t}.$$
(3.6)

Magnetic Moment

Historically, the concept of magnetic moments was explained using models of magnetic charges or poles, in analogy to the electric charge. These models are still used for calculation purposes; however, the question of whether or not single magnetic poles actually exist in nature is still debated. In the present day, the magnetic moment is described as the limit of a closed loop of electric current (see Figure 3.1). The magnetic moment $\vec{\mu}$ is a quantity that is related to the torque $\vec{\tau}$ it experiences in a magnetic field $\vec{H}$, defined by:

$$\vec{\tau} \equiv \vec{\mu} \times \vec{H}. \tag{3.6}$$

The potential energy of a dipole is given by [3.1]:

$$U = -\vec{\mu} \cdot \vec{H}. \tag{3.7}$$

The work done by the magnetic field in this case is non-zero, as the torque tends to align the moment in the direction of the field, which is the minimum energy configuration. A permanent magnet with a north and south pole possesses a magnetic dipole moment, which tends to align its moment vector in the direction of the field vector. Likewise, a circular loop of current produces a dipole field.
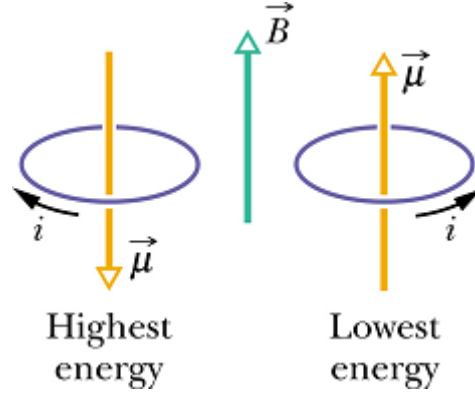
Figure 3.1 Dipole alignment in presence of magnetic field. The highest configuration of potential energy occurs with anti-parallel alignment with respect to the magnetic field, while parallel alignment yields the lowest energy.

Magnetization

Bulk magnetic materials are typically comprised of a large number of magnetic dipoles. The dipole moments can be induced in the case of *diamagnetic* or *paramagnetic* materials, or permanent in the case of *ferromagnetic* materials. The response of a material to an external magnetic field is defined by the magnetization $\vec{M}$, or magnetic moment per unit volume. It is related to the magnetic field $\vec{H}$ and magnetic induction $\vec{B}$ by [42]:

$$\vec{B} = \mu_0(\vec{H} + \vec{M}). \tag{3.8}$$

Furthermore, it is useful to define the *volume magnetic susceptibility* χ_v as a dimensionless constant that gives a measure of the degree to which a material is magnetized in the presence of an applied magnetic field, given by following relationship [42]:

$$\vec{M} = \chi_v \vec{H}. \tag{3.9}$$

In general, the magnetic susceptibility is a tensor quantity in materials that exhibit magnetic anisotropy. The value of χ_v is zero for a vacuum and, depending on the type of magnetic material, its value can be positive or negative (see Microscopic Properties of Magnetism).

Spatio-temporal Characteristics

The strength of a magnetic field can vary from point to point in space. Such fields are labeled as inhomogeneous. In this case, the motion of a particle will depend on the gradient vector that points instantaneously in the direction of the greatest increase in field strength. Like homogeneous fields, inhomogeneous fields exert forces on moving charged particles and torques on magnetic dipoles. The difference lies in the fact that no work is done by a homogeneous field. Consider a magnetic moment in an external field with a potential energy U given by equation 3.7. If $\vec{H}$ is uniform, the force exerted is (assuming that $\vec{\mu}$ does not change)

$$\begin{aligned}
\vec{F} &= -\vec{\nabla}U \\
&= \vec{\nabla}(\vec{\mu} \cdot \vec{H}) \\
\vec{F} &= 0
\end{aligned} \tag{3.10}$$

If $\vec{H}$ is non-uniform, however, the force will be non-zero and depend on $\nabla \vec{H}$. Hence, an inhomogeneous field can exert forces on magnetic dipoles. It was in fact in the Stern-

Gerlach experiment that an inhomogeneous magnetic field was used to deflect a beam of neutral silver atoms, showing that electrons possess an intrinsic spin [42].

Magnetic fields can be constant or changing with respect to time. Static magnetic fields are constant in time, as in the case of permanent magnets and electronics powered by direct currents (DC). Time-varying fields are capable of also inducing currents in conductive materials, which in turn generate their own magnetic fields. A familiar example is an alternating current (AC) that varies periodically with time at a peak voltage with a characteristic frequency (see Figure 3.2). As charges are accelerated back and forth, energy is emitted in the form of electromagnetic radiation.
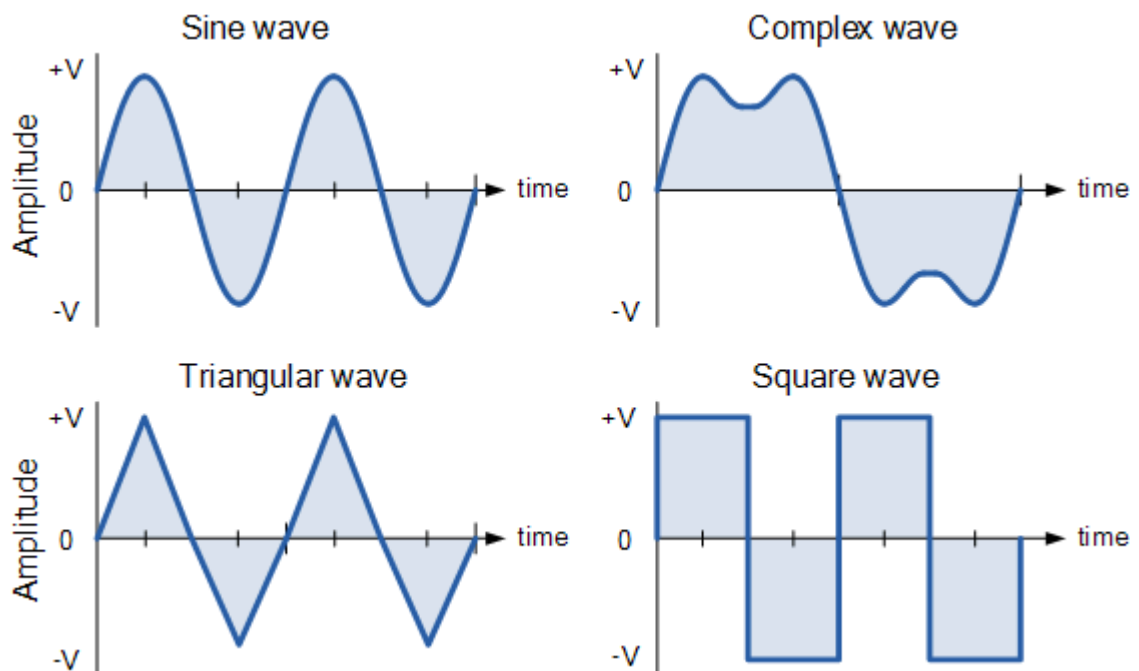
Figure 3.2 Various periodic waveforms.
https://www.electronics-tutorials.ws/accircuits/ac-waveform.html

Microscopic Properties of Magnetism

On the atomic level, electrons behave like microscopic currents due to their orbital motion. Additionally, they possess intrinsic magnetic moments due to the quantized nature of their spin angular momenta. Hence, the bulk magnetization in a material is inherently a quantum phenomenon. Several forms of magnetism are known to exist [41], three of which will be introduced here.

Diamagnetism

Diamagnetic materials respond to an external magnetic field by creating an induced magnetic field in the opposite direction, causing a repulsive force. The phenomenon arises due to the orbital motion of paired electrons, and hence is a property of all materials; for materials that show other forms of magnetism (i.e. paramagnetism, ferromagnetism) the contribution of diamagnetism is negligible. Diamagnetic materials have negative magnetic susceptibilities (see Table 3.1). Purely classical systems cannot exhibit diamagnetism; however, the classical theory of Langevin diamagnetism is a sufficient model as it makes the same predictions as the quantum theory [44].

Material	χ(SI) unitless	χ (cgs) Unitless	μ Unitless	Type of Magnetism
Bi	-165×10^{-6}	-13.13×10^{-6}	0.99983	Diamagnetic
Be	-23.2×10^{-6}	-1.85×10^{-6}	0.99998	
Ag	-23.2×10^{-6}	-1.90×10^{-6}	0.99997	
Au	-34.4×10^{-6}	-2.74×10^{-6}	0.99996	
Ge	-34.4×10^{-6}	-5.66×10^{-6}	0.99999	
Cu	-9.7×10^{-6}	-0.77×10^{-6}	0.99999	
Si	-4.1×10^{-6}	-0.32×10^{-6}	0.99999	
Water	-9.14×10^{-6}	-0.73×10^{-6}	0.99999	
Superconductors (only in superconducting state)	-1.0	$\sim -8 \times 10^{-2}$	0	
β-Sn	$+2.4 \times 10^{-6}$	$+0.19 \times 10^{-6}$	1	Paramagnetic
W	$+77.7 \times 10^{-6}$	$+6.18 \times 10^{-6}$	1.00008	
Al	$+20.7 \times 10^{-6}$	$+1.65 \times 10^{-6}$	1.00002	
Pt	$+264.4 \times 10^{-6}$	$+21.04 \times 10^{-6}$	1.000026	
Low carbon steel	$\approx 5 \times 10^{3}$	3.98×10^{2}	5×10^{3}	Ferromagnetic
Fe-3%Si (Grain Oriented)	4×10^{3}	3.18×10^{3}	4×10^{4}	
Ni-Fe-Mo superalloy	10^{6}	7.96×10^{4}	10^{6}	

Table 3.1 Different magnetic materials and their susceptibilities.
http://nptel.ac.in/courses/113104005/75

Paramagnetism

In paramagnetic materials, the presence of an applied magnetic field induces a net magnetization in the same direction of the applied field, giving rise to a weak attractive force. In this case, the magnetic susceptibilities are small, but positive. Paramagnetism arises due to unpaired electrons in atomic or molecular orbitals which carry a net spin angular momentum and hence non-zero magnetic moment. For high temperatures or low magnetic fields, paramagnetism is encapsulated by Curie's Law [44]:

$$\vec{M} = \frac{C}{T}\vec{B} \tag{3.11}$$

where T is the temperature and C is the Curie Constant. At lower temperatures or high magnetic fields, the underlying magnetic interactions will dominate and there will be a deviation from the linear relationship between M and B. Paramagnetic field strength exhibits a temperature dependence, whereas diamagnetic fields are temperature independent.

Ferromagnetism

Ferromagnetism is the strongest form of magnetism as it can produce forces strong enough to be felt, unlike paramagnetic and diamagnetic forces which require sensitive instrumentation. Iron, nickel, and cobalt are the common examples; unsurprisingly, these materials have large positive susceptibilities. Within a ferromagnetic material, there exists domains of magnetic moments which carry a net magnetization even in the absence of an external magnetic field. This behavior distinguishes ferromagnetic materials from diamagnetic and paramagnetic materials, which lose their induced magnetization upon removal of the external magnetic field.

