TEMPERATURE DEPENDENT WEAK MAGNETIC EFFECT ON BACTERIAL GROWTH

by

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EFFECT ON BACTERIAL GROWTH

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Dedication

Dedicated to my family and best friends,

thank you for all you've done to help me get to where I am today.

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First off, I would like to thank my family for being so supportive with my decision to undertake physics as a career path, even more so encouraging me and supporting me on my pursuit of a Master's degree, I would not be where I am without their support. I would also like to thank Juan Humberto Montemayor, my best friend and brother, I wouldn't have been able to survive the physics program or life without you.

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ABSTRACT

TEMPERATURE DEPENDENT WEAK MAGNETIC

EFFECT ON BACTERIAL GROWTH

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University of Houston-Clear Lake, 2018

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In this era of technology, exposure to weak magnetic fields is almost unavoidable and is always ignored. Short term exposure with weak fields may not influence anything. But long term effect of strong fields cannot be ignored. Cells are fundamental building blocks of life and are studied as biological systems traditionally. Cellular structure analysis, their growth rate and their molecular level studies are based mainly on experimentation and observations using biological techniques. Biophysics uses fundamental concepts of Physics to study the dynamics of biological systems in detail. Bacteria is the simplest form of single-celled life and is closely related to human life as bacteria is incubated in the human body and is strongly related to human life and health. Bacterial growth rate and the change in its behavior due to the exposure of bacteria with weak magnetic fields is directly related to human health and safety as well as it is the first step towards the impact of weak fields on molecular dynamics of cellular behavior and growth. In this

thesis we study the effect of small changes in temperature on bacterial growth in the presence of low magnetic field. Temperature effect on the growth rate of bacteria is known very well. However, in the presence of weak magnetic field the sensitivity of temperature is affected. We study the relative change of bacterial growth and its behavior with small change in temperature in the presence of different types of weak magnetic field. Two weak effects add up to give a different effect and is worth studying and interesting.

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INTRODUCTION

Current advancement in biological sciences is indebted to a detailed molecular level study of cellular mechanics [1-2, 31] and analysis of large data [45] in order to fix all sorts of ailments, coming from a biological standpoint and then branching out with overlapping disciplines sciences, like biochemistry and biomedical physics. This innovative approach in research is focused to answer unsolved mysteries of life. Man-made low-frequency electromagnetic fields have become a part of our biosystem [23, 30]. For example, magnetic fields are generated in the environment by high-voltage power lines and electrical appliances, during diagnosis by magnetic resonance imaging (MRI) in clinical medicine, during testing of super-conducting magnetically levitated trains (maglev trains), and during bone fracture therapy [46]. Physics and chemistry combined with biomedical sciences has yielded innovated results and led to the invention of very useful and efficient devices like Magnetic Resonance Imaging (MRIs) devices and Nuclear Magnetic Resonance (NMR) technology [30], among other others to help solve medical issues Interdisciplinary studies in biology has led to the significant improvements in the understanding of biomedical physics from theory standpoint as well as designing the relative experiments associated with the real-world applications. This has made more efficient diagnostics and improved medical treatment. Cellular level study is challenging and performing experiments on human body is not an option at early stage research. Eukaryotes are usually very complicated cellular structures with a great variation. Therefore, microbial study is a good starting point for cellular study, wherever it is possible [33]. Lot of studies has been carried out concerning different types of electromagnetic fields from microwaves to static magnetic field effects [38]. Bacterial cells are easily available cells and can be studied at the cellular level as they are single-celled organisms. In addition to that, several kinds of bacteria may reside in human body.

They not only play a key role in some body mechanisms but also their existence after certain limits, may cause diseases in human body [4]. Therefore, bacterial growth study in the presence of weak magnetic fields is a good starting point for the study of weak magnetic field effect on human body [9, 15].

In the biophysics lab of UHCL, an experiment is designed [25-29, 36-38, 41-43] to focus on the comparative study of the growth of gram positive and gram-negative bacteria of different shapes under varying magnetic fields in different temperature conditions. Effect of nano-ripple structures on the bacterial growth is also studied [38]. Average doubling time of most of the common bacteria is already known. However, the effect of weak magnetic field on bacteria is not studied in detail yet and the effect of temperature in the presence of magnetic field is not studied in literature. The experiment investigates the thermal effects along with the weak field effects. For this purpose, different bacterial species are grown at different temperatures in types of magnetic fields. Combined effect of temperature and the magnetic field gives some interesting results on distinct species of bacteria based on temperatures, magnetic field strength and type of magnetic field.

CHAPTER 1: CELLULAR STRUCTURE AND BACTERIUM

With the advent of molecular science, and an advanced understanding of the living world around us, we undertake the task of learning about units to their most miniscule scale, down to cellular scale. To understand the bacterium behavior and growth experimentally, the structure and processes of living cells were explored to further understand the behavior of the common bacterial cells. Through the efforts of biologists, we know that cellular structure is rather complex, regardless of its size. Although we know that all living matter is composed of a multitude of cells, cellular structure is much more complex than molecular structure. Over the course of landmark studies in history, the inner workings of a cell structure have been further analyzed with the creation of a microscope to electronic microscopy along with other advancements, and we now know about varying types of cells and their configurations.

1.1 - Cellular Membrane

When considering the overall structure of a cell, understanding the cellular membrane yields a closer view of the complexity that are living cells. Much like the integumentary system, cellular membranes envelop the system and give it a coating of protection to its structure. To appreciate the membranes activity and its dynamics within the cell, its components are studied. The membrane has a phospholipid bilayer which contains lipid-soluble proteins. Overall, the membrane serves two kinds of functions: it contains the cytoplasm within the external medium, mediating transport between the two; and it carries many -proteins with specific functions, such as biosynthetic enzymes and environmental signal receptors [40].

Phospholipid molecules have an interesting setup, where it contains a phosphate group, which is referred to as the “head,” and two chains of fatty acids, which make up the tails of the phospholipid. The head of the molecule, or the phosphate group, is negatively

charged, so it is polar and hydrophilic, which makes the phosphate group attracted to water, so they are attracted to both extracellular and intracellular environments. Since the phospholipid bilayer consists of two layers of phospholipid molecules arranged with their tails pointed to each other and the phospholipid heads point both inside and outside due to their hydrophilic properties, the phospholipids can operate on either the inside or outside environment of the cell. While the heads of the phospholipids have their own charge (Figure 1.1), and the tails are electrically neutral and are hydrophobic in nature, which is described as "water fearing" due to the tails repelling and being repelled by water. Some lipid tails consist of saturated fatty acids and some contain unsaturated fatty acids.

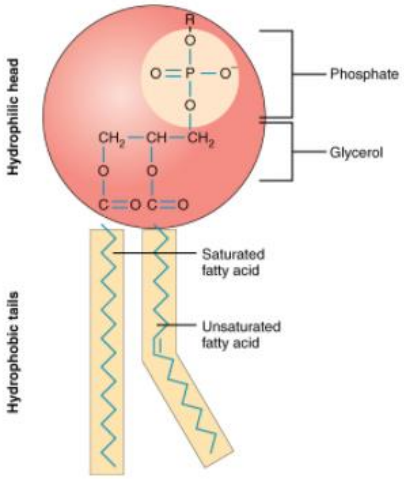


Figure 1.1: Phospholipid Structure [32]

This combination adds to the fluidity of the tails that are constantly in motion [32]. Since phospholipids have both a hydrophobic and hydrophilic section to its structure, it is then considered an amphipathic molecule. Due to the polarity of the phosphate groups, the phospholipids are attracted to intracellular fluid (ICF) and extracellular fluid (ECF), which are the fluid on the inside of the cell and the fluid outside of the cell membrane's environment, respectively. By the hydrophilic (water loving) properties considered, it is

the driving reason as to why the structure of the phospholipid bilayer turns out to have its heads pointed both inwards and outwards, and why the hydrophobic tails of the phospholipids orient themselves inwards to themselves. The cell membrane has many proteins, as well as other lipids (such as cholesterol), that are associated with the phospholipid bilayer [32].

Throughout the cell membrane, there are two types of proteins, integral protein, which is embedded in the membrane, and peripheral proteins, which allow ions or other particular matter go in or out of the cell (Figure 1.2). Peripheral proteins can exist either inside or outside of the surface of the phospholipid bilayer, and while some serve as a sort of transport control, some act as enzymes to break down nutrients in matter to pass through cells. In relevance to integral proteins in the cellular membrane, they have a subsection named cell recognition proteins, which allow cells to be differentiated from one another, such as receptors and ligands.

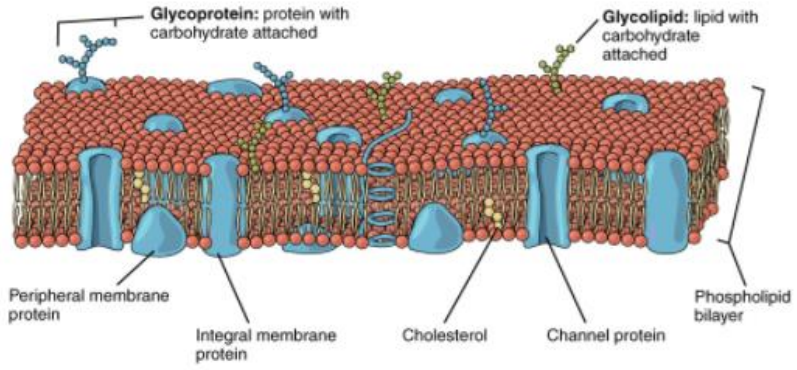


Figure 1.2: Cell Membrane Structure [32]

Receptors are proteins which can bind to a ligand molecule outside the cell, and this ligand, which binds to the receptors and activates them, induces a chemical reaction within the cell. While the integral protein serve their set functions, some proteins are able to perform a role as both a receptor and ion channel; an example of interaction between the receptor and ligand acting* upon nerve cells and binding neurotransmitters.

Another subsection of integral membrane proteins are glycoproteins, which are units of protein that have attached carbohydrate molecules, which help the glycoprotein in cell recognition. The carbohydrates that extend from the membrane proteins and even from some membrane lipids collectively form the glycocalyx [32], which consists of glycoproteins and other carbohydrates that are attached to the membrane, which surround the cell, and serves several functions for the cell membrane. For example, it may have molecules that allow the cell to bind to another cell, it may contain receptors for hormones, or it might have enzymes to break down nutrients [32]. With the help of the signal that glycocalyx produces, they give cells an identity to recognize one cell from another, which is one of the main processes that allows the body to function, since it might consider some bacterial cells as foreign matter and attack itself. While the membrane itself can have an identity with the help of proteins, it can also regulate the types of substances to go in and out of the cell; these substances include ions such as Ca++, Na+, K+, and Cl-, nutrients including sugars, fatty acids, and amino acids, and waste products, particularly carbon dioxide (CO_2), which must leave the cell [32]. When considering the phospholipid bilayer, we must remember the hydrophobic layer in between the bilayer and hydrophilic interior and exterior, which causes the membrane to be selectively permeable, allowing in certain substances with or without aid. The polarity of water solubility of materials have an influence on whether or not it can be transported in or out of the membrane due to the nonpolar hydrophobic tails inside the bilayer. Examples of nonpolar media that can pass through without any additional assistance are lipids, oxygen, carbon dioxide gases, and alcohol, while water soluble materials like glucose, amino acids, and electrolytes do need assistance to cross the hydrophilic cross section of the cellular membrane.

To differentiate the types of transport, it can be simplified to passive transport, which allows substances to move across the membrane without any additional energy, and active transport, which the equivalent of passive transport, but requires energy from adenosine triphosphate (ATP). To describe the motion of how substances can move across the cell membrane, we must recognize how diffusivity and concentration gradient play a role in the action of transporting materials across the bilayer.

The concentration gradient is the difference in an amount of a substance within a specified space, and diffusion is the movement of a substance across a concentration gradient from a higher amount to a lower amount by trying to spread a substance evenly across an area. When a substance, that is small and nonpolar like oxygen and carbon dioxide, is present in a system, it can easily diffuse throughout the phospholipid bilayer of the cell membrane, and when faced with a large concentration in the extracellular fluid in comparison to the intracellular fluid, cellular membranes will try to diffuse any matter without using any additional energy.

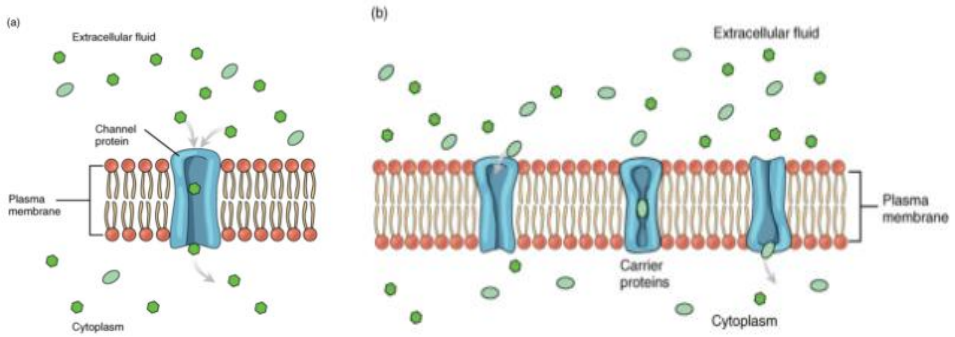


Figure 1.3: Facilitated diffusion using (A) channel proteins. (B) carrier proteins [32]

When nonpolar molecules can move throughout the lipid bilayer without using any energy from a large concentration to a lower concentration, it is considered a form of passive transport called simple diffusion. This is not always the case, as there are large polar molecules, which cannot simply pass through via simple diffusion due to the

hydrophilic tail section of the cellular membrane, so it needs another process to be granted passage into the cell. Facilitated diffusion is the alternate process that molecules need to undertake due to their size, charge or polarity, so the materials will cross from the extracellular fluid to the interior using channel proteins or carrier proteins.