Above a critical temperature known as the Curie Temperature, ferromagnets undergo a phase transition and begin to exhibit paramagnetic behavior [41].

Effects on Bacteria

Review of Experiments

A variety of magnetic field experiments have been conducted on bacteria. This section will focus on studies involving the effects of static and low-frequency magnetic fields on bacteria for which the mechanism of magnetoreception is still unknown.

Static Fields

Kohno et al. conducted an experiment with ferrite magnets (30 – 100 mT) on three species of bacteria: *S. mutans, S. aureus, and E. coli* [45]. They reported strength-dependent decreases in the growth rate and maximum number of *S. mutans and S. aureus* grown for 24 hours under anaerobic conditions; however, no effects were observed under aerobic conditions nor were there any effects detected on *E. coli* cultures. Another study tested the effects of strong homogenous and inhomogeneous magnetic fields on the viability of 8 different species of bacteria, two of which were *E. coli* and *S. aureus* [46]. Even with exposure times from 10 min to 1440 min, they observed no significant changes in the cell number.

While only a few studies have reported no effects of magnetic fields, many studies have reported the opposite. Mousavian-Roshanzamir et al. investigated the effects of static fields ranging from 0 mT to 20 mT on two different strains of *E. coli* [47]. They observed a significant reduction in the CFUs of samples exposed to 18 mT and 20 mT after 90 min, with the results being independent of the strain. Another study reported that static fields inhibited the growth of *E. coli* and *P. Aeruginosa*, while increasing enzymatic activity and ATP levels [48]. There are also a couple of studies that report magnetic field effects on antibiotic activity in *P. Aeruginosa* [49] and *E. coli* [21].

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In addition to effects on bacterial growth rate, there are some studies showing magnetic field influence on cellular and molecular level structures. Mhamdi et al. compared the effects of parallel and perpendicular static magnetic fields (500 mT) and found a decrease in adhesion and orientation of *E. coli* [50]. Another study detected inhibitory effects on growth and adhesion of *S. epidermidis* and *E. coli* along with changes in cell wall integrity and cell membrane permeabilization [51]. Using biochemical assays, the authors saw an increase in membrane permeability with longer magnetic field exposure due to the release of intracellular material. She et al. used Fourier transformation infrared spectroscopy (FTIR) to probe secondary protein structures in *E. coli* and *S. aureus* with exposure to an ultrastrong static magnetic field. Their results showed significant changes in the composition in the protein molecules in *E. coli*, while hardly any effects were detected in *S. aureus* [52].

A particularly interesting study conducted by Saleem et al. made use of nano-ripple substrates that were synthesized by oblique angle gas cluster ion beam [27]. Figure 3.3 shows an image of the nano-ripple substrate surface was generated using an atomic force microscope. Two rod-shaped gram-negative bacteria (*E. coli* and *P. Aeruginosa*) were grown on these substrates and compared to samples grown on regular glass substrates. The samples were also exposed to static uniform and non-uniform magnetic fields of 0.5 mT. A noticeable difference in colony size was observed among the samples on the different substrates (Figures 3.4, 3.5, 3.6, 3.7). Furthermore, exposure to the static fields seemed to decrease the colony forming capability of the bacteria. These results indicate that there may be an effect of a magnetic field on motile bacteria confined to nano-scale environments; however, further studies need to be done in order to understand these effects.
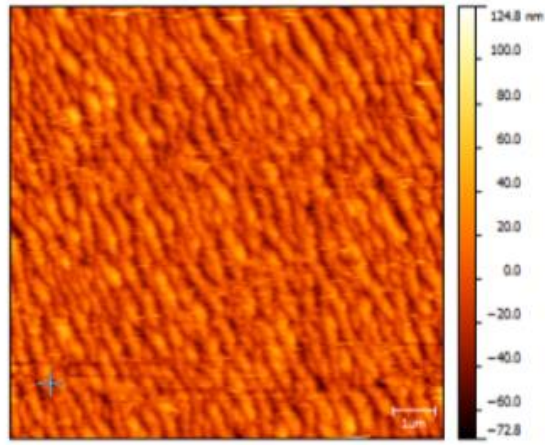
Figure 3.3 AFM image of nano-ripple substrate surface [27].
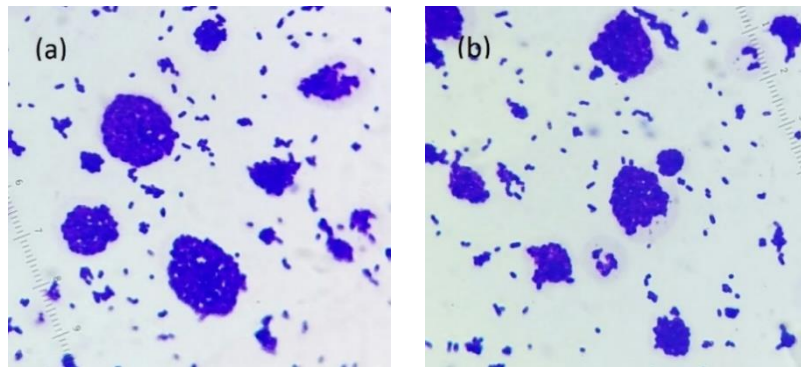
Figure 3.4 E. coli grown on nano-ripple substrates in zero magnetic field [27].
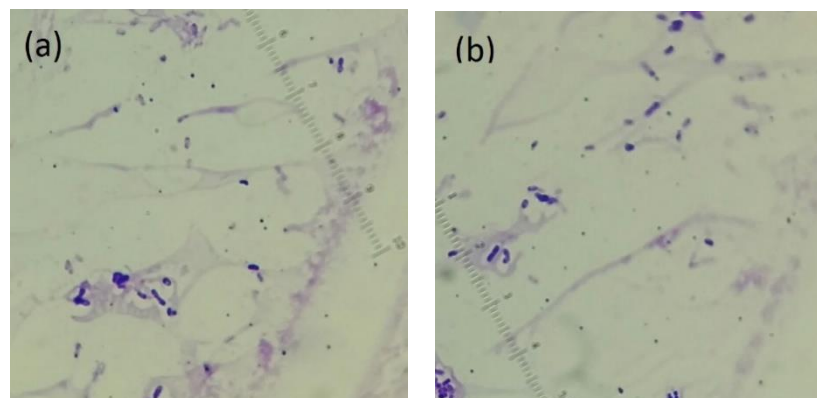
Figure 3.5: E. coli grown on plain glass substrates in zero magnetic field [27].
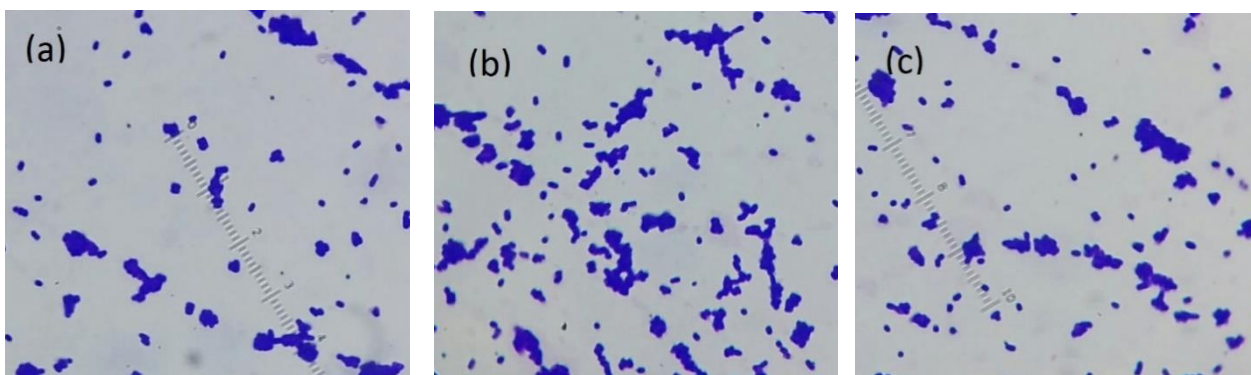
Figure 3.6: E. coli grown on nano-ripple glass substrates in Bar magnet field [27].
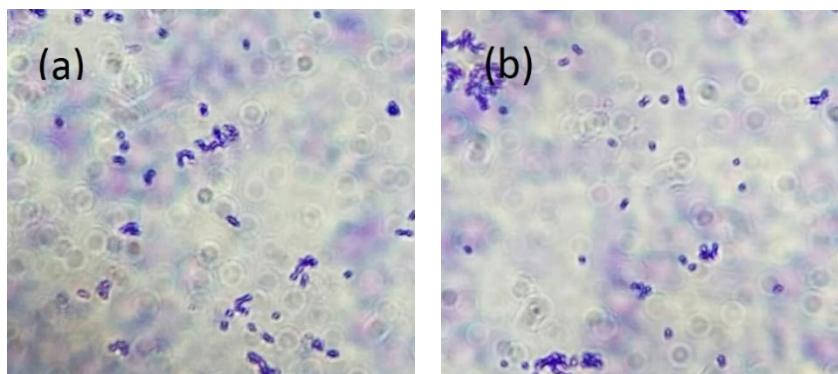
Figure 3.7: E. coli grown on nano-ripple substrates in Helmholtz magnetic field [27].

In addition to studying the growth on the nanostructured substrates, a complementary study was also conducted by Saleem et al. to quantitatively investigate the changes in growth in *E. coli* and several other bacteria grown on solid and liquid nutrient media [25]. They exposed colonies of the bacteria on solid agar plates to various configurations of magnetic fields for 36 hours before transferring isolated colonies to Luria-Bertani (LB) broth media and growing them for another 36 hours outside of the field in a shaking incubator at 37 °C. A decrease in the OD (650 nm) of samples previously exposed to a uniform solenoidal magnetic field of 0.5 mT was detected,

compared to the control samples that were exposed to no magnetic fields. Their experiment indicated that a post-exposure effect was detected.