Looking at (Figure 1.3), we can see at the process of facilitated diffusion take place with varying types of proteins. Channel proteins (Figure 1.3A) are less selective than carrier proteins, and usually mildly discriminate between their cargo based on size and charge [32], whereas carrier proteins (Figure 1.3B) are more particular and will only allow a certain type of molecule to pass through the cellular membrane. Since this process is still passive, it does not require any energy to be used by the cell as it all only uses passages already in the cellular membrane.

The previous transport methods need no energy, and are considered a passive transport, whereas active transport will require the use of ATP (Adenosine triphosphate) in order to move a substance across the cellular membrane with the assistance of protein carriers and will typically move against the concentration gradient. The sodium-potassium pump, which is also called the Na+/K+ pump is an important ion pump found in the membranes of different types of cells [32].

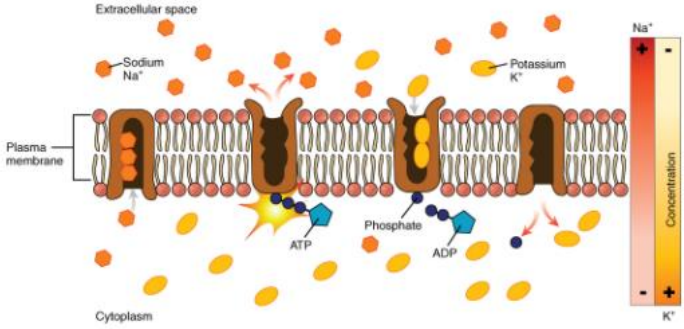


Figure 1.4: Sodium – Potassium Pump [32]

These Sodium-Potassium pumps, (Figure 1.4), pump out sodium ions and pulls in potassium ions in order to keep an electrical gradient across the cell membranes.

Electrical gradients, like concentration gradients, are characterized by the difference in electrical charge within a certain area and are critically important in nerve cells. These pumps requires a constant gradient in charge because it is responsible for the majority of the ATP usage in a cell. This process of active transport can also collaborate with passive transport systems in order to move substances to the intracellular fluid media of a cell. By using the active transport pumps, a concentration gradient can be conducted by keeping a high cluster of sodium ions outside of the cell, therefore, if the cell needs sodium ions, all it has to do is open a passive sodium channel, as the concentration gradient of the sodium ions will drive them to diffuse into the cell [32]. By doing this process, the sodium-potassium pump will then power the passive transport of sodium ions with the concentration gradient with the difference in concentration gradient in the intracellular and extracellular fluid.

1.2 - Prokaryotic and Eukaryotic Cells

Knowing the basics of the cellular structure, we can then differentiate some of the distinct types of cells based off their structure. From the cells that have been studied, we can have a base classification system for all types of cells; cells are separated based upon the lack or presence of a nucleus, which is then named as a prokaryote or eukaryote, respectively. Conversely, prokaryotes, in the absence of a nucleus, instead have a chromosome organized within the cytoplasm as a system of looped coils called the nucleoid [40]. While both are quite similar, they have distinct differences that set them apart; the complexity of eukaryotes is far more advanced than prokaryotes, as they have membrane bound organelles, such as the mitochondria, lysosomes, and endoplasmic reticulum (ER), which allow eukaryotes to operate at a higher level than prokaryotes. Eukaryotic cells also have a cytoskeleton, which assists the cell in maintaining its cellular shape, allowing it to keep its organelles and other structural pieces in plane, thereby giving it support.

The eukaryotic cell reproduction cycle is also different due to the structural differences; eukaryotic cells (Figure 1.5) first perform mitosis then cytokinesis; the cell membrane breaks apart and the chromosomes orient themselves to separate in to where each daughter cell will have two sets of chromosomes, and then the cytoplasm in the cell divides to create two identical daughter cells. Conversely, prokaryotic cells go through a different replication process, fission, which includes DNA replication, chromosomal segregation, and ultimately cell separation splitting the parent cells and producing two daughter cells.

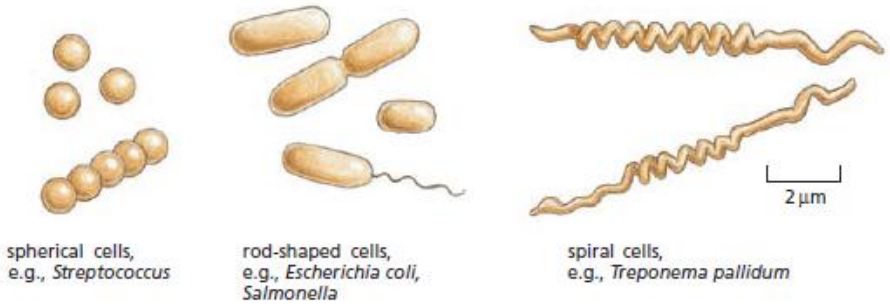


Figure 1.5: Varying types of prokaryotic structures [42]

Eukaryotes are more complex than Prokaryotes and prokaryotes are more convenient for initial study and easily available for experiments. We focus on *Staphylococcus Aureus* and *Escherichia Coli* for this experiment. Since bacterium and prokaryotes are similar in structure, they are sometimes considered quite alike, but they cannot be considered interchangeable, due to the fact that there are two branches of prokaryote types, bacterial cells and archaea, which is not included in this study.

Prokaryotes are single-celled organisms that do not have a nucleus or any other membrane-bound organelle and can take on several forms (Figure 1.5); spherical like *Streptococcus*, rod-shaped like *Escherichia coli*, or spiral like *Treponema pallidum* [42]. These prokaryotes vary structurally and behave differently from their eukaryotic counterparts.

Prokaryotes have a tough peptidoglycan cell wall that surrounds the contents as it helps maintain the shape and prevent dehydration, and typically has a polysaccharide capsule which assists the prokaryote in attaching itself to its surroundings. Different attachments, (Figure 1.6) can surround the capsule of the prokaryote, such as flagella, which are mechanisms used help prokaryotic cells to move around, pili, exchanging genetic material, and fimbriae, which bacteria use to attach to another cell, among other bacterial cell structures.

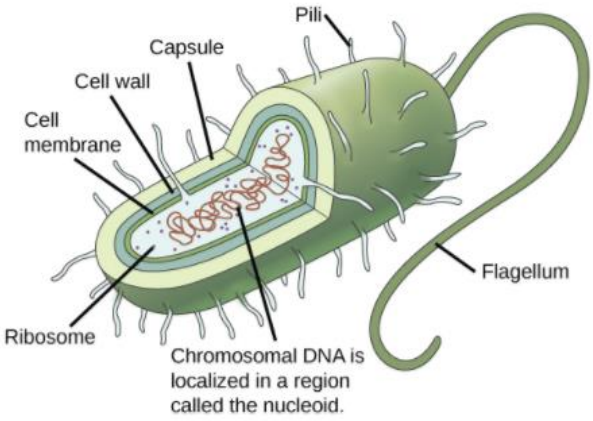


Figure 1.6: General Structure of a Prokaryotic Cell [42]

The prokaryotes, like all cells, still retain a plasma membrane, which contains the cytoplasm, which is the cytosol inside the cell where the other components are found, ribosomes, where the protein synthesis occurs, and DNA, the genetic information of the cell, but due to the lack of a nucleus within the structure, the DNA will simply float within the cytoplasm. The size of prokaryotic cells is smaller in scale in comparison to eukaryotic cells due to the lack of sufficient structure in prokaryotic cells in comparison to the complexity of eukaryotic cells.

Prokaryotic cells can range from 0.1 μm to 5.0 μm in length, whereas eukaryotic cells' length can range from 10 μm to 100 μm. The smaller size of the prokaryotic cells attributes to their lack of capabilities; when considering the rate of diffusion, a larger cell

will increase its surface are, therefore the plasma membrane will not be as efficient as it can be. To increase bacterial growth efficiency is to divide; another way is to develop organelles that perform specific tasks, and these cells' behavior of becoming more proficient in their processes is what ultimately led to the evolution of more complex cells like eukaryotic cells.

Although prokaryotes may not be as advanced as eukaryotic cells, they still are versatile with their habitats, they can exploit an enormous range of habitats, from hot puddles of volcanic mud to the interiors of other living cells, and they vastly outnumber other living organisms on Earth. Due to the adaptability of the prokaryotic structure, the variance in temperature along an electromagnetic field influence will be the primary focus of my thesis experiment. While archaea have more resistance towards critical climates, bacteria are more susceptible to environmental influence like temperature. Throughout several experiments from biologists and physicists alike, we know there is an effect that influences the growth of bacteria with the presence of a magnetic field [1,5,8] and the variance in temperature will either heighten or lessen its effect.

1.3 - Bacterial Structure

In regards to the complexity of the bacterial cells, we must delve further into what comprises the structure of the cells, primarily what keeps it together. Prokaryotes consist of the plasma membrane, which was previously discussed, the cytoplasm within the cell, ribosome, and genetic material, such as DNA and RNA, and, depending on the bacterium we are observing, prokaryotic cells may have other components such as a cell wall envelope, pili and flagella, which were also briefly mentioned. In the majority of prokaryotic cells, a cell wall envelope wraps around the cell membrane of a bacterial cell, which gives the cells the stability for the inner structures and allows it to withstand the environment where it may be. The cellular wall structure is referred to as the sacculus

(Figure 1.7A), which consists entirely of peptidoglycan polymers, (Figure 1.7B) is connected to one another and wrapping around all the cell.

Peptidoglycan is a polymer of peptide-linked chains of amino sugars. “Peptidoglycan” is synonymous with murein (“wall molecule”) [40]. Peptidoglycan exists primarily on bacterial structures, although a few species of archaea can construct similar structures that serves the same purpose. The peptidoglycan is made up of parallel polymers of disaccharides, which consist of two monosaccharide residues, and are referred to as glycan chains, also linked with peptides of four amino acids.

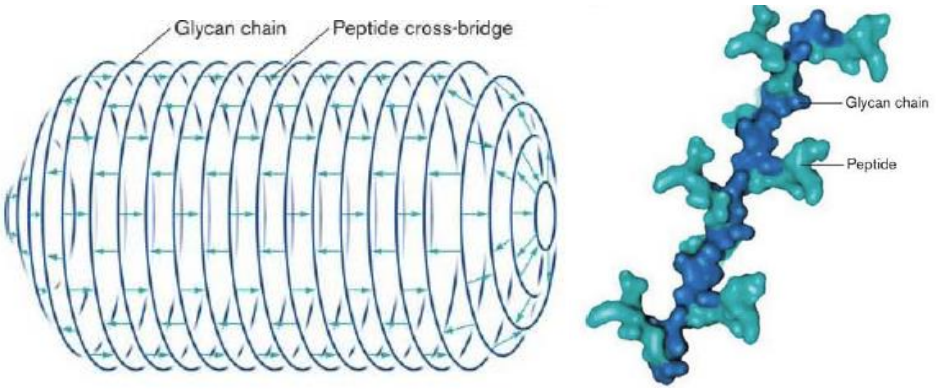


Figure 1.7: A. Sacculus structure of a prokaryotic cell consisting of glycan chains and peptide cross bridges. B. Peptidoglycan Structure [40]

By checking (Figure 1.7A) and (Figure 1.7B), we can see that the glycan chains are linked to one another via the peptide cross-bridges, and these peptidoglycan strands that supply the structure for the sacculus. The long chains of the peptidoglycan consist of repeating units of the disaccharide composed of *N*-acetylglucosamine (an amino sugar derivative) and *N*-acetylmuramic acid (glucosamine plus a lactic acid group) [40].

With these strands of peptidoglycan, the outwards peptide components create the cross bridges that then connect the glycan strands in the molecules, thereby constructing the cohesive unit that is the sacculus. The peptides in the peptidoglycan consist of two amino acids which are set in different mirror configurations: o-glutamate and o-alanine, which is

due to fact that we are referring to amino acids in microbes. The o- form in amino acids refers to those in prokaryotic systems, whereas the D and L amino acid formulation is used when describing ribosomes in protein.

While the formation of the sacculus remains the same, the peptidoglycan structure can possibly change based off the type of bacterium species we look at. Some Gram-positive species, such as *Staphylococcus aureus* (a cause of toxic shock syndrome), have peptides linked by bridges of pentaglycine instead of the *D*- alanine link to the *m*-diaminopimelic acid. In Gram-negative species, the *m*-diaminopimelic acid is linked to the outer membrane [40].

1.4 - Bacterium Identification

In order to tell the difference between different types of bacterium, Dr. Hans Christian Gram created a method to distinguish bacterium based upon the structure and retention of a violet dye and allows for the bacterium to be classified into two distinct types, either Gram positive or Gram negative. Gram-positive and Gram-negative cellular structure is the lack of an outer membrane and the other technicalities, so to differentiate the bacterium, the Gram staining method helps us separate the types based off their dye retention. Gram positive cells retain the dye whereas negative cells do not, due to their cell walls being more resistant to the antibodies. Gram positive bacteria retain the dark purple color from the CV, whereas the Gram negative will be stained pink due to alcohol used in the procedure. With the different dyes used in the staining process, we can classify different bacterium being inspected.