Oscillating Fields

An experiment conducted by Inhan-Garip et al. investigated the effects of a 50 Hz magnetic field of 0.5 mT on six species of bacteria, three of which were gram-negative and three that were gram-positive [53]. All of the field-exposed samples showed a decrease in growth at OD600 during 6 hours of exposure compared to the control samples. Afterwards, samples were transferred to a fresh medium and grown for 4 hours outside of the field. Five out of the six species exhibited a continued decrease in growth, indicating that the effect of the magnetic field persisted (Figure 3.9). Qualitative data obtained through transmission electron microscopy (TEM) revealed morphological alterations such as cell wall thickening and disintegration and cytoplasmic heterogeneity as compared to control samples (Figure 3.10). The authors propose that the decreases in growth rate may be due to morphological or metabolic changes due to the oscillating field. They speculate that hydrophobic and electrostatic interactions in the cell membrane may be affected. In a similar study, morphological changes were detected in *E. coli* (ATCC 700926) with 50 Hz fields (0.1, 0.5, and 1.0 mT) with exposure times of 20 min and 120 min [54]. The authors detected no significant difference in the colony counts. However, increased cell viability was observed for samples that were re-incubated for 24 hours after exposure to 120 min of the 0.5 mT field. TEM, optical, and fluorescent microscopy images depicted abnormal lengthened cells and coccoid morphotypes (Figure 3.11)
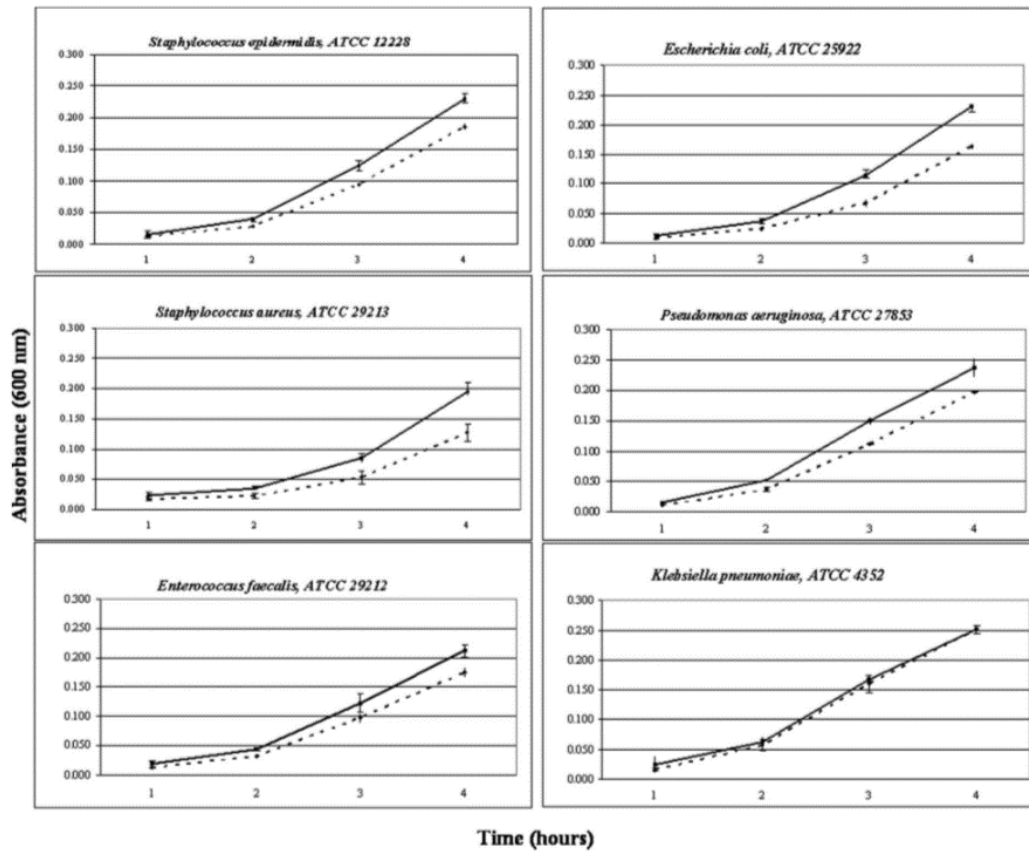
Figure 3.8: Growth curve comparisons at OD 600 nm. There were decreases in bacterial growth after field exposure [53]
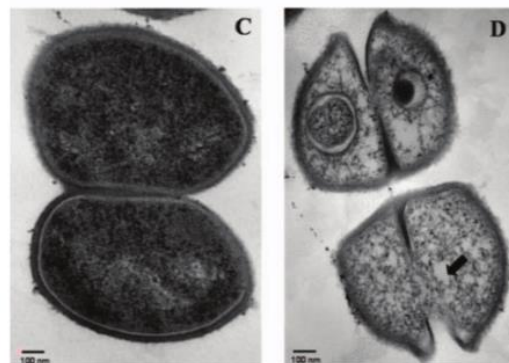
Figure 3.9: S. aureus microscopy comparison. (left) S. aureus control. (right) S. aureus exposed [53].
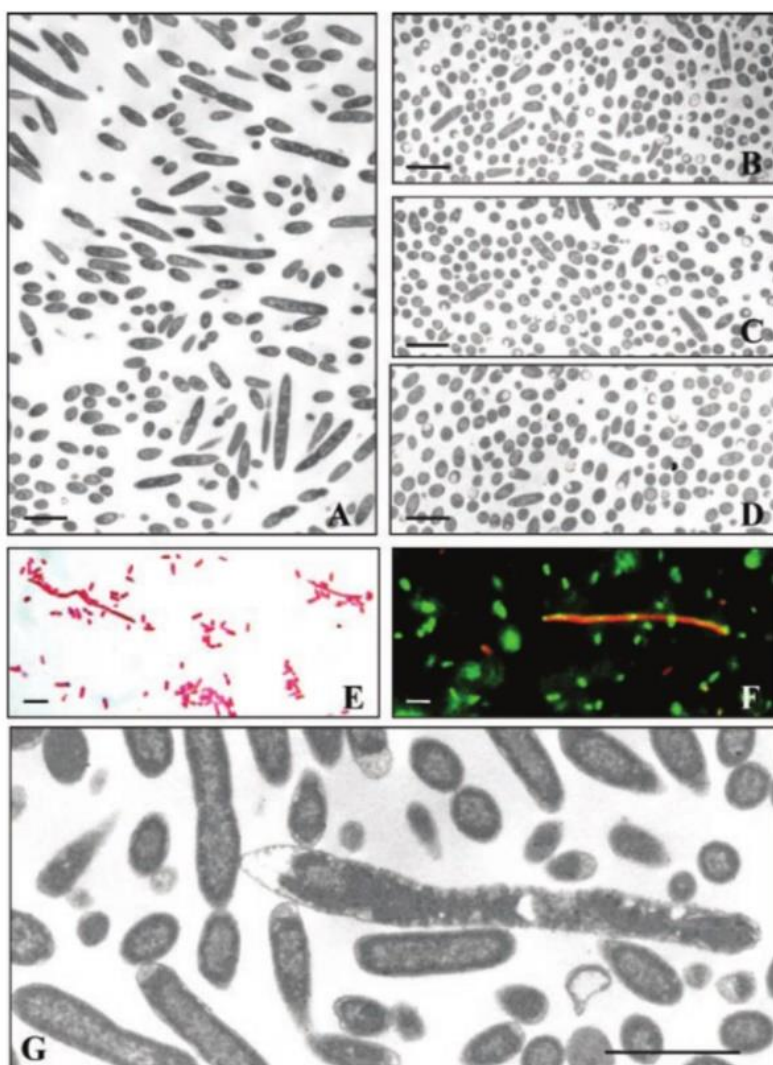
Figure 3.10: E. coli microscopy comparison. (A) E. coli control, (B) (C) (D) exposed to (0.1, 0.5, 1.0 mT) 50 Hz fields for 20 min, respectively, (E) optical stain (F) fluorescent stain (G) Exposed to 1.0 mT and 50 Hz field for 120 min [54].

Strašák et al. also examined the effects of 50 Hz fields on *E. coli*, but at higher field inductions (2.7 – 10 mT) and shorter exposure times (0 – 12 min). Their observations indicated a decrease in colony forming ability with increasing field intensity and time of exposure [55]. Bayir et al. investigated the effects of different intensities (2 mT and 4 mT), frequencies (20, 40 and 50 Hz), and exposure times (1 – 6 hours) on *S. aureus* and *E. coli* (O157:H7). Their results indicate that the longest exposure time had the most pronounced effects on both strains, whereas the magnitude of the effects for different frequencies and field intensities was dependent on the species [56]. The same species were also exposed to a 10 mT, 50 Hz field in a study by Fojt et al. for exposure times up to 30 min. Their results also showed a species dependent magnetobiological effect, with the greatest inhibition on *E. coli* [57].

There are some studies detailing the effects of square wave form oscillating fields. Aarholt et al. observed differences in the mean generation time of *E. coli* cultures in fields of 16.66 Hz and 50 Hz [58]. Re et al. compared the effects of a pulsed-square wave field and sinusoidal field of 50 Hz (0.1 – 1 mT) on *E.coli* [59]. They found that the pulsed-square wave field increased transposition activity and reduced cell viability, while the sinusoidal field reduced transposition and enhanced cell viability. Saleem et. Al used a time-varying solenoidal field configuration that alternated between 0.05 mT and 0.5 mT every 60 seconds [25]. They observed a difference in the OD (650 nm) of previously exposed samples as compared to the control, indicating the existence of post-exposure effects.

Towards a Mechanism of Action

The amount of experimental data garnered on biological effects of WMFs is staggering. Despite the evidence, progress towards a mechanism of action has been slow. This should be expected, as there still is no general agreement on the interpretation of experimental results. In this section, a brief introduction will be given on the state of the theoretical progress in magnetobiology.

A Paradox

One of the biggest issues hindering the development of a physical mechanism underlying the biological effects of weak magnetic fields has become known as the '$k_B T$ problem' (k_B is the Boltzmann constant and T is the temperature). The problem can be defined in the form of an important question: how can a weak, low-frequency magnetic field signal cause coherent biological effects in the presence of thermal fluctuations and biochemical transformations that have energy on the order of $k_B T$? According to Binhi and Rubin, the $k_B T$ problem raises a paradox, as the energy of weak magnetic fields are many orders of magnitude smaller [60]. Living organisms such as magnetobacteria and even some human tissue [61] are known to possess magnetite crystals called magnetosomes which act as magnetoreceptors. The magnetic moment of a magnetosome complex can be of the order $k_B T$ or even greater, and as such are able to be oriented by weak magnetic fields [62]. However, such effects are outside of the scope of magnetobiology, which studies the effects on organisms of which the identity of the magnetoreceptor is unknown. There is still ongoing debate as to whether the weak magnetic fields have any biological relevance [61] [63].

Ion cyclotron Resonance

One of the more prominent classical models is the ion cyclotron resonance (ICR) hypothesis, which was first suggested by Liboff [64]. In a uniform magnetic field B, an ion with charge to mass ratio $\frac{q}{m}$ will move in a circle due to the Lorentz force. From equation 3.3b, it follows that an excitation signal having a frequency f will resonate with ions that have a charge-to-mass ratio given by

$$\frac{q}{m} = \frac{2\pi f}{B} \; .\qquad\qquad(3.12)$$

A list of ion cyclotron frequencies for biologically relevant ions is shown in Table 3.2. It has been suggested that ICR plays an important role in biochemical signaling, such as the effect of ELF magnetic fields on cellular calcium concentrations [65]. The essence of the model suggests that resonance conditions can affect the flow of ions through the cellular environment. This could, in principle, explain the frequency dependence of magnetobiological effects as seen in the literature.

ION	Q/M (C/kg) x 10^{-6}	f/B$_0$ (Hz/μT)
H$^+$	95.76	15.241
Li$^+$	13.90	2.212
Mg^{2+}	7.937	1.263
H$_3$O$^+$	5.066	0.807
Ca^{2+}	4.814	0.766
Zn$^+$	2.951	0.470
K$^+$	2.447	0.393
arg^{2+}	1.235	0.197
asn$^+$	0.838	0.133
glu$^+$	0.747	0.119
tyr$^+$	0.591	0.094

Table 3.2: Biologically Active Ions. Charge to mass ratios and ion cyclotron frequencies are shown. [65]

There are several difficulties that arise with this classical model, however. Thermal effects must be accounted for, as ions are typically found in water solutions at physiological temperatures and will undergo many collisions with other molecules in the cellular medium. Moreover, the effects of charge shielding by water molecules will act to reduce the effective charge of the ion target. Another model proposed the idea that ICR could occur in ion channels of cell membranes, where thermal collisions with molecules could be ignored [66]. There are fundamental difficulties with this model as well. An ion would have to have a near zero initial velocity prior to entering an ion channel in order to have a small enough cyclotron radius, which is implausible [67]. Nevertheless, ICR serves as a foundation for more advanced classical models.

Proton Tunneling in Biological Systems

Quantum mechanical tunneling is a fundamental property of the microscopic world. Classically, when a particle encounters a potential barrier, it can surpass the barrier if it has a sufficiently high energy. If the particle's energy is too low, then it will be unable to pass the barrier and its momentum will simply be absorbed or redirected away from the barrier. According to quantum mechanics, a particle has a finite probability of tunneling through the barrier, even with a kinetic energy that is lower than the potential energy. This phenomenon arises due to the wave and particle nature of matter on the quantum level. For a simple system that consists of a particle incident on a finite square potential barrier, it is straightforward to obtain a solution for the tunneling probability [68]. In more realistic physical systems—such as atoms or molecules—the spatial dependence of the potentials is typically complex and most of the time approximations must be made when analytical solutions cannot be found. In general, the probability of tunneling depends on the mass and energy of the particle, the size of the potential, and external interactions.

Quantum tunneling is essential for many physical processes that life depends on, such as nuclear fusion in stars and radioactive decay [69]. More recently, there have been claims that tunneling is one of the most important non-trivial quantum phenomena in biological processes [70] [71]. As early as the 1960s, there was speculation that magnetic fields could have an effect on the genetic code [72]. Recall that a DNA molecule is made of four nucleotide bases that are linked together via hydrogen bonds. Adenine (A) shares a double bond with thymine (T), while guanine (G) shares a triple bond with cytosine (C) (see Figure 2.3). The hydrogen bonds are formed by protons which are shared by lone electron pairs on the nitrogen or oxygen atoms of the bases. As the sharing of the proton is due to an attractive force, the interaction can be represented as a double asymmetric

potential well (see Figure 3.11). Each of the wells represents the equilibrium position that the proton can be in (i.e. a bound state), where the proton will have the largest probability of being in the lowest-energy level of the deepest well. There is a finite, but small probability that the proton can tunnel through the central barrier and into the other well.
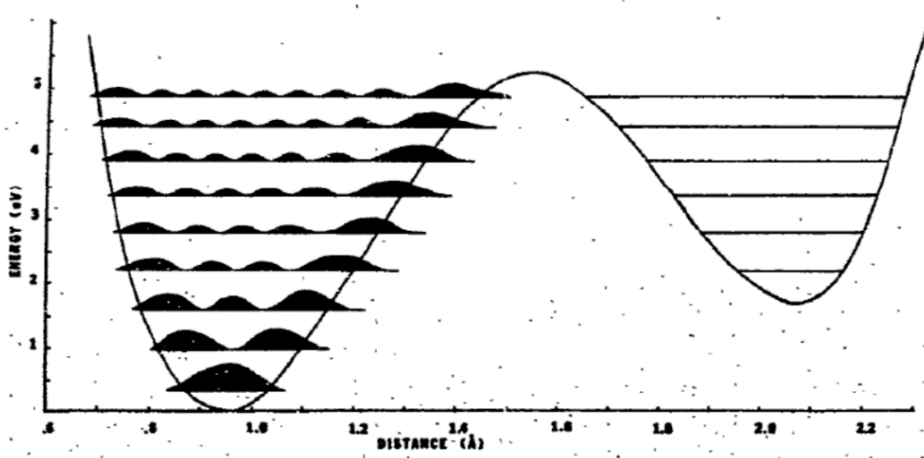
Figure 3.11 Asymmetric double-well potential [3.40]
http://einstein.drexel.edu/~bob/Term_Reports/Megan_Wolfe.pdf

In the DNA replication process, mutations can occur when the wrong complementary bases are attached to each other. If the complementary bases have an equal charge, the tunneling of a proton in one direction is followed by the reverse tunneling of a proton in the second hydrogen bond, which puts the original bases in a tautomeric state, where protons have been re-located [72]. Over time, the tunneling will occur again and the bases will return to their original states. During DNA replication, hydrogen bonds are broken in order to unzip the DNA strands to be read by mRNA. If these bonds are broken before bases can return to their original states, then different complementary bases will be attached to each other and cause a mutation in the genetic

code. Although mutations are rare, any changes that go unchecked by the DNA proofreading mechanisms (which can happen) will result in irreversible changes.

How then, can magnetic fields play a role in this process? The proton is an elementary particle that carries an intrinsic spin. An inhomogeneous magnetic field may be able to affect the spin orientation. Also, the presence of an external magnetic field can split the energy levels of the bases or even affect the charge distributions on the base pairs, which could alter the shape of the potential well and hence influence the tunneling rate [72]. It is important to keep in mind that these are still speculations, and as of today there is little to no experimental evidence that support these claims.

Magnetic Isotope Effect

In the field of spin chemistry, the effects of magnetic fields on chemical reactions is well-known [73]. Such reactions involve the spin evolution of the excited states of chemical species whose electron spins are in a well-defined spin-state. As per the radical pair (RP) mechanism (Figure 3.12) two species A and B are excited to a spin-selective state (i.e. singlet or triplet state), after which singlet-triplet intersystem crossing occurs. Depending on the magnetic interactions of the spin states and the external magnetic field, a proportion of singlet states will decay into singlet products, triplet states to triplet products, or there will be back conversion to a non-selective product. The important feature of the RP mechanism is understanding how the spin states evolve between the singlet and triplet states, as this will determine the reaction yields. Furthermore, weak magnetic field interactions of energies much less than $k_B T$ are able to cause significant effects in the singlet product yields [73]. The transitions from singlet to triplet states arise due to the interactions of the magnetic moments of electrons via external fields and

hyperfine interactions; for a rigorous treatment of the quantum theory underlying spin dynamics, the reader is referred to Sakurai [43] and Shankar [74].
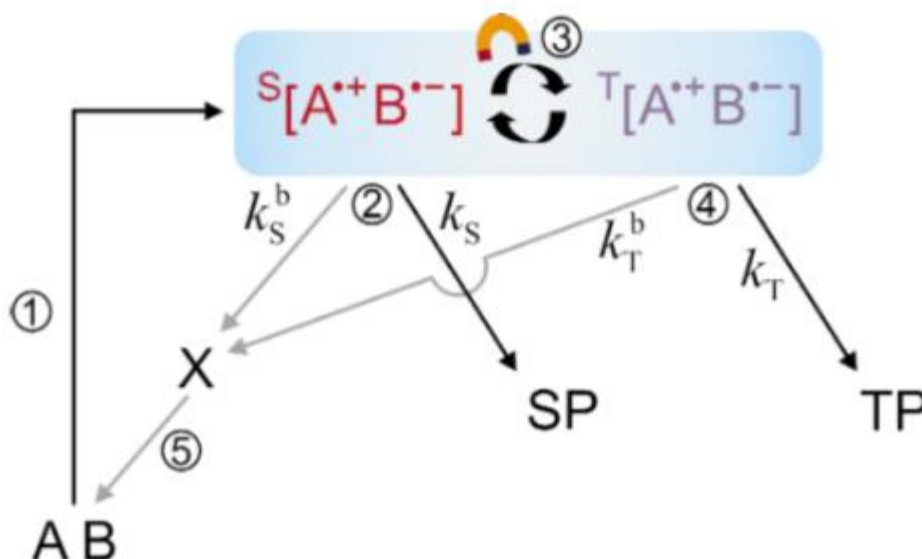
Figure 3.12 The radical pair mechanism [73]

A multitude of metabolic reactions occur in biological systems that involve enzymes. In a recent paper by Buchachenko and Kuznetzov, ATP synthesis was discussed as a candidate for the nuclear-magnetic isotope effect of magnesium by phosphorylating enzymes [75]. The idea is that the first step is an electron transfer process involving the formation of an ion-radical pair with Mg^{2+} and the adenosine diphosphate (ADP) anion. The singlet and triplet spin states were shown to have different yields for the products of ATP synthesis, controlled by an external magnetic field. In a study conducted by Letuta et al, three isotopes of Mg (^{24}Mg, ^{25}Mg, and ^{26}Mg) were introduced to cultures of *E. coli* [76]. The bacterial samples were exposed to static fields ranging from 0 mT to 96 mT. According to their results, the colony forming ability of

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cultures enriched in ^{25}Mg increased significantly in the 76 mT—93 mT range (see Figure 3.13), as compared to cultures enriched in the non-magnetic isotopes. They attribute the findings to an increase in the rate of ATP synthesis due to the role of the nuclear spin of ^{25}Mg in the enzymatic ion-radical reactions, confirming predictions made by Buchachenko and Kuznetsov. The significance of these results may play a role in the understanding of spin sensitive biochemical reactions in biological systems.

Figure 3.13 Colony forming units as a function of magnetic field strength. Samples enriched in ^{25}Mg showed significant increases in CFU/ml[76].
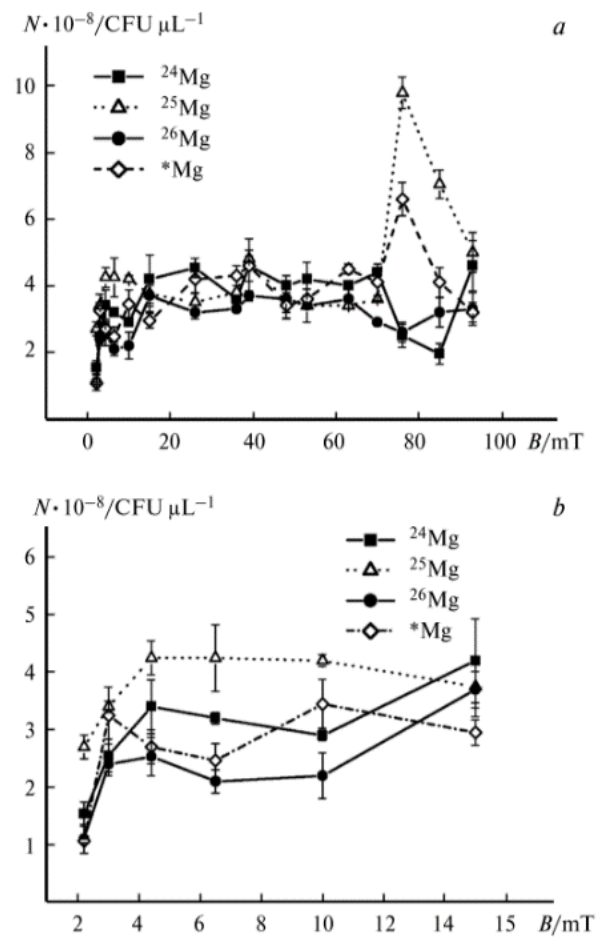
Closing Remarks on the Literature

The current experimental evidence seems to indicate that biological effects can be influenced by many factors such as the bacterial species, magnetic field characteristics, exposure times, and frequencies. For the most part, quantitative data has come from population level assays such as viable cell counts and optical density measurements of cell suspensions. Morphological changes were detected in field-exposed cells using various microscopy methods [53][54]. As the absorbance of cellular suspensions depends on light scattering, it is likely that changes in the cellular shape or size distributions can influence optical measurements. On the other hand, it is discussed in the study by Cellini et al. that bacteria may enter a viable but non-culturable (VBNC) state due to the weak magnetic field acting as a stress factor [54]. The switch to this low-metabolic state can affect the measurements of viable cell counts and cause disagreements with absorbance measurements. Further research must be done to confirm that bacteria enter the VBNC state in the presence of WMFs. It may be worth considering the limitations of these methods on an individual basis and their effectiveness in comparing the growth of bacterial populations in the presence of magnetic fields.