What makes the difference in the classification of the Gram staining method is the peptidoglycan layer surrounding the bacterial cell membrane. The thick peptidoglycan layer in Gram-positive bacteria absorbs and retains the CV dye, while Gram-negative bacteria has a thinner cell wall in its structure, and it is surrounded by an additional

liposaccharide membrane. The Gram staining process begins with the selected media being washed with an aqueous solution of crystal violet stain. Crystal violet is an organic dye which dissociates in CV+ and Cl- ions, penetrating the cell wall and the cell membrane of both Gram-positive and Gram-negative cells, staining the cells purple. After the cells are stained, a mordant, typically Iodine, is used in order to form large complexes of CV within the inside and outside layers of the cell. Afterwards, a decolorizer, usually alcohol or acetone, is applied to the system and it interacts with the lipids of the cell membrane, and due to the thin peptidoglycan layer, which consists of one to two layers depending on the bacterium, and an additional lipopolysaccharide layer, the layers dissolve when impacted by the alcohol. Since the layers are wiped out, the Gram-negative bacterium is not able to retain the complex that was grown with the mordant interaction. While the Gram-negative cells have the CV stripped away, the Gram-positive cells experience dehydration from the interaction with alcohol, closing the pores in the cell wall and does not let the CV stain exit the cell.

Since Gram-positive cells have such thick cell walls that have up to 40 layers of peptidoglycan, the cells can retain its dye, allowing for the gram straining examination to keep its hue. With the assistance of teichoic acids, which provide support to the cell wall by keeping the structure secure, threaded throughout the many layers of the peptidoglycan, the Gram-positive bacteria must retain their Gram stain. After the alcohol, a counterstain is applied to the system, usually safranin, in order to give the decolorized Gram-negative a pink hue to it, and it will allow for an observer to check for the results of the examination for Gram-positive and Gram-negative bacterium under a microscope based solely upon the color. As we can see on (Figure 1.8), we see the different type of coloration after the Gram staining of two different bacterium.

Staphylococcus aureus and *Escherichia coli*, the two bacteria under examination on this experiment are a size of 0.9 μm and 2.5 μm, respectively, and in order to differentiate them, we can look at their distinct differences. *S. aureus* is Gram-positive, retains the purple dye, and can see the colonies of cocci bacteria clumping together, and *E. coli* is a rod shaped, Gram-negative bacterium, so the dye is washed off by the solution, giving the bacterium its pink hue.

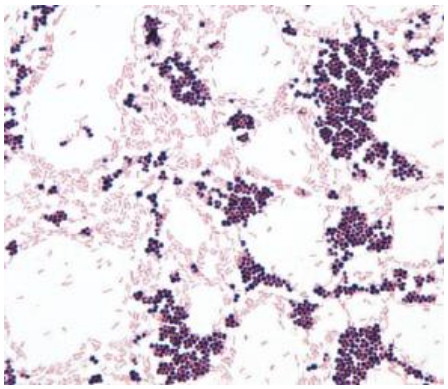


Figure 1.8: Proteus (Gram Negative) and Staphylococcus (Gram Positive) identified after Gram Staining [53]

Running bacteria through an oxidase test is also useful for this purpose in that it will identify if bacteria produces cytochrome c oxidase which is an enzyme located in the bacterial cell membrane processes and is needed for electron transport chain (ETC), essentially proving with the examination where or not there is a preference for oxygen. If bacteria are oxidase positive, it may be either aerobic or facultative due to the use of oxygen in the respiration process, whereas oxidase negative could prove to be aerobic, anaerobic, or facultative anaerobic due to it not using oxygen in the respiration process or it doesn't produce the cytochrome c oxidase enzyme in the bacteria.

Another identifying marker for bacterial cells is catalase production, where catalase is described as an enzyme that detoxifies the cell by converting hydrogen peroxide produced in the ETC to water and molecular oxygen [40]. The electron transport chains

on both aerobic and facultative anaerobic bacterial cells are proficient in their ability to transfer or accept electrons as the climate of the cell requires down to the electron acceptor. The energy shift resulting from the electron transfer pushes the ATP to be produced from ADP, a phosphoryl group, with produces the energy.

Through the use of a carrier molecule in the ETC called flavoprotein, it has the ability to bypass the next carrier in the chain and transfer electrons directly to oxygen [40]. By using this alternative, it also consequently creates two toxins, hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-), and bacterial cells which are able to produce these two are able to make enzymes that can break them down. Superoxide dismutase, which is an enzyme whose purpose is to break down the harmful toxins, consequently catalyzes the superoxide radicals into hydrogen peroxide, and the catalase inside the cell changes the hydrogen peroxide toxin to water and oxygen. The types of bacteria that make catalase can be detected using hydrogen peroxide added to the area of potential bacteria for testing.

Figure 1.9: Catalase Slide test, showing catalase-positive bacteria on the left and catalase-negative bacteria on the right [40]

Whenever the solution is added to bacteria, it will either have no effect, or it will make oxygen bubble on the surface (Figure 1.9). Therefore, the catalase test, which may be done in either a slide or test tube, attempts to identify the presence of catalase enzyme

within a bacterial cell, proving if the bacterial cell under inspection is either catalase-positive or catalase-negative.

A key factor into the survival of these types of cells is the presence of oxygen, so identifying under what conditions bacteria will thrive will help encourage the growth of bacterial cells; some cells are obligate aerobes, which require oxygen to survive, while other cells are obligate anaerobes that surviving without the presence of oxygen and will die upon contact to any exposure, or a mixed possibility where bacterial cells can be facultative anaerobic, which allows them to survive with or without the presence of oxygen. Obligate anaerobes use oxygen in aerobic respiration, which produces ATP, and is more efficient than anaerobic respiration due to the difference in oxygen.

The oxygen in the system acts as an electron acceptor for the transition in the respiration process, yielding a more efficient ATP production system. If obligate anaerobes exist with a lack of oxygen, it will only lead to the bacterial cells' death, so they are only respiratory, so no fermentation takes place, and they also have the enzymes superoxide dismutase and catalase.

Obligate, or strict, anaerobes, opposite their aerobic counterpart, cannot survive in a system with the presence of oxygen. They have never developed a strategy for coping with this toxic compound or its byproducts, are catalase and superoxide dismutase-negative and catalase, and can be found in nature only where little or no oxygen exists [52]. The obligate anaerobes can use respiration, just like obligate aerobes, but these use anaerobic respiration, which serves the same purpose, just at a slower rate, or they can use fermentation. Fermentation occurs in bacterium that exist in oxygen deprived system, where energy is released from glucose regardless if oxygen is not present in the system. Facultative anaerobic bacteria, however, have a metabolism capable of both respiratory processes and fermentation; in the presence of oxygen, it can continue ATP production

via aerobic respiration, or if there is no oxygen present in the system, then it may switch to either fermentation or anaerobic respiration to make up for the lack of oxygen in the system. Regardless of the fact that they may operate with or without oxygen, facultative anaerobic bacteria will still have a tendency to gather to the top to use oxygen to help with the aerobic respiration, since it works much more efficiently than the anaerobic respiration counterpart.

Within these three systems, there are two more specialized branches; aerotolerant anaerobes and microaerophiles. Aerotolerant anaerobes do not concern themselves with the presence of oxygen in the system, they can live in the presence or lack of oxygen, but do not have the capability of using it to their advantage and use only fermentation for their metabolism. These types of bacteria are catalase-negative, although the majority do contain superoxide dismutase. As far as microaerophiles, they require a precise set amount of oxygen in order to continue living. If these types of bacteria have either too little or too much oxygen, then they will die out. In order to test bacteria for their ability to grow without oxygen, an anaerobic culture chamber is required. Oxygen is removed from the chamber when it reacts with hydrogen gas to form water [52].

When testing bacterial growth inside of a test tube, we can see the differences more clearly as far as where the bacterium gathers in relevance to their oxygen preferences. As we can see on (Figure 1.10), obligate aerobe bacteria will gather to the top of the tube to seek oxygen rich broth, obligate (or strict) anaerobe bacteria will gather near the bottom to keep away from the oxygen and try to reach the oxygen depraved area of the broth, facultative anaerobic bacteria is well dispersed throughout the tube, although it will have a tendency to gravitate towards the top in order to allow the oxygen in the tube to better facilitate the metabolism with aerobic respiration.

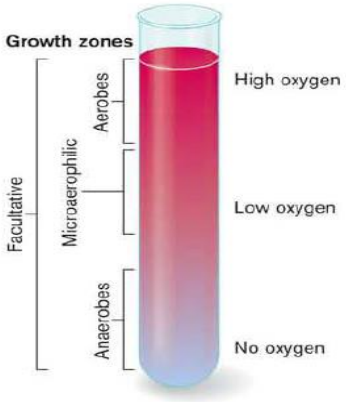


Figure 1.10: Bacterial growth patterns with different oxygen preferences [40]

Aerotolerant anaerobic bacteria, like facultative anaerobic bacteria, will be dispersed throughout the test tubes, but will be a true dispersion and will not gravitate towards the top or bottom since oxygen does not have any effect on the bacterium, and microaerophiles will gravitate towards the top but not all the way since they have to calculate where the perfect spot it needs to stay at in order to thrive.

1.5 - *Escherichia coli*

The thesis focuses on two types of bacterium, the first being *Escherichia coli*, shortened as *E. coli*, which is most commonly found in human body, and is the first of two bacterium used for this experiment. *E. coli* is a gram negative, rod shaped, facultative anaerobic bacterium. Since it is an easy bacterium to be cultured and manipulated for research purposes, *E. coli* is typically used for experiments [44]. Moreover, it is the most extensively studied bacteria and is most suitable for the comparison of results. When under inspection for an experiment, using *E. coli* is particularly useful due to its generation time, i.e. the time it requires for a new generation of bacteria to be produced, which allows the population in E. coli to double around every twenty minutes. The length of that interval varies with respect to many parameters, including the bacterial species, type of medium, temperature, and pH [40]. Also, E. coli was selected because of its wide use in molecular biological studies [33].

Generation time is also referred to as doubling time due to the population of cells double over a single generation, thereby giving an exponential growth rate after the passing of every generation. After the bacterium in a test tube is allowed to activate and acclimate to their environment, this exponential growth quickly overtakes the density of the respective broth solution. *E. coli* and *S. aureus* use doubling time as both will double their number of cell population per generation, but there is other bacterium which have the capability to produce much more per generation, such as the parasite *Plasmodium falciparum*, which is able to produce a cluster of 20 parasites every generation. Looking towards (Figure 1.11), bacterial size colonies can grow exponentially to a high amount quite quickly depending on the amount of progeny they are able to generate and how long they take to carry out their doubling time. Knowing their generation times, deducing when their growth rate will begin to collapse upon itself based upon the resources its environment has. In relation to the growth phases of bacterium, for example if contained to a system like a tube of broth or other medium, it will inevitably run out of essential nutrients to grow and divide, and the cells will begin to die out, so if there is more bacterial cells relative to the amount of nutrients in a set system, the population will crash.

The structure of *E. coli*, differing from the *S. aureus* structure, allows it to be more motile due to the flagellum along the surface of the cell. It contains most of the features of a prokaryotic cell, and in addition to the essentials, the flagella on *E. coli* (Figure 1.12), displays the size relative to the bacterial cell and shows how much influence on the motility it carries.

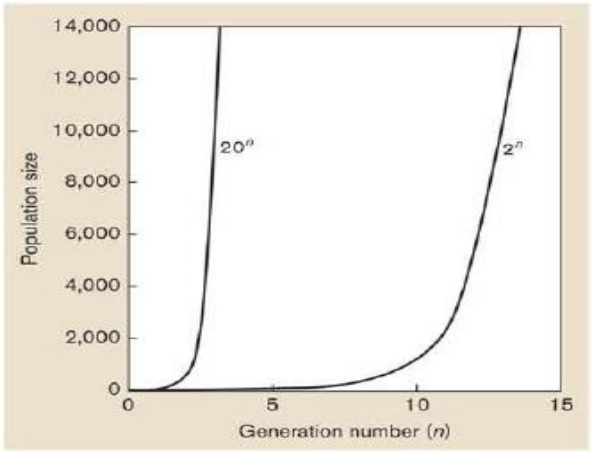


Figure 1.11: Population Size of Bacterium

Flagella are designed to move the entire cell, and instead of generating a current, they propagate regular waves along their length that drive the cell through liquid [42]. The flagella composition consists of an arrangement of microtubules, which are filaments of protein that form themselves as thick hollow tubes. The movement of a single flagella is performed by the bending of the microtubules sliding against one another, creating the wavelike motion propelling a bacterial cell, or *E. coli* in this case, forward to search for the optimal environment, allowing for a greater degree of motility with each additional flagellum strand.

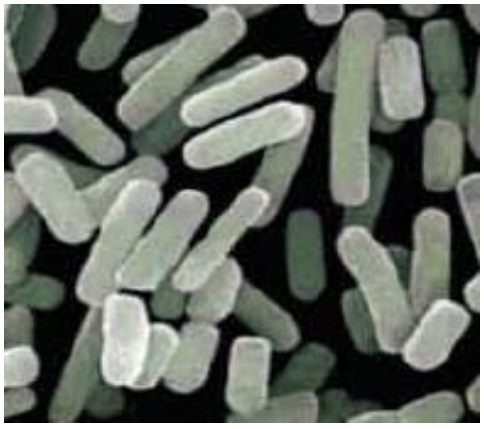


Figure 1.12: Escherichia Coli population [40]

A cross section of the flagella demonstrates a system of nine doublet microtubules arranged in a ring around a pair or single microtubules [42], which is applied in both

flagella and cilium. With the flagellum on the *E. coli*, it allows for the cell to have a high degree of motility, which shows when under inspection on a test tube, as the cells do not cause any sedimentation from the lack of motion and instead flow freely throughout the broth or other medium.

1.6 - *Staphylococcus aureus*

In this thesis, the variances on the effects of temperature in the presence of a magnetic field are observed on *Staphylococcus aureus*, shortened as *S. aureus*. *S. aureus* is a Gram-positive bacterium and, like *E. coli*, is also facultative anaerobic bacterium, capable of existing in an oxygen rich or poor system. It is also nonmotile and part of the cocci family and, as it doesn't have any flagella or any structures to facilitate motion, so *S. aureus* typically groups together in clusters when forming, therefore *S. aureus* has a tendency of gravitating towards the bottom of the tube and forming sedimentation due to the lack of motility of the bacterial cell. The doubling time for S. aureus is around 30 minutes, which is longer than *E. coli*, so comparing their population sizes requires that we take time into consideration to account for the delay. The growth rate factors of *S. aureus* depend on temperature, pH level, and more environmental factors that will be discussed later.

When undergoing a catalase test, it produces oxygen bubbles, so it tests as catalase-positive. *Staphylococcal* cell walls have a rather extraordinary type of structural design and belong to the most highly cross-linked type, but the walls of other Gram-positive bacteria exhibit a much lower degree of cross-linking and the muropeptide fraction of these walls does not contain long oligomeric chains [41]. The durable cell wall surface of *S. aureus* (Figure 1.13) consists of murein, components of the peptidoglycan structure as previously referred, teichoic acids, keeping the structure of the cell envelope firm and durable, and surface proteins throughout the cell surface. With such a rigid cell wall, it

bars any protrusions to the membrane, thereby protecting *S. aureus* from the surrounding environment and acts as exoskeletal element that prevents bacterial cell safe.

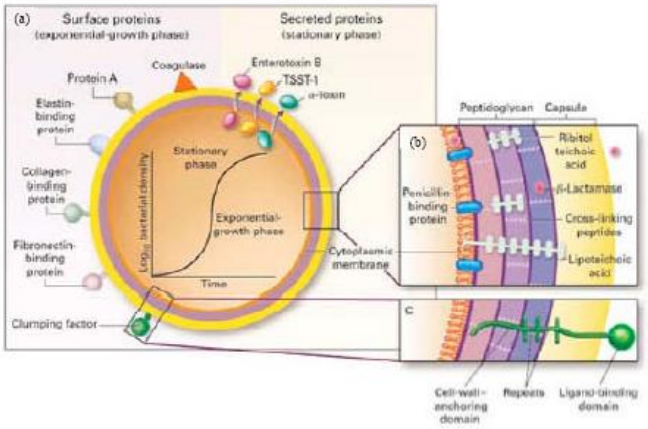


Figure 1.13: Structural Components of Staphylococcus Aureus [41]

The high intensity of security around the cell envelope is due to the high quantity of murein cross-linking supplying the degree of security to the sacculus. It is a distinctive feature of *staphylococci* that the observed degree of murein cross-linking which was determined as a ratio of bridged peptides to the total amount of all peptide ends in general, is extremely high, to the order of 80%-90% [41]. In addition to the high-density exoskeleton system *Staphylococcus aureus* has wrapped around it, it is hard to get rid of via normal methods like immune system or antimicrobial cells.

The additional defenses that *S. aureus* has is due to the coagulase enzyme it produces. Coagulase works in conjunction with normal plasma components to form protective fibrin barriers around individual bacterial cells or groups of cells, shielding them from phagocytosis and other types of attack [22]. These coagulase enzymes are constructed and applied as either free coagulase or bound coagulase. Free coagulase consists of enzymes being released from the coagulase producing bacterial cell and it reacts with the plasma component of the cell called coagulase-reacting factor (CRF) and the reaction from their interaction yields a clotting mechanism, whereas bound coagulase, which can

also be referred to as the clumping factor, sticks to the sacculus of the bacterial cell and interacts with the fibrinogen in the plasma, thereby precipitating it, allowing bacterial cells to then clump together.

By performing a Coagulase Test, one can see if the bacterial cell under inspection is capable of producing any coagulase enzymes, which also assists in classification purposes, distinguishing it from *Staphylococcus aureus* or other Gram-positive cocci bacterium. Due to the coagulase produced, Staphylococcus aureus always has attention from researchers in regard to testing how to better target coagulase producing bacterium with antibiotics, yielding better techniques to eradicate specific cells.

1.7 - Phases

Bacterial growth to fully function to the best possible scenario requires to be set under suitable conditions such as the nutrients in the system, temperature, and incubation time and other factors. Bacterial growth always has a stopping point, whether the amount of nutrients are depleted or toxic by-products of the cells slow the growth until it stops completely. In this experimental method of this thesis, batch culture methodology was used versus continuous culture. In batch culture, we have a closed system with no fresh medium for the bacterium under inspection, so the amount of nutrients in the medium is constantly being depleted and waste continues to gather while the bacteria populations continue to grow. In continuous cultures however, it uses an open system where a set amount of medium in the tube is removed and replaced with fresh medium, thereby constantly keeping the bacteria in the system alive and growing at a steady rate.

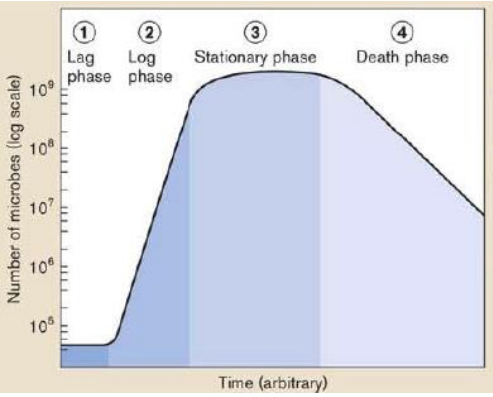


Figure 1.14: Bacterial growth phases [40]

When the conditions are set efficiently in order for the experiment to be run, the lifetime of the bacteria will go through four distinct phases, consisting of a lag phase, log or exponential growth phase, stationary phase, and a death phase (Figure 1.14). In the standard diagram of bacterial growth, bacteria cultures will undergo the lag phase, the bacterium tested will try to settle into their system and adjust. During this this time, the bacteria will not divide, due to a couple factors: they may be from an older culture and require time to repair themselves, changes in the temperature, difference in the quality and quantity of nutrients in the medium, and other factors.

Once the bacteria has sufficiently prepared and had time to acclimate to the environment, then it will move onto the log phase, which is the period where the bacterium will go through an exponential growth rate and the amount of new bacteria doubles and is proportional to the amount of the population present in the system. At this stage, cells are growing and dividing at the maximum rate possible based on the medium and growth conditions provided (such as temperature, pH, and osmolarity) [40]. Referring back to (Figure 1.14), looking at the log, or exponential, phase, it is expressed as linear although it divides at an exponential rate. The reasoning for it is while *E. coli* and *S. aureus* divide at a doubling rate, not all cells experience their division and make a new generation at the

same rate, so integrating the step growth produces a straight line for this phase in the bacterial growth cycle.

After a certain point, the bacterium will use up all of the nutrients in the system or have too much waste buildup in the system, which will lead towards the stationary phase; once the bacterium reaches this equilibrium point, the rate at which new bacterium being created is the same rate that it is dying. As a result of the disparity in size in prokaryotic and eukaryotic cells, the eukaryotic cells will reach the stationary phase much quicker than their prokaryotic counterpart since the larger cells require more nutrients. Due to the lack of nutrients for the bacterium to absorb, the population will stagnate and keep a roughly similar population. Finally, the death phase will begin, where the population of viable bacterium population will begin to decline and it will also decrease at a negative exponential rate, just like the log phase.

In the death phase, the amount of dying cells is proportional to the initial inoculation of the bacterium into the medium, and this rate is important into examining food preservation and antibiotic development [40]. Considering the death rate is logarithmic, the exact calculation of the amount of surviving cells is difficult to surmise on account of mutations that bacterial cells adopt in order to survive, so the death phase will continue to go on for an extended period of time. Since the model of this thesis adopts the batch culture methodology, *E. coli* and *S. aureus* will adopt this model, while an experimental model using continuous culture would allow for the bacterium to undergo constant growth and never quite reaching the death phase thanks to the constant supply of fresh medium in the system.

Using (Figure 1.14) as a reference and comparing to (Figure 1.15), the size of the original inoculation of the bacterial cell will allow for much quicker growth over time.

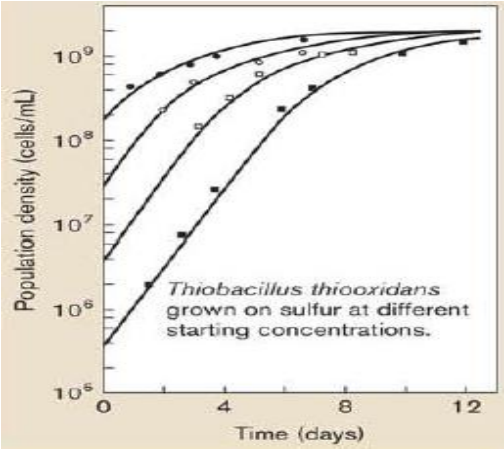


Figure 1.15: Exponential Growth of Thiobacillus thiooxidans [40]

Using *Thiobacillus thiooxidans* as an example, the growth rates comparing their environment and limited amount of essential nutrients. We can note that the cultures with the lowest initial cell density will inevitable reach the level of the higher cell density cultures and begin to slow down, while the culture with the highest cell density inoculation will have its population go into the stationary phase quicker than any lower tube. Once *Thiobacillus thiooxidans*, an acidophil that oxidizes sulfur to sulfuric acid runs out of sulfur, it will enter the stationary phase [40]. Knowing their generation times, the initial population density of the bacterium, and other key factors, we can make our own conclusions once the population will begin to have its decline depending on the environment the bacterium is being tested upon.

CHAPTER 2: ELECTROMAGNETIC INFLUENCE

Bacterial growth is studied in different types of weak magnetic fields (of the order of a few Gauss), may be due to the field effects on the ion channels in the cell, which controls the flow of nutrients of the cell, and has major impact on the generation time between the mother and daughter cells in the colonies. Effect of high magnetic fields on bacterial cells depends on the growth phases of the cells suggests that a magnetic field has the potential to be used as a controlling factor in each growth phase [46]. While planning to study how the variation in temperature affects the growth rate of bacteria while it is put in certain types of magnetic fields. This way, the combined effect of thermal perturbation and the magnetic field perturbation will be estimated to figure out that if the rise in temperature enhances the field effect or reduces it. Now that we have a general idea how the substances behave, we move to the magnetic fields that will be affecting the system.

2.1 - Magnetic Field

Magnetic fields are created under the influence of moving charges in electrical current or from magnetically charged materials objects like magnets and the like. These fields can be explained mathematically through a vector field notation and can express their influence physically (Figure 2.1). These field lines below represent how the lines interact within the area of influence and behave similar to electric field lines but have distinct differences.

Magnetic materials have a sphere of influence where the magnetic field strength is dependent on $1/r^2$, causing inversely proportional relationship, thus the strength will decrease with increased distance and increase strength with decreasing distance, described via Ampere's law. The magnetic field lines also do not cross as their have an ongoing closed loop and where their influence does not start or stop, it simply has its influence and continues on its own track. The direction of the magnetic field also has a

convention of adding a North and South to indicate in which the direction of the field is moving in. The magnetic field will be influence both by the strength, calculated in either T (Tesla) or G (Gauss), and direction of the flow of charge.

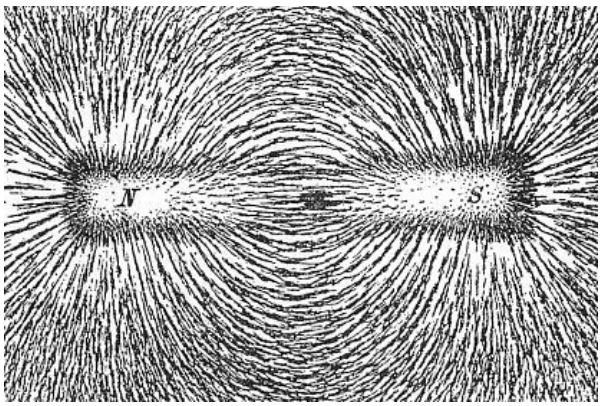


Figure 2.1: Magnetic field lines [30]

These fields influenced by the strength uses the amount of charge put in motion sways the potency of the magnetic field that will be made. The primary way to conduct an efficient magnetic field for any experiment is to generate an electric current in a wire. Current flows through the wires, and as we increase the current (amount of charge in motion) the field increases proportionally, as we move further away from the wire, the field we see drops proportionally with the distance [30]. The magnitude of the magnetic field of the wire can be calculated using Equation 2.1, using the current I, radius r, and constant μ_0, representing the permeability of free space. In order to decipher in what direction the magnetic field is pointing, the right hand rule is used, wrapping around the wire in the scenario and pointing your thumb in the direction of the current, and the rest of the fingers give the direction in which the magnetic field is wrapping around.

$$B = \frac{\mu \mathrm{oI}}{2\pi r} \tag{2.1}$$

For this experimental method, Helmholtz coils are used, so the relationship in Equation 2.1 influences the field inside of the coils. In order to prevent any bacteria tubes having a

stronger or weaker influence to a magnetic field, a stack of Helmholtz coils are used in order for a magnetic field gradient to be as small as possible so the bacteria will have a uniform influence. Magnetic fields applied to the system must be uniform in order to verify that there is an interaction between the two to secure a relationship between a controlled setting without an applied magnetic field and an experimental setting with an applied magnetic field. Since we are not only looking at the effects of magnetic fields but also the diffusivity of the E. coli bacteria and Staphylococcus aureus, we need to consider the structure of bacterium to run the experiment efficiently and to understand how the system is being affected.

2.2 - Experimental Research

When we see the growth of the bacterium affected by magnetic fields, we see that the *E. coli* is grouped together and elongated, whereas the *E. coli* unaffected directly by magnetic fields is scattered throughout, which suggests a correlation with the electromagnetic field and the adhesion property of bacterium. This change can be described as a result of the induced gradient inside the membrane, which could explain the elongation of the structure of the cells [3]. This relationship between the bacterium inside the magnetic field could be explained through fluid dynamics and electromagnetism [39].