Few studies have detailed the post-exposure effect of a weak magnetic field. When bacteria are subject to external stresses, their natural tendency is to adapt to these changes. For instance, bacteria can develop resistance to antibiotics by passing on the appropriate genes that code for it. Studies that investigate the growth behavior of subsequent generations of bacteria may be crucial to understanding the extent through which they can adapt to the long-term exposure of various magnetic field conditions.

It is clear from the literature that there is a lack of consistency in the results of magnetobiological experiments on bacteria. Much research still needs to be done towards reconciling the current experimental data before a mechanism of action can be deduced.

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On the theory side, the $k_B T$ paradox is an obstacle that still needs to be addressed. There exist many more theoretical models of magnetobiological effects—both classical and quantum—that are not discussed in this thesis. For more detail, the reader is referred to an exceptionally in-depth review and analysis of the literature by Binhi [67].

CHAPTER IV EXPERIMENTAL METHODOLOGY

Does the bacterial response to a WMF depend on the duration of exposure? Furthermore, are there observable effects on subsequent generations of bacteria—and if so—what can they tell us about the biological response? Bacteria can adapt to all sorts of conditions that induce stress, from nutrient deprivation and temperature changes to antibiotic treatments. If the presence of a WMF acts as an environmental stressor, it may be enlightening to study the growth response of a population with continuous long-term exposure. In this chapter, the details of a growth experiment will be discussed in which the bacterial adaptability to WMFs is tested. The subject of study is *Escherichia coli*, a rod-shaped, gram-negative bacterium. It is facultatively anaerobic and possesses the ability to transfer genes via bacterial conjugation. In industry and many biologically related fields of study, *E. coli* serves as a model organism. In this study, three generations of *E. coli* were continuously subjected to weak static and oscillatory magnetic fields. The growth response of each generation was recorded and compared using absorbance spectrophotometry.

Materials & Instrumentation

The exposure system consisted of two solenoidal configurations of stacked Pasco 200 turn Helmholtz coils (21 cm diameter, 1.2 kΩ). Each configuration had eight coils stacked vertically to a height of 21.5 cm and connected in series. Both solenoids were elevated 6 cm above a wooden table, each encircling a centered Styrofoam test tube rack situated 4.5 cm off the table holding open disposable glass tubes. The control test tube rack was placed away from the solenoids at the same elevation. Figure 4.1 shows the arrangement of the set-up. Static magnetic fields were generated at 0.9 ± 0.3 mT in the exposure volume of the solenoids using a Pasco DC power supply. The oscillating magnetic fields were produced by Pasco function generators that delivered sinusoidal currents (25 Hz and 50 Hz) with a strength of 0.5 mT. Tables 4.1 and 4.2 display the spatial variation of the AC fields at various points within the solenoids along with various statistics and values. The culture tubes were centered within the solenoids such that the amount of liquid media would be exposed to small variations in the magnetic field ($y = 13$ cm to $y = 20$ cm). The center of the coils was located at $x = 10.5$ cm, and the center of the test tubes were spaced 2.5 cm apart.
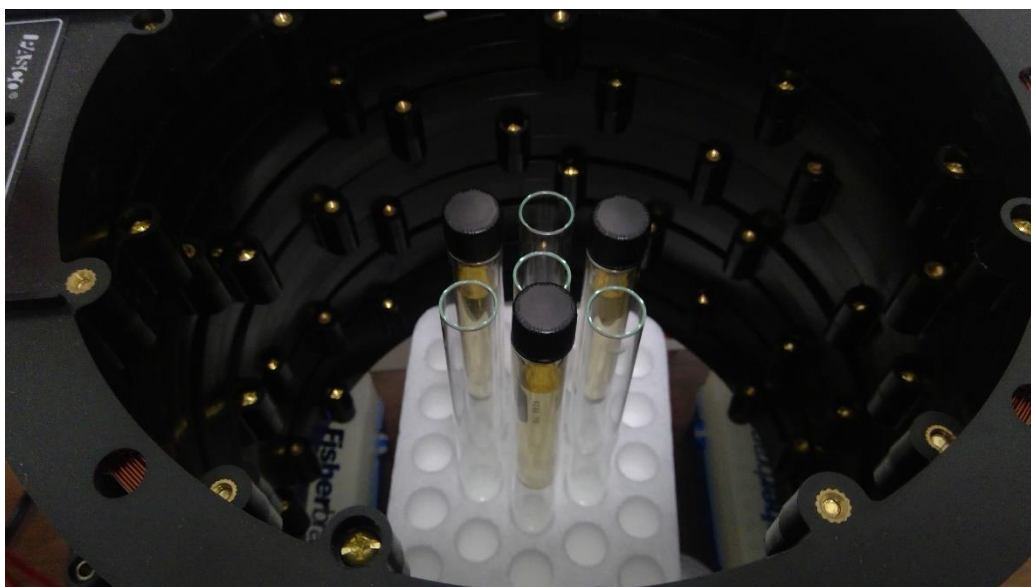
Figure 4.1: Magnetic field exposure apparatus. Set up consists of two solenoids with 8 stacked Helmholtz coils connected in series. Styrofoam racks are centered inside the solenoids. The control group is the same without magnetic field exposure.

Table 4.1: 25 Hz Oscillating Field ($V_p = 6$ V, $I_{rms} = 0.04$ A)*

x (cm)	y (cm)	ΔB (mT)	B_{min} (mT)	B_{max} (mT)	B_{avg} (mT)	StDev (mT)
10.5	13.0	1.058	-0.530	0.528	0.001	0.370
13.0	13.0	1.073	-0.542	0.532	-0.003	0.374
10.5	17.0	1.085	-0.542	0.543	0.002	0.380
10.5	20.0	1.038	-0.522	0.516	0.001	0.362
13.0	20.0	1.042	-0.526	0.516	-0.002	0.364

Table 4.2: 50 Hz Oscillating Field ($V_p = 10$ V, $I_{rms} = 0.04$ A)*

x (cm)	y (cm)	ΔB(mT)	B_{min} (mT)	B_{max} (mT)	B_{avg} (mT)	StDev(mT)
10.5	13.0	1.081	-0.526	0.555	0.020	0.375
13.0	13.0	1.097	-0.538	0.559	0.012	0.383
10.5	17.0	1.116	-0.546	0.571	0.010	0.390
10.5	20.0	1.077	-0.530	0.547	0.010	0.376
13.0	20.0	1.077	-0.534	0.543	0.009	0.375

*   *B = magnetic field strength*
V_p = peak voltage
I_{rms} = root mean square current
StDev = standard deviation

Preparation of *E. coli* Cultures

A lyophilized strain of *Escherichia coli* ATCC 10536 (Microbiologics, St. Cloud, MN) was used in the experiments. The solid growth medium used was tryptic soy agar (TSA, Teknova, USA) which consisted of tryptone, soytone, NaCl, and agar; liquid medium used was tryptic soy broth (TSB, Criterion Dehydrated Culture Media, Santa Maria, CA) which was composed of casein peptone, soy peptone, NaCl, dipotassium phosphate, and dextrose. Bacteria were incubated on TSA plates overnight at 37 °C. Samples were harvested from isolated colonies, transferred to TSB, and then incubated (V.I.P CO2 Incubator 417, Lab Line Instruments, Inc.) at 37 °C. During the late log phase of growth OD 0.7 (~ 10^9 CFU/mL), 50 μL of the parent culture was inoculated into each of 3 aliquots (7 mL TSB) for each field configuration. The samples were immediately placed in a shaker for 10 min before being relocated to their respective conditions. Bacterial growth was determined by optical density (OD) measurements (Spectronic 20D+) taken at 600 nm (every 0.5 – 1 hrs) for 7 – 10 hours. The subsequent generations were prepared by transferring 50 μL of the previous generations into 7 mL of fresh nutrient broth. All samples were grown in Pyrex culture tubes (1.3 cm diameter) at ambient room temperature (24.5 ± 0.4 °C). Control samples were grown in the same conditions without magnetic fields.

Data & Analysis

Raw data was input into Excel 2016 and statistical analysis was carried out using the Excel Analysis ToolPak add-in. Ambient temperature and magnetic field strength were monitored using Vernier temperature probes and a Vernier low magnetic field sensor connected to a 3-port Vernier Lab Quest Mini, while data and statistics were acquired using Logger Pro. Single-factor ANOVA (Analysis of Variance) was used for simultaneous comparison of the different groups. Any *p-values* less than 0.05 were considered significant.

CHAPTER V: RESULTS AND DISCUSSION

Effects of the DC Magnetic Field

A total of three groups were studied in the DC magnetic field (see Table 5.1). Each group contained three replicate samples, and three generations were grown in their respective conditions. In the DC magnetic field, it was expected that the rate of growth would be inhibited as it was observed in previous studies [ ]. The data indicated, however, that the populations grown in the presence of the field (F1-S, F2-S, and F3-S) had an increase in growth (see Figure 5.1). Interestingly, the samples tested for post-exposure (F2-S*, F3-S*, see Figure 5.2) showed no signs of difference in growth from the control (FC-2, FC-3).The differences in OD were significant with $p < 0.05$, according to the one-way ANOVA test.