Temperature may have a positive or negative effect as compared to the magnetic field effect. By describing both mediums as fluids, we can describe the motion of the interactions. If we consider both interacting fluids, we can calculate what it takes for diffusion to take place, which is dictated by the viscosity of the fluid and the mean free path of the particles [23]. Throughout past experiment runs, the bacterium would make a population jump once the lag phase turned to the log phased and began replicating itself,

and bacteria under a magnetic field present would then grow similar to a controlled population, suggesting that a magnetic field influences the growth of microorganisms. Many studies conducted prove that there in fact is a correlation between the two, such that magnetic field affects DNA synthesis and transcription as well as ion transcription through all membrane [35, 45] Mohammad et al reported that exposure of the microorganism S. typhi to the magnetic field caused change in growth characteristics and the number of cells at the stationary phase increased. [33] These results of impact on the bacterial growth is due to the interactions the plasma membrane bilayer and effectively improving the activity on all enzyme properties.

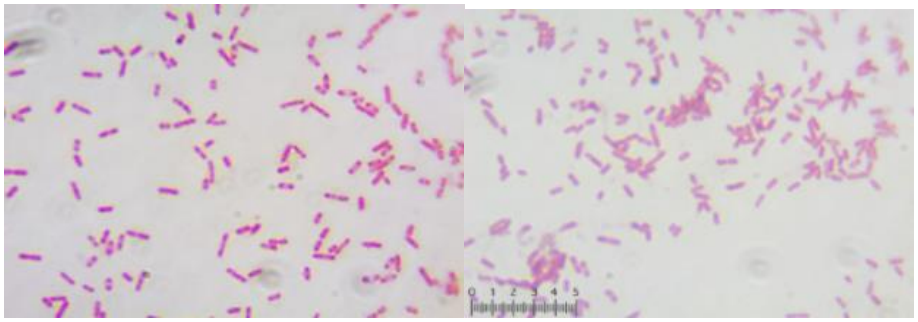


Figure 2.2: E. coli bacteria growing A. Outside of a magnetic field. B. Inside a present magnetic field [30]

Bacteria treated under a magnetic field or lack thereof have physical and grouped effects as well; control bacteria (Figure 2.2A) were sporadically grown and separated, while the magnetic field exposed bacteria (Figure 2.2B) was in fact grouped together and elongated. When the bacterium was observed under a microscope, the physical structure of the bacteria changed, the magnetic effect caused the elongation of the bacteria [38]. The bacteria under this experiment, like the data on this thesis experiment, experienced bacteria that grew at a quicker rate than the control samples. Due to the influence of the present magnetic field on (Figure 2.2B), the speed of replication sped up, apparent in the beginning hours of exposure. After that, the bacteria shows that it adapts to the magnetic

environment present and alters its replication cycle accordingly [5, 38]. The data demonstrates a correlation between the magnetic field present and the morphology changes in bacteria [21].

CHAPTER 3: THERMODYNAMICS

Knowing the growth rate, the bacterium will take with our phase knowledge, we have the general idea of the lifetime of the system, with all the elements taken into consideration. Treating this experiment from a quantum experiment, we can view the process as a Hamiltonian, knowing our original result without any perturbation, and using previous research gives us a sense of what to expect with an induced magnetic field applied to the system, and treating that as our first perturbation to the system. As for my research, I'd like add yet another perturbation to the system to see what kind of effects the temperature variance plays into the growth rates of the bacterium [34]. Due to the effects of constant heat having an effect on growth and inhibition rates [24], I'd like to see how adding the magnetic field would vary the growth rates of the bacterium.

3.1 - Temperature Influence

Prokaryotic cells, namely bacteria, have the ability to adapt to its environment, and each strain actively searches constantly for the environmental conditions where the amount of bacterial growth will be at its peak, and through this evolution, various strains have found their sweet spot at critical points. Differing arrays of bacterial cells are capable of growth at various temperature conditions, ranging from extreme to extreme, aptly going by extremophiles. These microbial cells can grow at high or low pH levels, freezing or boiling temperatures. With the initial conditions known for the peak growth point for any bacterium under inspection, any variations applied to the environment will have a distinct effect on the growth patterns of the cells.

Temperature does affect the function of the enzymes, so the doubling rate of microbial cells grows along with the increase of temperature. Remarkably, the relationship between the maximum growth temperature and the growth rate constant k (number of generations per hour) obeys the Arrhenius equation for simple chemical reactions:

$$\log k = C/T \tag{3.1}$$

Where T is the absolute temperature in kelvins (K), and C is a second constant that combines the gas constant and the average activation energy of cellular reactions [40]. By calculating the Arrhenius equation at two differing points, the constant C can be evaluated using equation 3.1 to reach the result:

$$\log (k_2/k_1) = C/(T_1/T_2) \tag{3.2}$$

Which thereby proves that the growth rate in microbial cells will generally double for every 10° C, but the effect does have its bounds, like all systems do. Plasma membranes are manipulated through the effects of temperature and react according to the extrema they approach; higher temperatures cause the proteins and enzymes in bacterial cells to denature, while lower temperatures decrease membrane fluidity and limit the conformational mobility of enzymes, thereby lowering their activities [40].

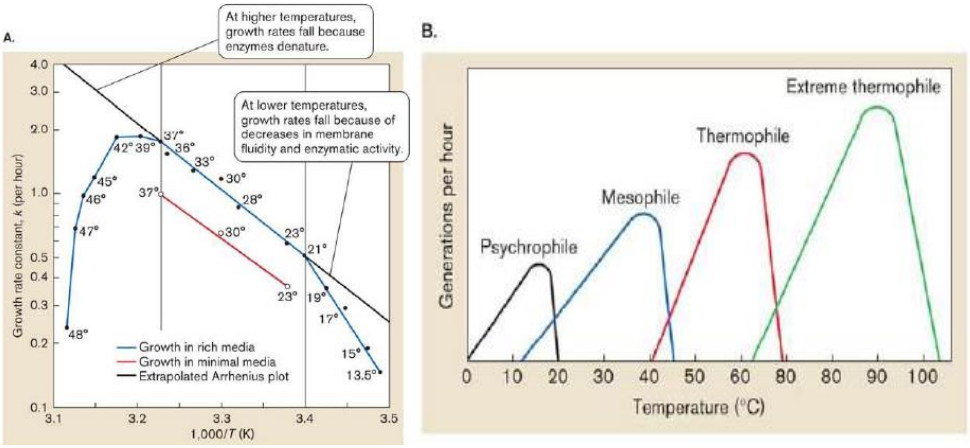


Figure 3.1: A. Growth rate constant versus temperature. B. Temperature ranges for a variety of microbial cells [40]

Each bacterium has their operating range and their peak growth temperature, so noting their range will influence the behavior of the enzyme interactions within cells, so cellular proteins allow bacteria to operate within a set tolerance range, or else they begin to break down in either temperature direction. Looking at (Figure 3.1A), we see the Arrhenius

effect taking place in an *E. coli* culture, showing the linear relationship between the growth rate in bacteria and the temperature levels, and the crash of the growth rates once going past the ranges the proteins and cellular membrane can handle.

Though each bacterial cell has the optimal range it can operate, there's an abundance of bacterium that can handle a myriad of environments, capable of living in temperatures as cold as 0° C or as hot as 100° C, and seeing (Figure 3.1B), these types of cells are all categorized by their tolerance levels. *E. coli* and *S. aureus* are both mesophiles, which typically operate within a range of 20° C to 40° C, whereas psychrophiles grow at low temperatures and thermophiles and extreme thermophiles grow at much higher temperatures in comparison to mesophiles. Referring to the graph, overlap between the groups occurs, demonstrating the Arrhenius effect leaking from one group of microbes to another, validating when one group begins to break down outside its normal limits, another group of microbes can thrive.

The bacterium does have a last saving grace when approaching an inhospitable environment, allowing bacteria to temporarily acclimate to their environment in order to survive resulting in the heat- shock response. Rapid temperature changes experienced during growth activate batches of stress response genes, and the protein products of these heat-activated genes include chaperones that maintain protein shape and enzymes that change membrane lipid composition [40]. The response to the sudden temperature change in bacteria was discovered by Yamamori and Yura in 1982 in E. coli bacteria and it has been observed to hold true in all bacterium.

Bacterium must also be grown in a pressure within its bounds, or else it will have a negative effect on its growth rate. Pressures in barosensitive bacteria, like E. coli, must be kept in pressures lower than 60 MPA or it will slow its growth rate and begin to die off, so the membrane of the bacterial cells must be able to handle the environment it is in.

In addition to the pressure and temperature conditions, pH levels also play a role in the viability of bacterial cell growth and how well it is capable of thriving in a system. The varying amounts of hydrogen ions (H^+), specifically hydronium (H_3O^+) and hydroxyl ions (OH^-) ions will inflict an effect on growth pattern on bacteria in a solution. Although cells can live in a plethora of environments, like the effect on the critical temperatures had on cells, too much or too little pH levels will hurt the growth and inevitably kill any bacteria populations. Because H^+ concentration affects the protonation of these ionizable groups, altering pH can alter the charges on these groups, which in turn changes protein structure and activity [40].

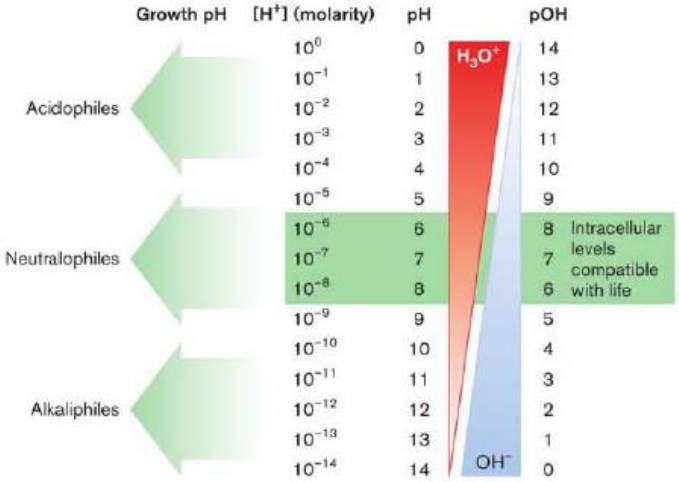


Figure 3.2: Organisms grouped by optimum growth pH [40]

Though temperature causes changes to the enzymes inside of the bacterial cell, the extracellular and intracellular fluid will keep different pH concentrations, extracellular media will be relative to the environment, whereas intracellular pH levels will remain the same to the structure of the cell. Due to homeostatic mechanisms they carry, therefore the intracellular pH will not have a pronounced change inside of the bacterium.

The plasma membrane is quite impermeable in regards to allowing protons to pass through the surface, which allows it to retain the pH inside the cell but can allow it under

set conditions. In general, the disparity between the internal and external pH can be quite different, but if the difference becomes too high, then protons may find their way in either directly or through the proteins on the surface of the membrane, and if the internal pH changes too much can cause problems to the cell. The cells can thrive in several different pH ranges due to the need for survival in different environments, and via (Figure 3.2), we can be differentiate any cells by their respective category: neutralophiles, acidophiles, and alkaliphiles.

Acidophiles are organisms that lean towards an acidic pH environment from pH 0 to pH 5 and have intracellular fluid that has a higher pH than its environment conversely, alkaliphiles go to a basic pH environment from pH 9 to pH 11. Neutralophiles, which is our focus as it includes both E. coli and S. aureus, grow within pH 5 and pH 8, and typically adjust their metabolism to maintain an internal pH slightly above neutrality, which is where their enzymes work best, and they maintain this pH even in the presence of moderately acidic or basic external environments [40].

T (°C)	K_W (mol^2 dm^6)	pH
0	0.114×10^{14}	7.47
25	1.008×10^{14}	7.00
50	5.476×10^{14}	6.63
100	51.3×10^{14}	6.14

	0°C	25°C	50°C
Acid	2.01	2.00	2.00
Neutral (Water)	7.47	7.00	6.63
Basic	13.80	12.83	12.15

Table 3.1: A. Water ionization constant versus temperature. B. pH variance for solutions at different temperatures [47]

The bacterium typically try to maintain a pH difference of about +/-0.5 pH along the limits the cell can handle so that it may acclimate to their environment while maintaining the integrity of the plasma membrane intact. As the temperature effects the cell and pH remains distinctly different from the intracellular and extracellular fluid pH, and yet the temperature variances does affect the pH level. Noting the temperature variances, the molecular vibrations increase in the pH differences, resulting in water to ionize hydrogen

ions, resulting in the pH drop. With the change in temperature affecting the pH level, we can see in (Table 3.1A) that testing the changes in temperature affects the water ionization constant, or K_W, and the pH levels. The constant will grow inversely to the lowering of the pH as the water exchanges protons and ionizes itself.

Since water is a neutral solution, verifying the effects of the inversely proportional relationship temperature and pH carries, (Table 3.1B) proves how prevalent the effect may carry based on how high the initial conditions of the pH is and where it ranks as temperature goes up. Acidic solutions do not carry a pronounced effect as the pH level is already quite low, so higher temperatures does not affect it much. A neutral solution like water uses the same data as (Table 3.3A), showing there is a perturbation to the pH level much more apparent than the acidic solution values. Basic solutions on the other hand has a relationship with the change in temperature; with rising temperatures, there is a clear correlation of the inverse relationship the two carry, demonstrating that the temperature influence has the most influential effect on basic solutions. From this data, we can conclude that pH levels decrease when temperature heightens, so we can assume the temperature and pH level variances in the data will have an effect on both the *E. coli* and *S. aureus* cultures.

3.2 - Temperature Gradient

Microbiological organisms like bacteria are able to live in a wide array of environments, but each have their preferred conditions in which bacteria tend to approach, temperature being a key factor in deciding how the organism will react. When swimming in a chemical (or thermal) gradient, bacteria continuously detect changes in the concentration of chemicals (or temperature) [8]. The reaction the bacteria are acting upon, choosing a better place in the media within seconds to grow the population is defined as chemotaxis or thermotaxis (also referred to as thermophoresis, the Soret effect, or thermodiffusion),

depending on what type of change in environment the bacteria sense. Bacteria will consistently search for an improvement in the environment, but if too minimal a change, then the bacteria will not respond accordingly. In the case of temperature for example, when bacteria are exposed to a temperature gradient with steepness < 0.02°C/μm that extends over short distances (~100 μm), no response or directed migration is observed [8].

The effects of thermodiffusion and chemotaxis on (Figure 3.3) notes the interactions on bacterial populations and stacked upon one, allowing us to differentiate how pronounced the reactions may be.

The temperature gradient impacted the environment using an infrared laser with wavelength λ=1480 nm and heated up the media from the room temperature of ~24°C, and the center section was heated by the laser to about ~27°C, which caused the gradient to be around 0.02°C/μm as the shallow gradient and made took the rest of the data under ~34°C.

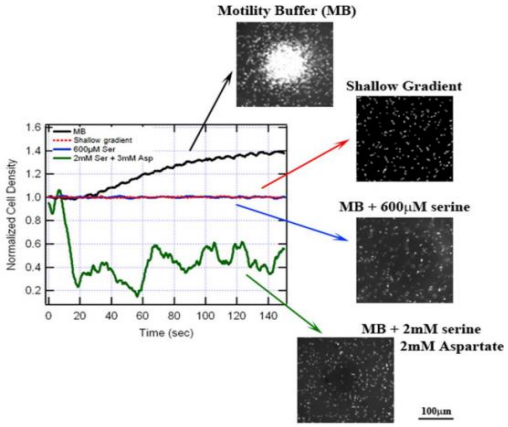


Figure 3.3 Effect of chemical environment and thermal gradient [8]

When bacteria are exposed to the motility buffer, the bacteria accumulate towards the heated point, experiencing a thermotaxis effect on the populations. In the shallow temperature gradient, the thermotaxis effect is not noted due to the low threshold on the gradient influencing the bacteria field, so they have no reason to populate the shallow

field, same as the motility buffer + 600μM serine. This differs in the motility buffer + 2mM serine and 3mM aspartate field as the cell population density disperses from the focal point of the heated region proving that the opposite of thermotaxis also holds true. We describe thermodiffusion, the molecule drift along temperature gradients, in liquids with a general, microscopic theory [43]. While the migration pattern of bacteria can be influenced by the temperature and chemical gradients, temperature also is capable of raising the rate of enzymatic activity, biochemical reactions, proteins' conformation and binding affinity, as well as the viscosity and pH of the environment [36]. Chemically speaking, chemotaxis also may affect enzymatic activity and also change the pH of the environment. Motile bacteria like Escherichia coli rely on the flagella to get around, and the influence of thermotaxis and chemotaxis continues to present and shape its actions. The operation of the flagella is dependent upon the flux of protons across the membrane. To maintain this flux, the cell needs to sustain a pH difference between the interior and exterior of the cell, which in turn requires energy [8], emphasizing the importance of the temperature and chemical gradients upon the environment of a bacterial organism.

CHAPTER 4: EXPERIMENTAL RESULTS

For the examination of this thesis, the procedure viewed the bacterial growth at various temperatures in a both a fixed and oscillating magnetic field and compared the results with the bacteria grown under controlled conditions with no magnetic field present. The experiment was repeated with *Escherichia coli* and *Staphylococcus aureus*, comparing the results between Gram-positive and Gram-negative bacterium and applying both a set and fluctuating magnetic field. It has been pointed out (Pavlovich 1971) that cells may contain magnetic structures, so that their enzyme structures and RNA systems may well be influenced by applied magnetic fields. If this is so, it is clearly probable that the growth rate, mutation frequency, spore germination rate and other parameters of cells will be affected by exposure to magnetic fields [25]. While previous research has proven a correlation between bacteria growing under the effect of a magnetic field present, I continued my research while presenting my data with a thermal conductivity aspect added to my theory.

4.1 - Experimental Method

The procedure for this thesis was carried out through microbiology techniques and shaped to use physics ideas to produce the results. Both LB agar plates and TSB (Tryptic Soy Broth) were prepared prior to the inoculation of the bacteria. Once the preparation was finished, in order to prevent any contamination, the media was autoclaved (Figure 4.1).

Figure 4.1: Autoclave used to disinfect materials

To grow our mother culture for the bacteria, LB agar plates and fresh medium for bacterial growth were required; using dehydrated *E. coli* and *S. aureus* and a rehydrating medium, the bacterium was then mixed and vortexed, and allowed time to populate the tubes with freshly grown bacteria. Once sufficiently blended, the solutions with the rehydrated bacteria had a small amount of solution transferred to a plate of solid LB agar and gave the plate time to grow bacterial colonies.

After enough time elapsed, a single colony of bacteria was isolated from the plate and streaked onto a new plate. From the defined colonies that were formed from the growth on the plate, using a loop, a single colony from each plate that showed good colony growth was removed into a tube of TSB and a repeated for several tubes in order to allow for a selection with the best possible growth per tube and choose a tube in which to inoculate bacteria from.

Figure 4.2: E. coli and S. aureus cultures

Via (Figure 4.2), the agar plates with the respective bacteria shows the streaks with lines populated with bacterial growth along their quadrants; several bacterial populations were grown from these to derive what would allow for the best growth of the cells. Once the best tube was chosen, the bacteria solution would be used as the mother culture, from which the next generation of bacteria would be grown; using a micropipette, both the *E. coli* and *S. aureus* mother cultures would then populate a small amount of media to autoclaved TSB broth tubes, used in preparation for the initial inoculation of rehydrated bacteria.

Due to the doubling time factor difference in the two bacterium, various concentrations of initial inoculations were used to grow the tubes. For *E. coli*, the initial inoculation was 25 μL, whereas for *S. aureus* used an initial inoculation of 50 μL. For the sake of both bacterial growth rates and time spent, various levels of concentrations of both bacteria were observed, and a good growth rate without the bacteria hitting the stationary phase too quickly or not enough growth inside the tubes, these points in the initial inoculation were best served to reach a population boom while keeping both in a steady growth rate.

Figure 4.3: Incubators used for experiment, A) G24 ENVIRONMENTAL INCUBATOR SHAKER B) V. I. P. CO. INCUBATOR 417

Once the few tubes were inoculated from the mother colony tube, they were all vortexed and labeled to the temperature orientation they would be set to grow for 12 hours using two incubators (Figure 4.3), and were left to grow under three temperatures, in 24° C, 30.5° C, and 37° C. As a redundancy check, after all the tubes were inoculated and vortexed, they would be placed in the G24 incubator for ten minutes without a temperature influence. Since the G24 incubator (Figure 4.3A) was an older model than what is currently available, there was more room for error in the temperature variation in the incubation period. The G24 would be sensitive to any interaction, so the temperature this group was grown under has an error margin of 30.5 ± 0.2° C, assuming for a general consensus of 30.5 ° C. The V. I. P. 417 incubator (Figure 4.3B) had a stable temperature and was better suited to keeping a constant temperature, so that one had no issues to its structure.

After the 12 hour incubation period, the best sample of the group in each respective temperature was taken out and used to inoculate autoclaved TSB tubes and placed under a specific magnetic field. The magnetic orientations used in this experiment are a static field of with a field strength of 9 Gauss and an oscillating magnetic field with 50 Hz with

5 Gauss (Figure 4.4A). It is well known that the effects of ELF-EMF generally depend on both physical and biological parameters, including field signal characteristics (frequency, amplitude, wave shape, etc.), duration of exposure, cell metabolic state, genotype, and how long cells are allowed to grow before, during, and after exposure [39]. The power supply and programmable wave function generator powered the Helmholtz coils (Figure 4.4B) to create the magnetic field the bacterium would be exposed to.

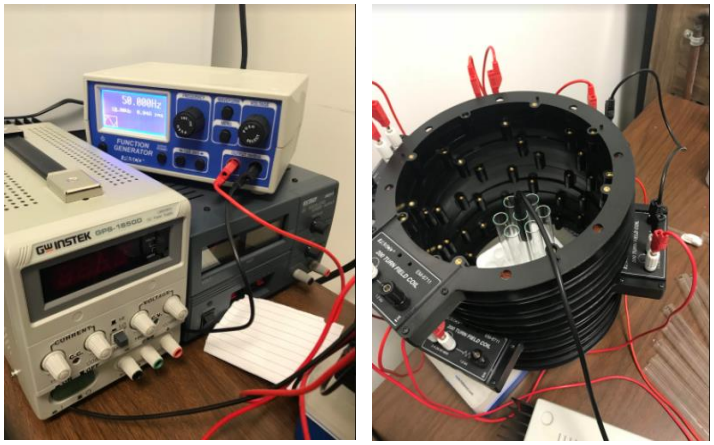


Figure 4.4: A. Power Output and Wave Function Generator B. Overhead view of magnetic coils used in experiment

While the experiment was conducted in room temperature, the coils experiencing a static magnetic field had heat dissipation of 1-1.5° C. Due to the heat caused by the coils, some influence can be said had effect on the growth of the bacterium, regardless of how small the perturbation to the system was. Tis issue was not fixed on time, so the data for static magnetic field can be considered to have been grown in 24-25° C rather than the 24° C the rest of the bacteria was grown under. The bacterium not exposed to a magnetic field would be set outside the range of any present magnetic field influence and allowed to grow elsewhere, whereas the bacteria exposed to a magnetic field would be placed in the series of Helmholtz coils. Through the course of the experiment, nine configurations of the experiment would take place simultaneously. The bacteria have been grown under the

three different temperatures and exposed to the three magnetic field circumstances, yielding various results based upon the growth conditions before the initial inoculation.

Figure 4.5: Spectrophotometer used to take readings

In order to check the growth of the bacteria populations in the tubes, a spectrophotometer (Figure 4.6A) was used. The spectrophotometer used optical density (OD) under a wavelength of 600 nm and was consistently calibrated with a blank from the autoclaved TSB tubes. Due to the marks and scratches that inserting the tubes repeatedly would cause, the blanks would be replaced with every round of TSB tube made, each tube was wiped thoroughly and checked to see what initial absorbance reading it would give; while no bacteria would be present a false absorbance reading would read out, thus presenting a scratch influencing the real absorbance of a tube with bacterium present.

Every TSB tube with an absorbance over the concentration of 0.015 would be set aside to not be used as the initial reading of a tube with bacteria present would be influenced. The tubes would be calibrated against each other using as a reference, so a negative tube of absorbance of -0.002 for example, would be clear, so that would be the new standard and the tubes would be subject to comparison for a true reading of the concentration on tubes with bacteria present.

Once the spectrophotometer was calibrated using a blank TSB tube with an absorbance near 0.000 relative to the other tubes, experiment (Figure 4.6B) would take place under the wavelength of λ=600nm. The tubes grown under a control would be subject to either no magnetic field, a static magnetic field (SF), or an oscillating magnetic field (OF), and each configuration would have tubes of bacteria that was grown under the three temperature variations of 24° C, 30.5° C, or 37° C.

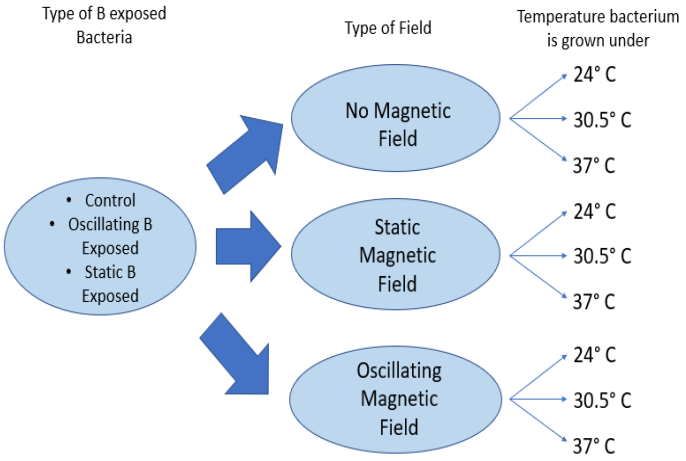


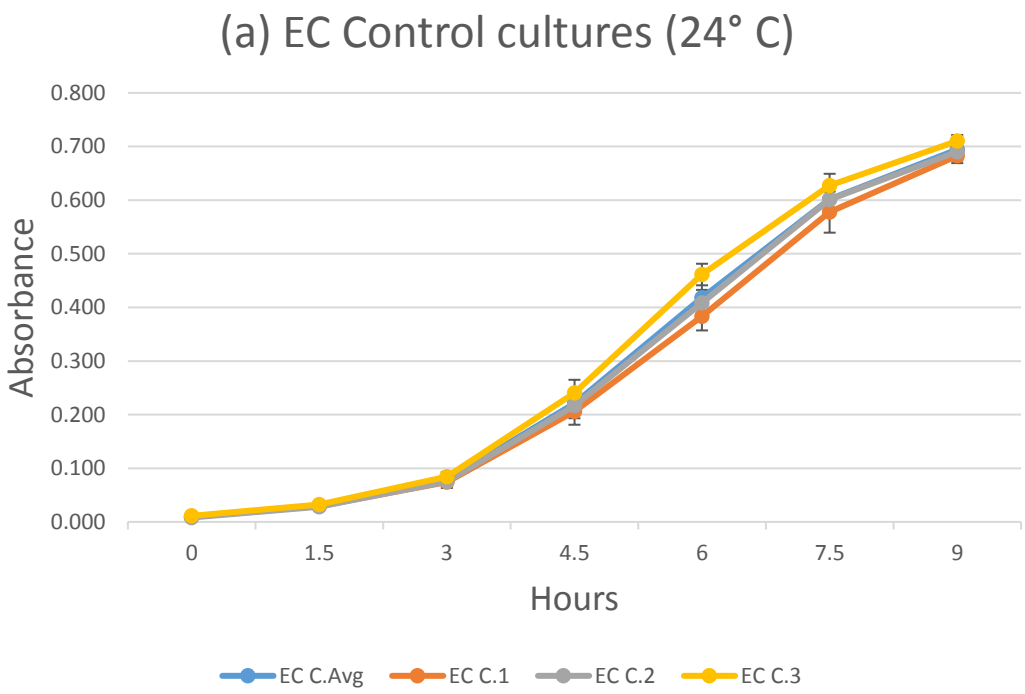
Figure 4.6: Flowchart of experimental methodology for bacterial growth.