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Table 5.1 DC Field Experimental Group Labels

Groups	Generation 1	Generation 2	Generation 3
Control	F1-C	F2-C	F3-C
Static	F1-S	F2-S	F3-S
Static*	N/A	F2-S*	F3-S*

Samples that were tested for post-exposure effects

(a)

F1 in DC Field (OD$_{600}$)

(b)

F2 in DC Field (OD$_{600}$)
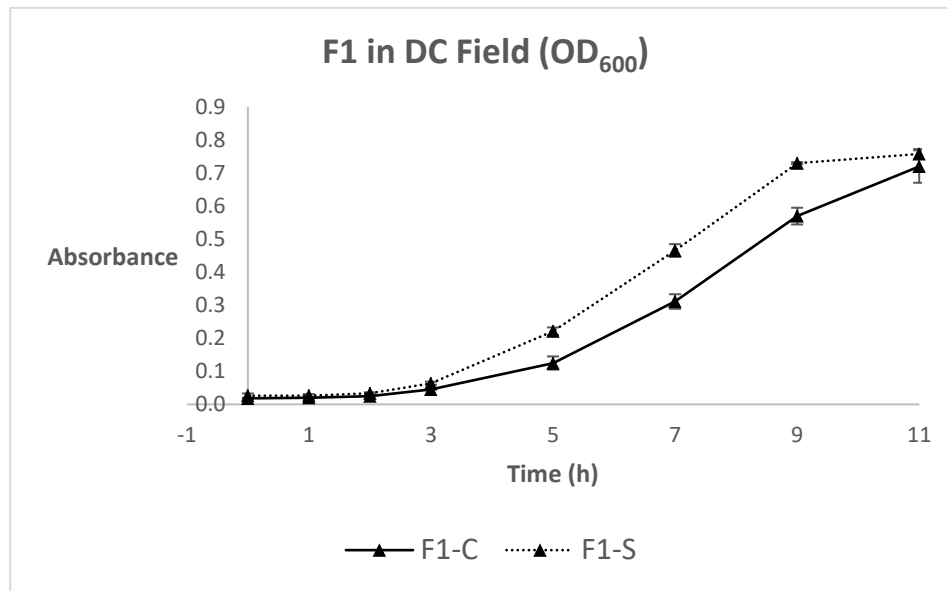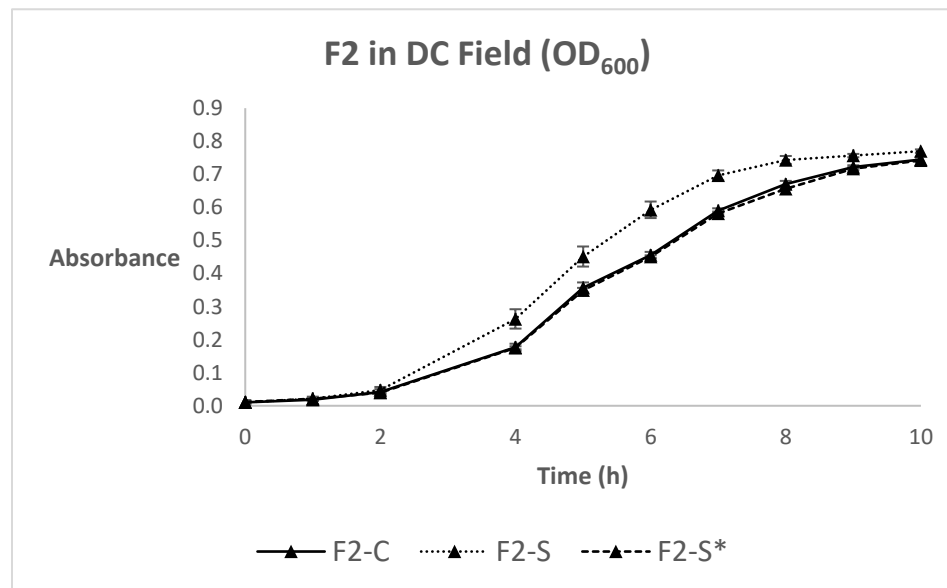
Figure 5.1 Growth curve comparison of F1 and F2 E. coli. (a) An increase in growth was observed in the F1 field exposed group as well as the (b) F2 field exposed group with p < 0.05. The group tested for post-exposure effects showed no significant differences compared to the control. Results are shown are the average ± standard deviation (SD) of three replicate samples per group.
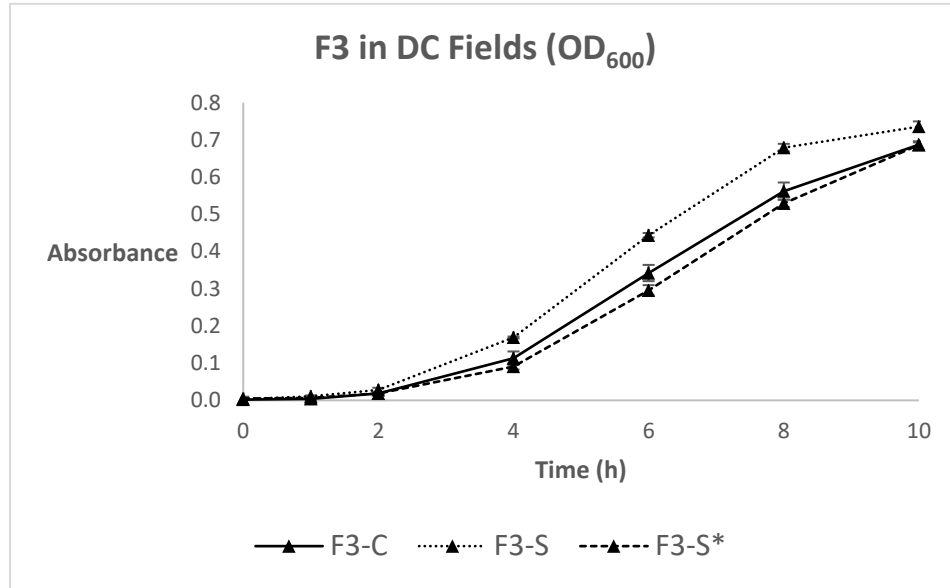
F3 in DC Fields (OD$_{600}$)

Figure 5.2 Growth curve comparison of F3 E. coli. An increase in the growth was detected with the field-exposed group, but no significant effects were seen in the post-exposure group.

Previous studies conducted by Saleem et al. [25] and Smith [18] reported growth inhibition of *E. coli* in static solenoidal fields. In contrast, our studies seem to indicate an increase in growth of field exposed samples compared to the control. Furthermore, no significant differences were seen between the control group and the groups previously exposed to the fields, which suggests that there was no post-exposure effect with regards to the OD values. However, there are a couple of points to address with this experiment in comparison to the previous experiments by Saleem et al. and Smith. This study used a uniform field of 0.9 mT instead of 0.5 mT, as well as a different strain of *E. coli*, which may have been a factor in the results. Another important factor was a significant ambient temperature (~ 1.5 °C) increase inside the exposure volume of the coils due to power dissipation. As the growth of *E. coli* is less and more sensitive at temperatures less than

the optimum temperature (37 °C), the increase in growth was likely due to the differences in ambient temperature between the field-exposed and control samples.

On the other hand, the possibility that the magnetic field could have played a role cannot be ruled out either. The static magnetic field has a constant value and is not usually expected to affect the bacterial growth. However, bacterial growth takes place by the absorbance of nutrients which is related to the mobility and motility of bacteria in its culture medium. The motility creates a relative change in the effective magnetic field sensed by the *E. coli* due to the shape of the cell and its charged nature [77], which could affect the growth rate. This effect is very small, but it increases the growth rate as seen in Figures 5.1 and 5.2. The changes in growth could be attributed to changes in the metabolic activities of the cell as reported by earlier studies in which there was increased enzymatic activity and ATP levels in *E. coli* [48].

Effects of the AC Magnetic Field

There was a total of five groups in this experiment, with three replicate samples in each group as depicted in Table 5.2. The samples were grown over three generations, in the same manner as in the DC field experiments, with some improvements to the exposure apparatus to mitigate temperature variations. No significant differences in the control and field-exposed samples were seen in the first (G1) generation of *E. coli* (see Figure 5.3).

Table 5.2 AC Field Experimental Group Labels

Groups	Generation 1	Generation 2	Generation 3
Control	G1-C	G2-C	G3-C
25 Hz	G1-25	G2-25	G3-25
50 Hz	G1-50	G2-50	G3-50
25 Hz*	N/A	G2-25*	G3-25*
50 Hz*	N/A	G2-50*	G3-50*

Samples that were transferred from previously field-exposed cultures and subsequently grown outside of the fields (post-exposure).
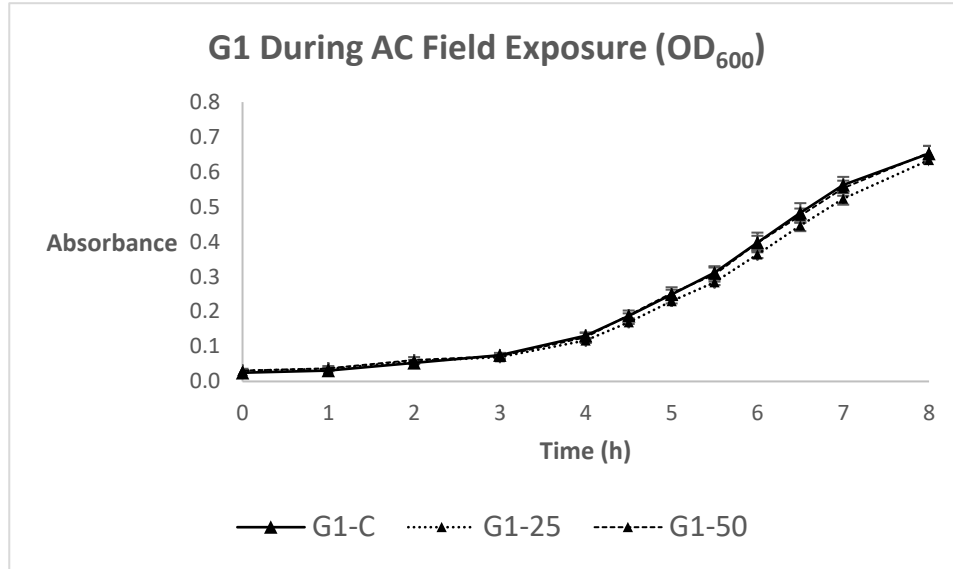
G1 During AC Field Exposure (OD$_{600}$)

Figure 5.3: Growth curve comparison of G1 E. coli cultures during field exposure. No significant growth inhibition was observed between control and field exposed samples. Results are shown are the average ± standard deviation (SD) of three replicate samples per group.

These results fall out of agreement with Inhan-garip et al., who reported immediate decreases in the OD of field-exposed *E. coli* [53]. They do agree with the results by Cellini et al. to a certain extent, who reported no significant changes in the OD with exposure. It is important to note, however, that they only had at total of two time points of observation (20 min and 120 min) which may not have been enough time to see any effects of the field [54]. Furthermore, since both studies mentioned carried out their experiments at 37 °C, there is a possibility of temperature playing a role in the results seen in our study. At lower temperatures than the optimal growth temperature, the metabolic activity of *E. coli* is significantly reduced. If increased metabolic activity correlates to an increase in the biological effect of the field, this could explain the difference in our results with the ones seen in the literature. The AC magnetic field in our experiment is a weak perturbation that changes direction continuously at a rate of many

cycles per second. If there exists a resonance frequency that causes an amplification of the biological effect, different temperatures could influence the resonance conditions, assuming the resonance is of a classical physical nature.

In the second (G2) generation, however, there were some interesting results. A significant difference ($p < 0.05$) was observed in the OD of the G2-25 and G2-50 groups from 3 h to 5 h (Figure 5.4). The fact that significant effects begin to take hold in G2 seems to suggest that there may be a time-related threshold of exposure to magnetic fields before any changes in growth occur. This threshold could also be dependent on the temperature, conditions of growth, and the characteristics of the oscillating field. If such a time threshold does exist, it may be due to a critical time it takes for small perturbations of the weak AC field to accumulate to certain levels that are detectable by the bacterial population.
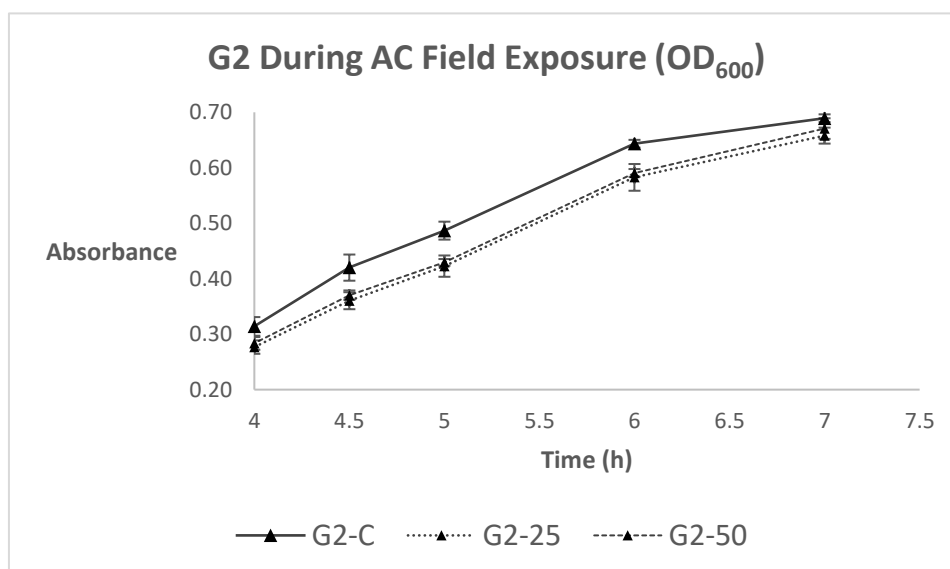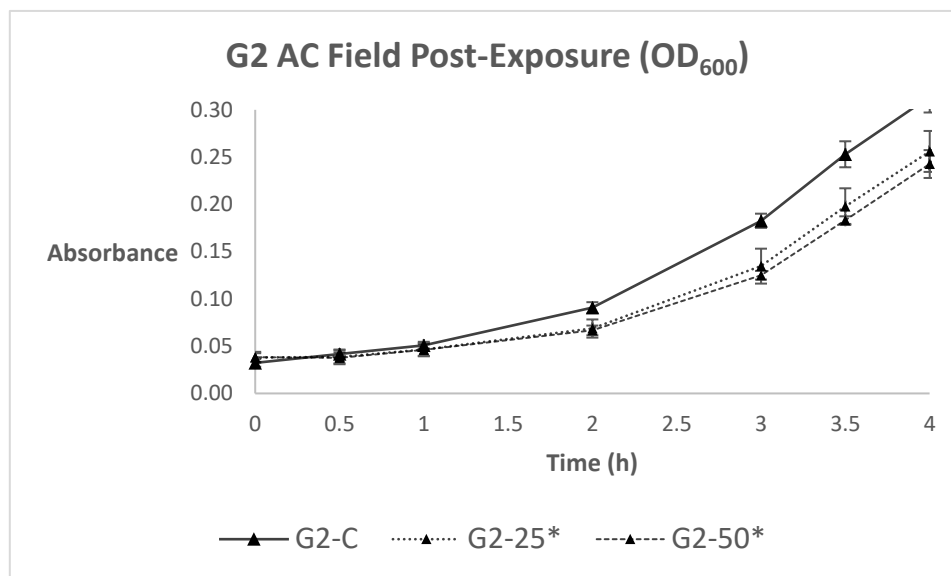
(a)
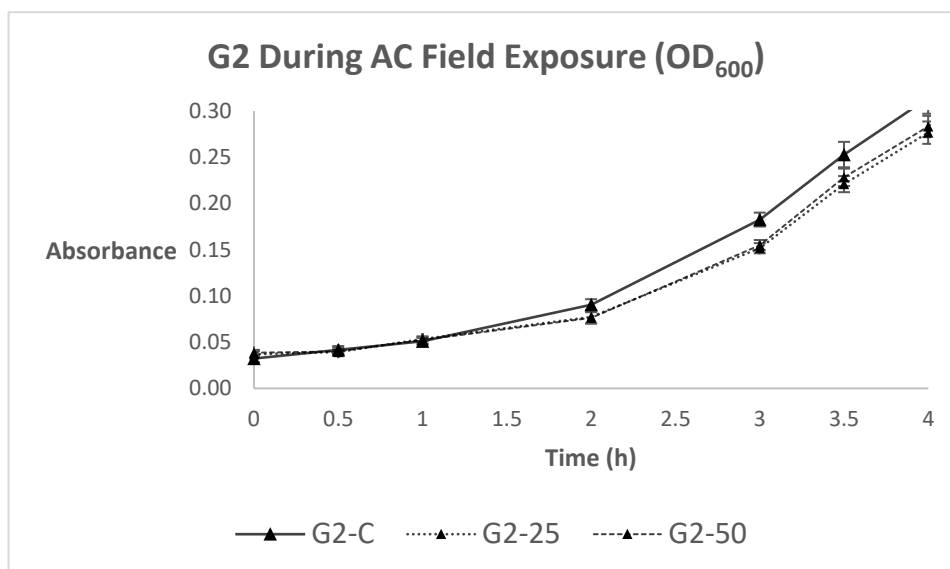
(b)

Figure 5.4 Growth curve comparison of G2 E. coli during field exposure. Significant differences (p < 0.05) in the growth inhibition were observed from (a) t = 0 h to t = 4 h and (b) t = 4 h to t = 7 h.

After reaching an OD of 0.7, *E. coli* samples that were previously exposed to the magnetic field were inoculated into fresh media and grown outside of the field influence to test for post-exposure effects (G2-25* and G2-50*). Significant decreases in the OD of the post-exposure samples were seen (see Figure 5.5). These results show that the effects persisted in the subsequent generations of cultures that were previously exposed to an oscillating field.

This data is contrasted with the DC field samples, where no sustained effects were detected in the subsequent generations. The difference in OD started at $t = 2$ h and continued until $t = 7$ h, where the growth curves began to coincide. In accordance with the data, it is hypothesized that the coincidence of the curves arises due to the faster rate of growth and hence nutrient consumption of the control group which causes the growth curves to saturate sooner. With a slower rate of growth and nutrient consumption, the field-exposed samples had sufficient nutrient availability and their growth curves saturate later than the control. It is speculated that significant changes in the metabolic activity of the *E. coli* is responsible for the slower growth rate of the field-exposed groups. Furthermore, the AC fields may have affected the lag phase in which the bacteria are becoming acclimated to their environment, which could delay the start of the exponential phase. These ideas are based on the evidence of the morphological changes as seen in the study by Cellini et al (see Figure 3.10). The transition to coccoid morphologies in *E. coli* may be indicative of the presence of an environmental stressor—which in this case could be the oscillating magnetic field—that can cause cells to enter a VBNC state [78].
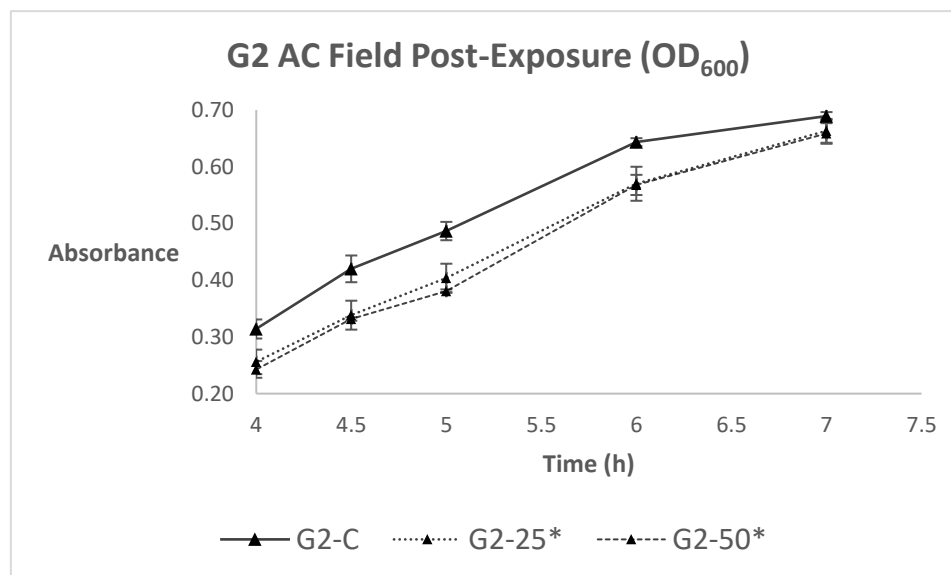
(a)

(b)

Figure 5.5: Growth curve comparison of G2 E. coli in post-field exposure. Significant inhibition of growth (p < 0.05) was observed from (a) t = 2 h to t = 4 h and (b) t = 4 h to t = 6 h.

In Figure 5.6, the relative change in the absorbance (ΔA) of the field-treated samples with respect to the control group was plotted over the exposure time t to compare the differences between the treated samples. Each of the samples exhibited similar trends; although the magnitude of the changes was larger in the post-exposure groups, the maximum change occurred at t = 5 h (shown in red) for all samples. The time intervals in which growth inhibition took place are from early log phase to the later log phase. Since each group shared common turning points in the ΔA curves, there is a question of whether the bacteria begin to acclimate to the field conditions after t = 5 h. It could be that the magnitude of the biological effects is dependent on the number density of bacteria that are present in the sample at any particular time in the log phase of growth.
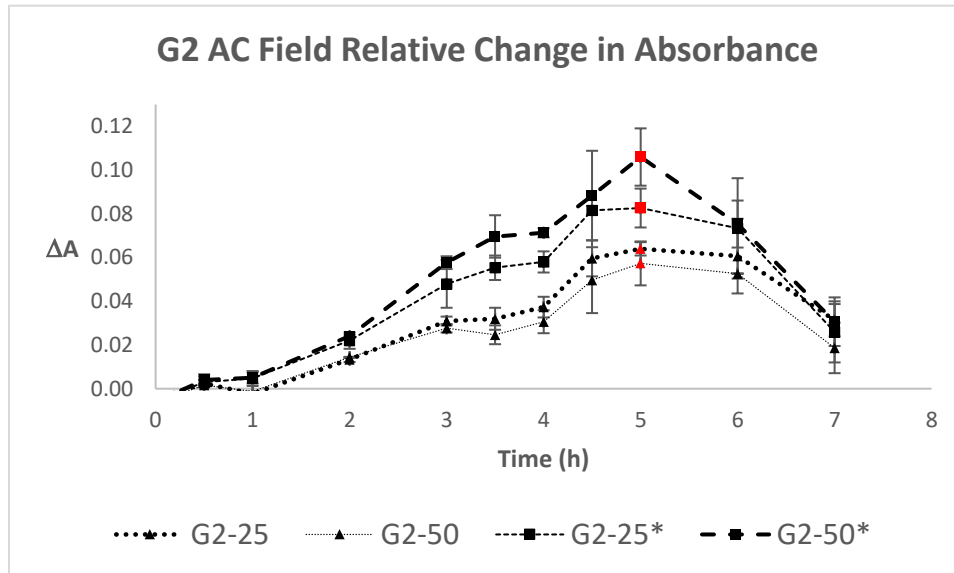
Figure 5.6: Relative change in absorbance. Change in absorbance of field-treated groups are compared with the control group. A maximum change occurs at t = 5 h.