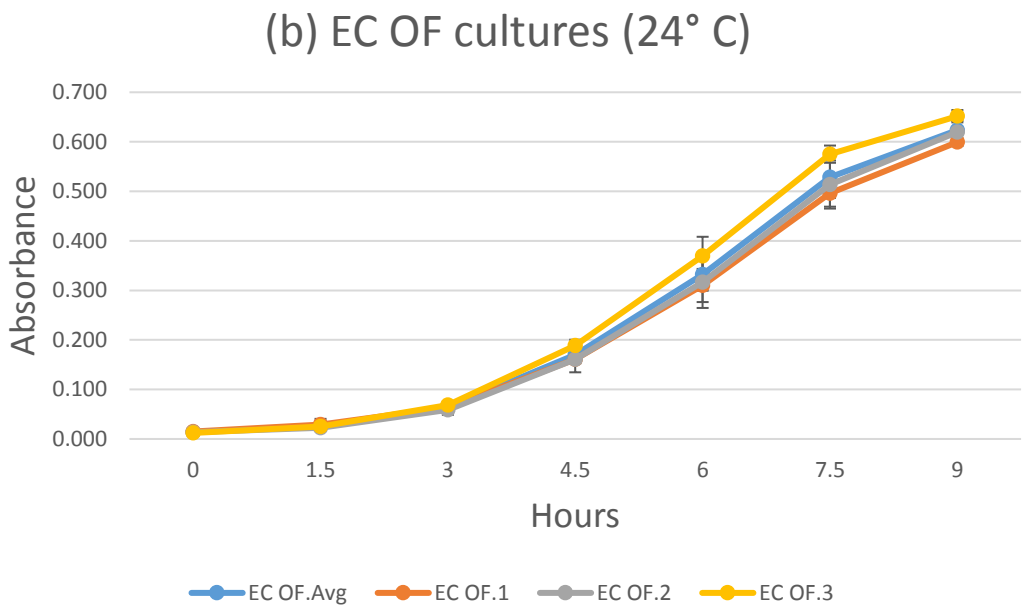
This method was repeated using only control bacteria, then repeated for bacteria exposed to a static magnetic field and bacteria exposed to an oscillating magnetic field. Each type of magnetic field exposed bacteria had 6 tubes in each temperature and each configuration for *Escherichia coli*, yielding 54 data sets for each type of magnetic field exposed, resulting in 162 data sets, and, due to the temperature influence affecting the growth of *Staphylococcus aureus*, only two temperatures, 30.5° C and 37° C were used, so 108 data sets were produced. In order to consolidate all the data, averages of the results were made to easier ascertain the examination of this procedure and thus analyze the outcome.

4.2 – Results

To perform this experiment in a consistent and unified manner, the data presented has some points to note. It was shown that magnetic fields can affect biological functions of organisms by changes of the concentration of hormones, by changes of the activity of enzymes or of the transport of ions by cell membranes, by changes in the synthesis or transcription of DNA [32]. The lines of growth patterns are illustrated below with different colors and noting the pattern is useful; the absorbance of the OD is graphed against time and grouped by temperature and previous growth pattern based upon the magnetic mode it was exposed to when inoculated. Control is the bacteria not exposed to a magnetic field, OF (Oscillating Field) is the bacteria that was exposed to the oscillating magnetic field and then inoculated into new media and passed through the experiment, and SF (Static Field) is the bacteria exposed to a static field prior to inoculation for further analysis. The graphs are then under a classification of bacterium (type of growth condition).orientation.

For example, the bacterium will fall under either *Escherichia coli* or *Staphylococcus aureus*, the type of growth condition will differ as either C (Control), OF (Oscillating Field), or SF (Static Field) as far as what conditions the bacteria grew under, and the orientation will have 1, 2, or 3, stating the type of field the bacteria was grown under. 1 represents bacteria grown under no magnetic field, 2 being bacteria placed in an oscillating field, and 3 serving as bacteria that was grown under a present static magnetic field.


(a) EC Control cultures (24° C)
Absorbance
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
1.5
3
4.5
6
7.5
9
Hours
EC C.Avg
EC C.1
EC C.2
EC C.3


(b) EC OF cultures (24° C)
Absorbance
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
1.5
3
4.5
6
7.5
9
Hours
EC OF.Avg
EC OF.1
EC OF.2
EC OF.3

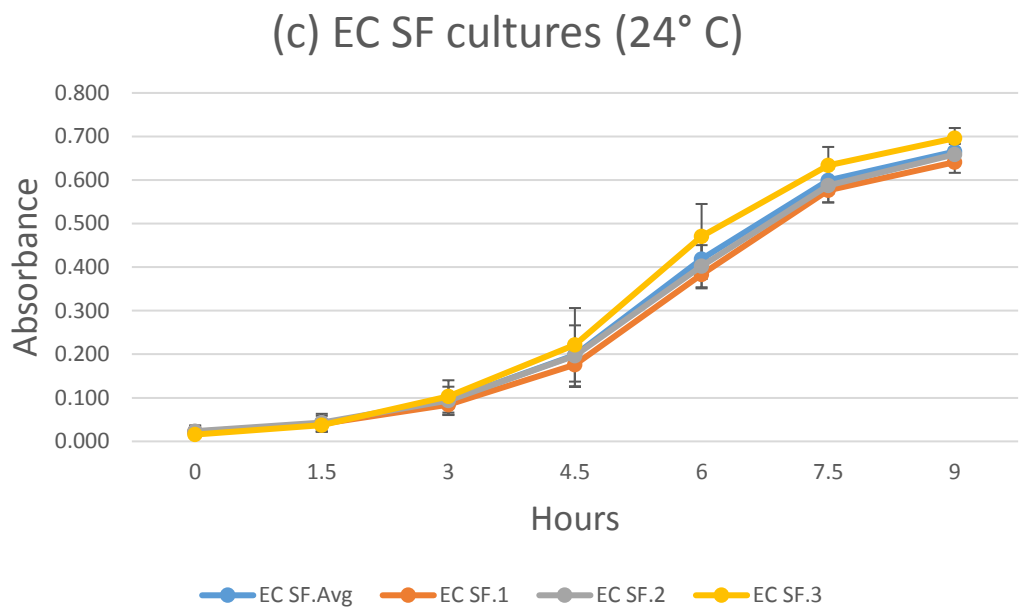


Figure 4.7: E.coli Cultures at 24° C (a) control (b) E.coli exposed to oscillating field (c) E.coli exposed to static field. Three samples of each conditions are used and then the corresponding average value is given in blue, just for comparison.

Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
EC C.Avg	0.010	EC OF.Avg	0.014	EC SF.Avg	0.020
EC C.1	0.009	EC OF.1	0.015	EC SF.1	0.022
EC C.2	0.008	EC OF.2	0.014	EC SF.2	0.024
EC C.3	0.011	EC OF.3	0.012	EC SF.3	0.016

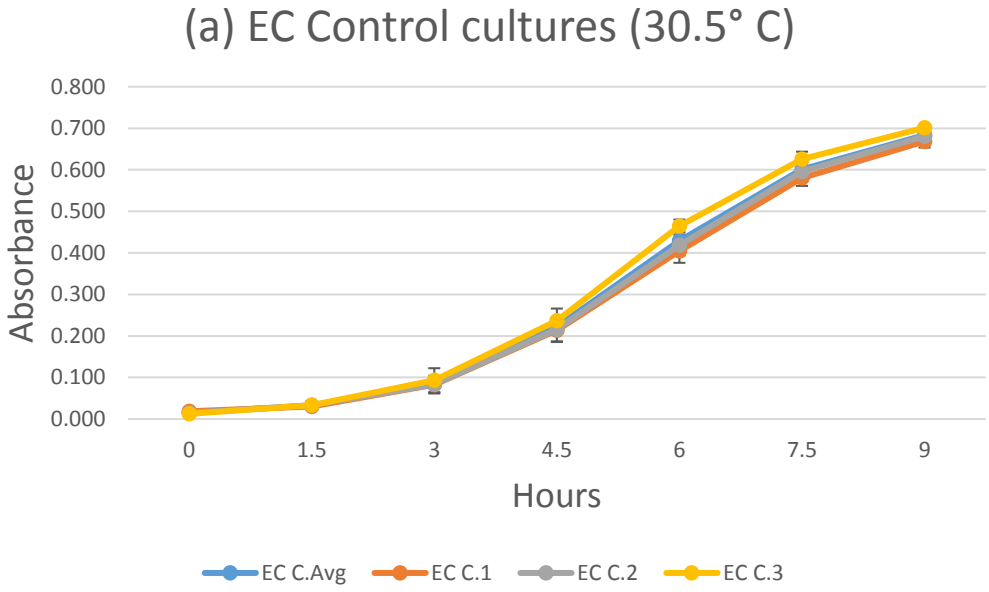
Table 4.1: Initial Values of EC (24°)

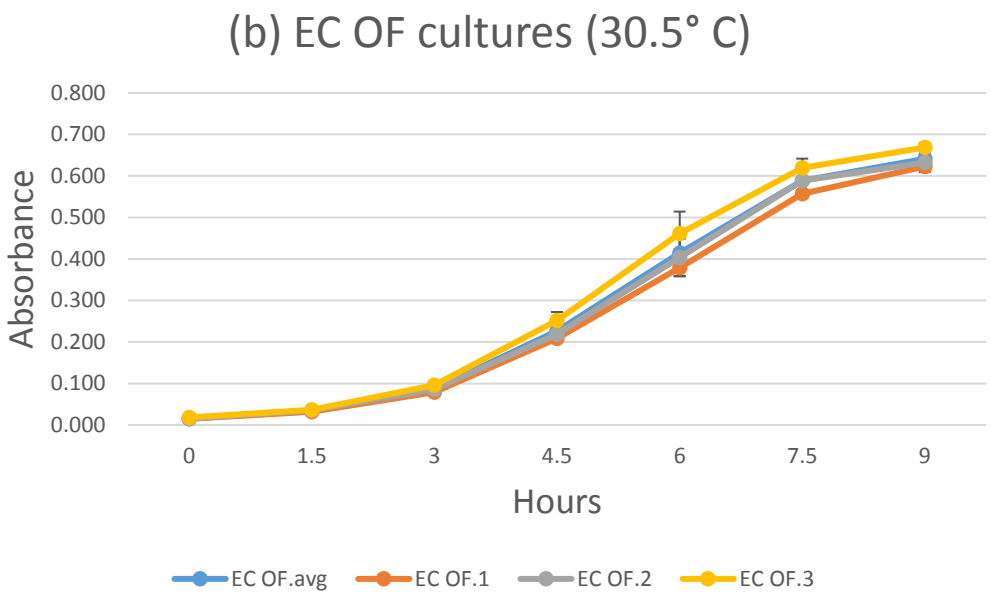
Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
EC C.Avg	0.694	EC OF.Avg	0.624	EC SF.Avg	0.665
EC C.1	0.683	EC OF.1	0.599	EC SF.1	0.641
EC C.2	0.690	EC OF.2	0.620	EC SF.2	0.659
EC C.3	0.710	EC OF.3	0.652	EC SF.3	0.696

Table 4.2: Final Values of EC (24°)

In this run, the *E. coli* is grown under 24° C and the bacteria grown under control conditions grows more than the OF and SF magnetically treated bacteria, suggesting that

the effect of a preexisting magnetic field bacteria and inoculation into new media yields lower growth rates on a second generation. Consistent upon previous experimental runs, bacteria grown under a magnetic field does grow at a higher rate and produce a higher optical density on the spectrophotometer, and but when previously treated under a magnetic field, the cultures final optical density varies with control conditions, yielding a question of whether the magnetic field either encourages or discourages bacterial growth. This effect is due to the magnetically treated bacteria reach the stationary phase quicker, producing a high optical density and then decreasing its growth rate afterwards. Based upon (Table 4.1), both bacteria treated with a magnetic field have an optical density greater than that of its control counterpart on the initial readings, but the final values (Table 4.2) seem that the bacteria that did not get treated with an initial magnetic field seem to pass its magnetically treated bacteria analogue, suggesting an effect on the growth on future bacterial generations.





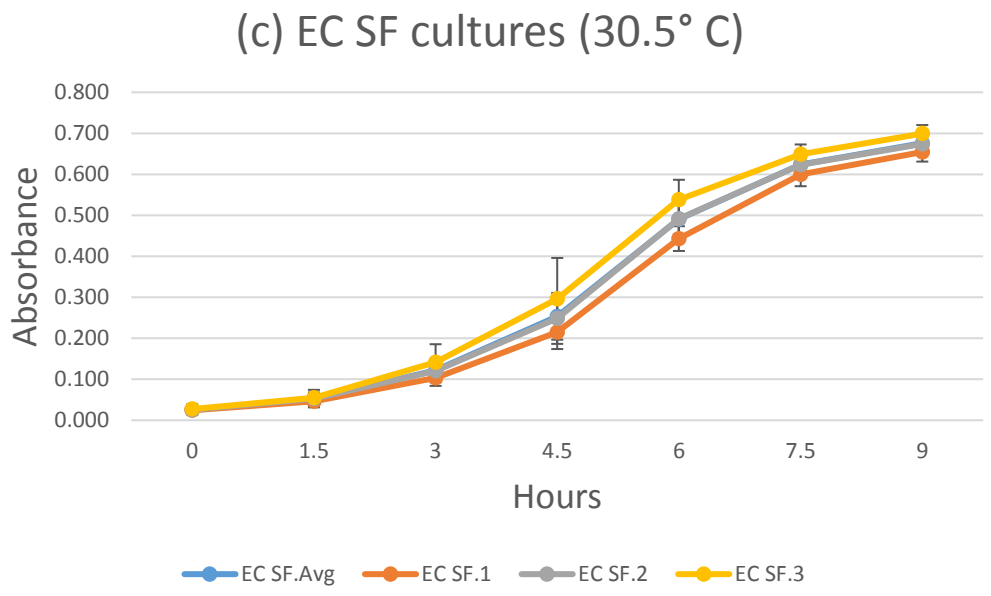


Figure 4.8: EC Cultures at 30.5° C (a) control (b) E.coli exposed to oscillating field (c) E.coli exposed to static field. Three samples of each conditions are used and then the corresponding average value is given in blue, just for comparison.