In the third generation, all of the field-treated groups (F3-25, F3-50, F3-25*, F3-50*) showed no significant differences from the control group. All of the growth curves followed the same trends. These results signify a possible mechanism for adaptation to the magnetic field conditions. Fojt et al. reported a post-exposure effect that was dependent on the strain of the bacteria [57]. In the study by Inhan-garip et al., all bacterial samples showed continued decreases in growth in the post-exposure conditions without a magnetic field. The exception was *K. pneumoniae,* which showed no further signs of growth inhibition [53]. It should be noted that their study did not test any further post-exposure effects. The sustained effect of the magnetic field could very well depend on the strain of the bacteria as well as the exposure time, and it is possible that a "relaxation" time can be detected in subsequent generations following exposure to the magnetic field. In our study, the exact amount of time for the *E. coli* to become acclimated to the field conditions is unclear.

(a)

G3 AC Field During Exposure (OD$_{600}$)

Absorbance

Time (h)

G3-C ......▲...... G3-25 ----▲---- G3-50

(b)

G3 AC Field Post-Exposure (OD$_{600}$)

Absorbance
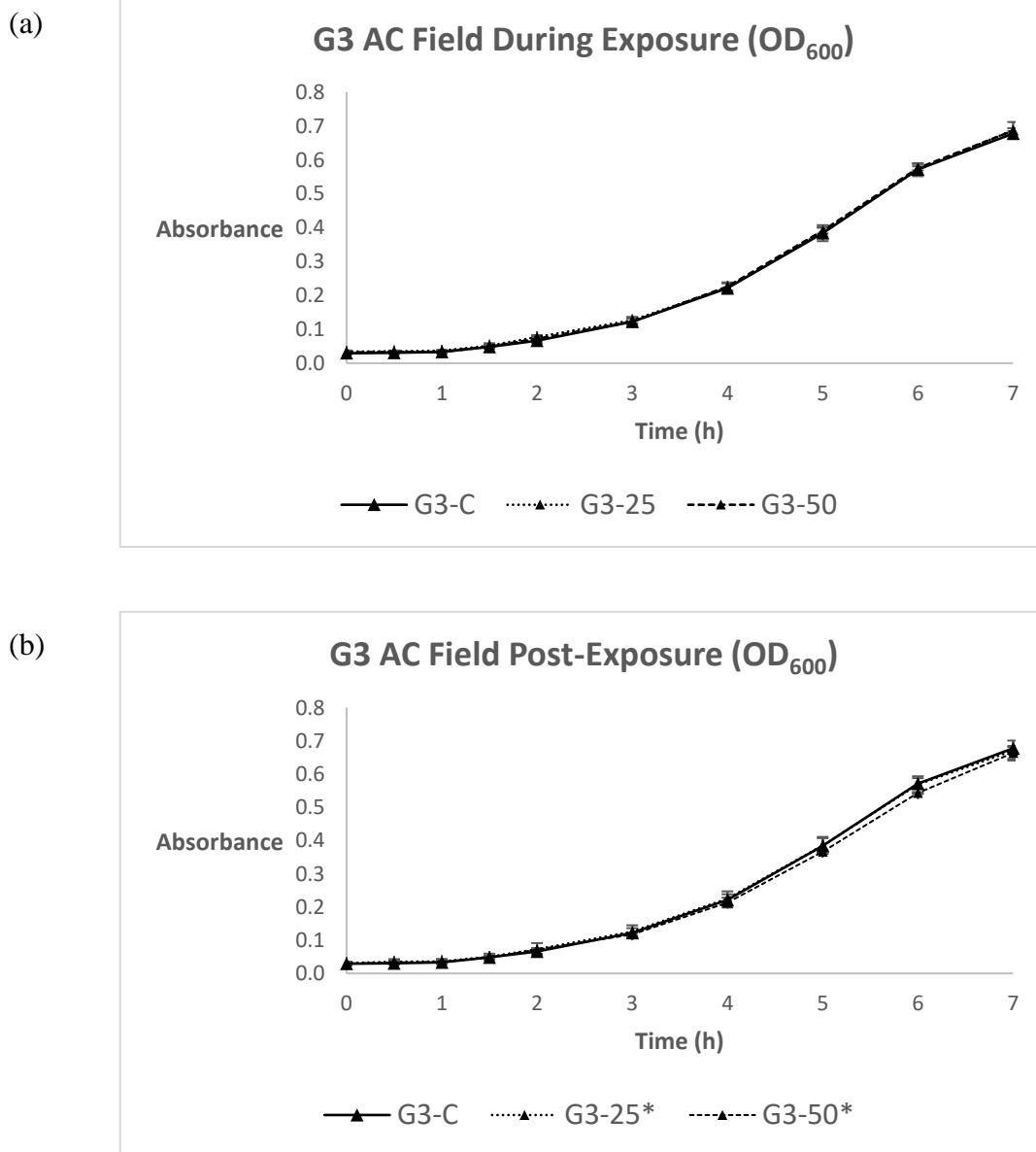
Time (h)

G3-C ......▲...... G3-25* ----▲---- G3-50*

Figure 5.7: Growth curve comparison of G3 E. coli. No significant differences in growth were detected in the groups (a) during exposure to the field or (b) post-exposure.

Concluding Remarks

In the DC field experiment, it was taken for granted that the ambient temperature inside the exposure volume of the solenoids had small variations. Temperature sensors were obtained after the experiment had already concluded and data was already taken. Measurements of the temperature as a function of time showed a significant 1.4 °C increase in the ambient temperature within the coil due to heat dissipation by a sufficiently high current. Conditions of higher temperature would be expected to increase the growth rate of *E. coli* up to the optimum temperature of growth (37 °C). Whether or not the increase in temperature obscured any magnetic field effects is still unclear. Future experiments should have better temperature control by either providing airflow or a cooling mechanism for the solenoids or by conducting the experiments inside incubators to decrease variations due to ambient room temperature. Additionally, the relationship of temperature and the magnetobiological effect could also be in interesting direction to pursue.

For the AC field experiment, the negative results in G1 and G3 and the positive results in G2 are particularly interesting. On the one hand, there is a possibility that the effects observed in G2 are due to unknown artifacts capable of causing significant differences in growth. For instance, impurities or contaminations in the culture tubes could be a cause of variation. There were also observed variations in the growth of the control groups from generation to generation, which is a source of error that should be investigated. On the other hand, it is possible that the positive results are indicative of a biological effect. The lack of an effect in G1 could be due to a time threshold of exposure before any effects can be observed. In G2, the OD difference starts as *E. coli* begins its exponential phase of growth and begins to vanish as the cell culture approaches the stationary phase, with the maximum difference occurring at t = 5 h. This result suggests

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that there is a possible dependence of the biological effect on the number density of bacteria in the sample at a given time. No significant OD differences are seen in G3 samples. It is speculated that there may an apparent 'relaxation' time after which bacteria will have adapted to the field conditions. It is unclear by what cellular mechanisms they adapt, however it could be related to changes in metabolic activity or changes in the gene expression. This time of relaxation could be dependent on the frequency and strength of the magnetic field as well as the physiological capability of the species. Static magnetic fields supply a constant energy perturbation, whereas oscillating magnetic fields have a periodic variation in the perturbation and hence their effects may take time to accumulate in biological systems. To further understand the role of energy of a magnetic field in cellular systems, it will be important to investigate prolonged exposure to static fields and high gradient fields and compare their effects with oscillating fields.

On the subcellular level, changes in growth could be associated with modulation of gene expression. These changes could arise due to the biological response of a magnetic field as a stress factor which can perturb certain cellular functions such as nutrient uptake, membrane transport, and alike. However, such effects can hardly be discerned using population level methods. Further studies involving metabolic assays and quantitative DNA analysis and will help to shed light on these effects.

Finally, it should be noted that complementary methods were not used in this experiment to confirm the optical density measurements. In chapter III, it was discussed that morphological changes as well as cells entering the VBNC state could be contributing factors in disagreements between OD measurement and viability or culture counts due to changes in the light scattering characteristics. Future growth rate experiments will include viable counts among other techniques for comparison. It may

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also prove useful to study multi-wavelength absorbance spectra of cell populations as opposed to the traditional single wavelength methods.

There are many improvements that could increase the effectiveness of this study. The findings in this study only scratch at the surface of understanding WMF effects in bacteria. More advanced studies must be done to confirm these preliminary results.

Outlook

It is evident that the effects of WMFs on bacteria are not well-understood. On the experimental side, difficulties arise in the interpretation of inconsistent results. This is one of the reasons for why there is still a fair amount of controversy in the field. Classical models have thus far been insufficient to explain the wide array of experimental results. Recently, there are proposed models that take on the more radical approach of quantum theory. Although current experimental approaches may lack the ability to probe quantum level effects, there is a possibility that magnetobiology will find solutions in the emerging interdisciplinary field of quantum biology.

What sort of understandings can come from studying the interactions of bacteria with magnetic fields? From a technological point of view, bacteria find a wide range of applications in the engineering and medical fields. Can we harness the utility of bacterial functions to develop more efficient and green technology? Furthermore, can we achieve these goals with electromagnetic control of biological and biochemical systems? From a biological standpoint, bacteria are inescapable. They are found in almost every corner and every niche of the planet and even inside our bodies. As far as we know, they predate almost every living organism on Earth and multicellular life forms owe their existence to

these unicellular entities. In a world of increasing technological advances, there is an inevitable increase in electromagnetic activity from artificial sources. How do these sources affect the biological activity of bacteria, or of living organisms in general? From a more fundamental perspective, it should be considered that the geomagnetic field has existed well before the dawn of the first living organisms. Would life have been possible if the Earth's magnetic field were much stronger than it is now, or if it didn't exist at all? Clearly, there are still many questions left to be answered.

On top of the roles that biology and chemistry play in understanding living organisms, there will always be a physical underpinning at a deeper level. In this sense, the progression of the field of magnetobiology should highly depend on multidisciplinary efforts. Currently, the field is still in its infancy. As more sophisticated biophysical methods are developed, it is likely that scientists will achieve better resolution and insight into the complex magnetobiological interactions of living systems.

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