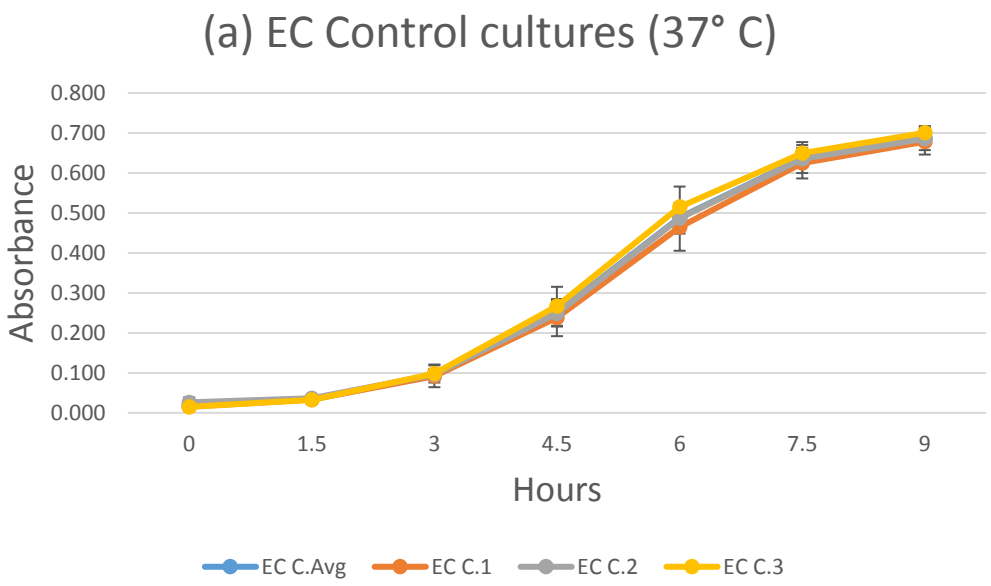
Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
EC C.Avg	0.015	EC OF.avg	0.016	EC SF.Avg	0.026
EC C.1	0.019	EC OF.1	0.015	EC SF.1	0.024
EC C.2	0.015	EC OF.2	0.015	EC SF.2	0.026
EC C.3	0.013	EC OF.3	0.018	EC SF.3	0.027

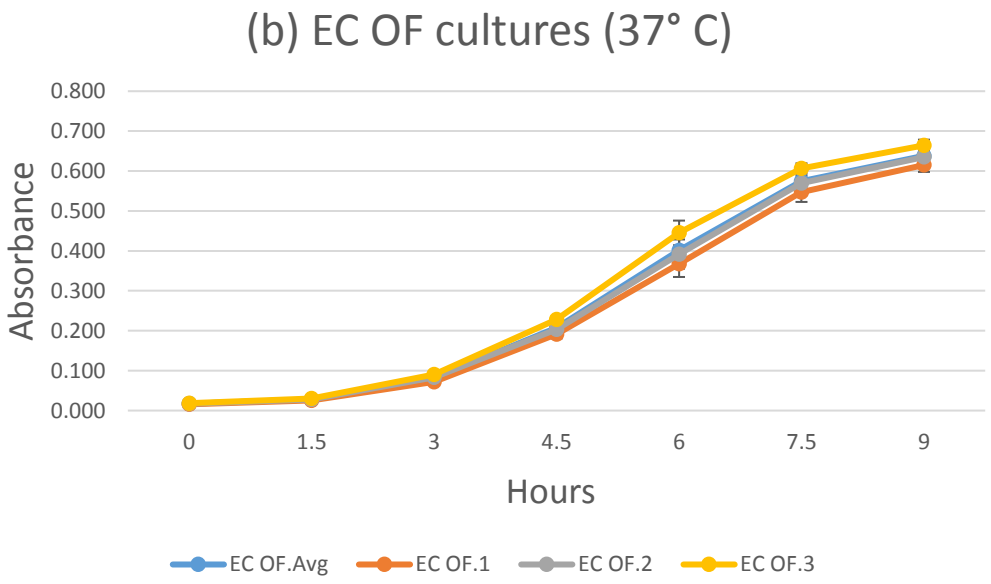
Table 4.3: Initial Values of EC (30.5°)

Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
EC C.Avg	0.683	EC OF.avg	0.641	EC SF.Avg	0.676
EC C.1	0.668	EC OF.1	0.623	EC SF.1	0.654
EC C.2	0.681	EC OF.2	0.632	EC SF.2	0.674
EC C.3	0.701	EC OF.3	0.669	EC SF.3	0.699

Table 4.4: Final Values of EC (30.5°)

These cultures were grown under 30.5° and proved to have greater initial optical density (Table 4.3), observing the enzymatic processes taking place across the bacterial membrane, thereby producing higher growth of the bacterial populations. With the greater temperature effect upon the inoculation of the bacteria, the process still yielded high absorbance quantities in comparison with the control bacteria and the magnetically treated bacteria in the final readings (Table 4.4). A significant deviation is present on the SF cultures, producing the expected effect, while the OF cultures still have a preference for growth on the static configuration, still retaining the OF bacteria as the data set with the smallest amount of optical density at the end of the nine-hour period due to the weaker magnetic field influence. While the control bacteria continue to have the greatest final optical density of the three types of exposed bacterium, the orientation which still boasts the highest numbers is the SF configuration.


(a) EC Control cultures (37° C)
Absorbance
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
1.5
3
4.5
6
7.5
9
Hours
EC C.Avg
EC C.1
EC C.2
EC C.3


(b) EC OF cultures (37° C)
Absorbance
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
1.5
3
4.5
6
7.5
9
Hours
EC OF.Avg
EC OF.1
EC OF.2
EC OF.3

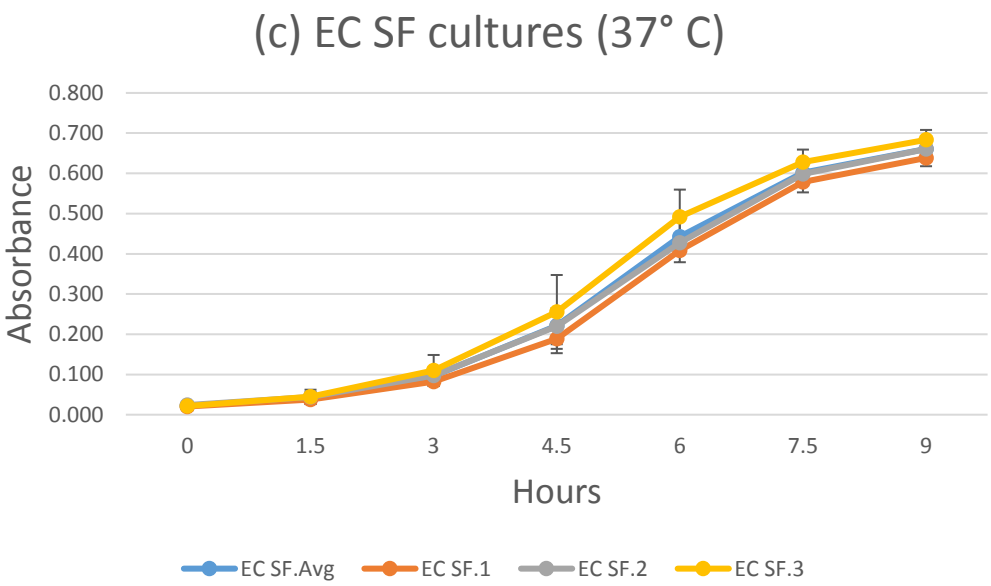


Figure 4.9: EC Cultures at 37° C (a) control (b) E.coli exposed to oscillating field (c) E.coli exposed to static field. Three samples of each conditions are used and then the corresponding average value is given in blue, just for comparison.

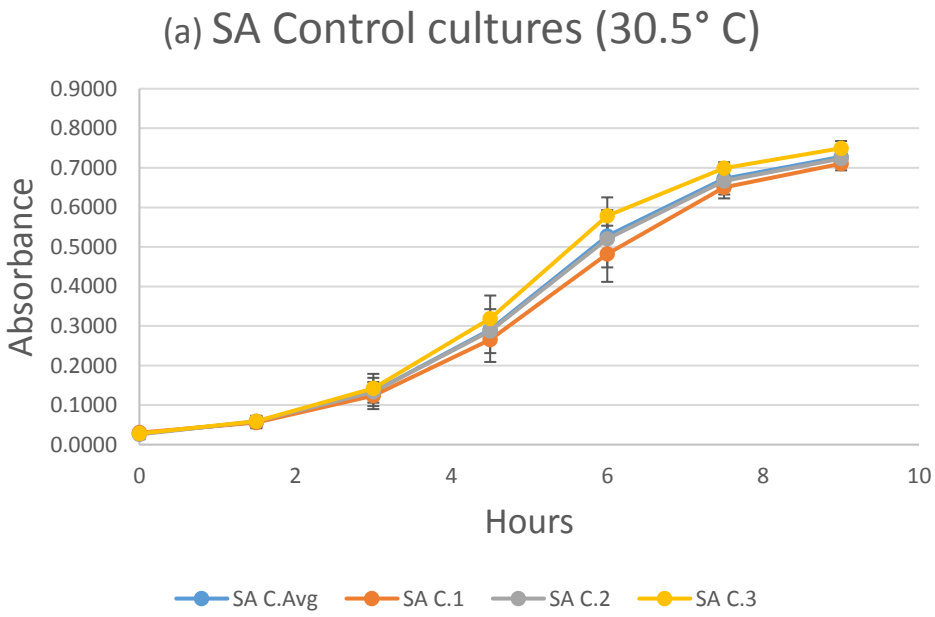
Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
EC C.Avg	0.019	EC OF.Avg	0.017	EC SF.Avg	0.022
EC C.1	0.015	EC OF.1	0.016	EC SF.1	0.020
EC C.2	0.026	EC OF.2	0.017	EC SF.2	0.024
EC C.3	0.015	EC OF.3	0.019	EC SF.3	0.022

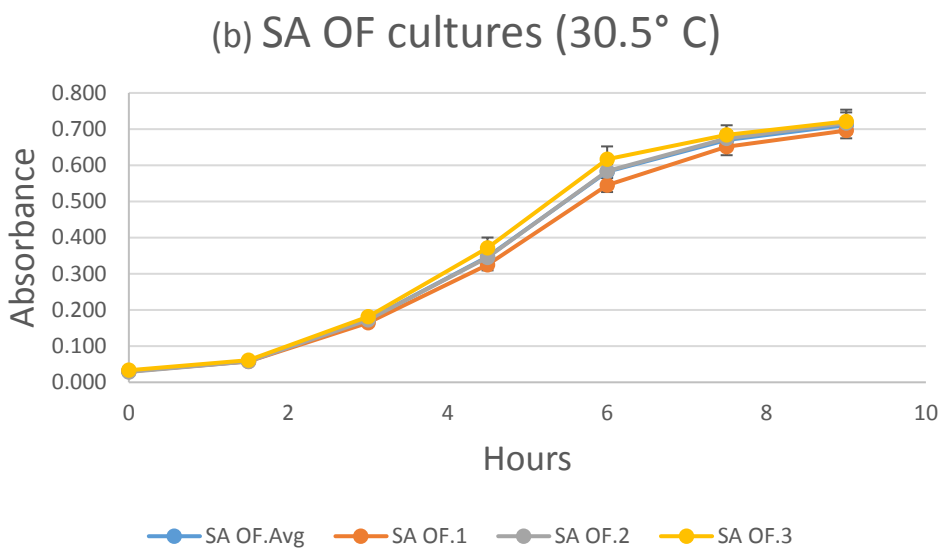
Table 4.5: Initial Values of EC (37°)

Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
EC C.Avg	0.688	EC OF.Avg	0.638	EC SF.Avg	0.661
EC C.1	0.678	EC OF.1	0.615	EC SF.1	0.638
EC C.2	0.686	EC OF.2	0.635	EC SF.2	0.660
EC C.3	0.700	EC OF.3	0.664	EC SF.3	0.683

Table 4.6: Final Values of EC (37°)

While C.# initial values consistently give the lowest optical density, on these runs it has the highest due to a high OD average outlier which influenced C.2, garnering EC C.Avg to have a higher initial optical density versus the norm. The control cultures growth data remained tight, perhaps due to EC C.2 rising the average of the data lines and aligning the .1 configuration data along with the .2 and .3 configurations. The OF and SF cultures have seemingly overlapping optical density readings with smaller deviation than the normal, colluding the temperature effects with the magnetic field, suggesting one effect may overpower the other. Bacteria initially grown outside of a magnetic field continues to have the highest final OD while still underperforming when we track its progress versus bacteria exposed to a magnetic field, suggesting that the morphology of the bacteria, while not necessarily proven in this experiment, may be affected by the magnetic field possibly influences the rate at which bacteria populations increase.





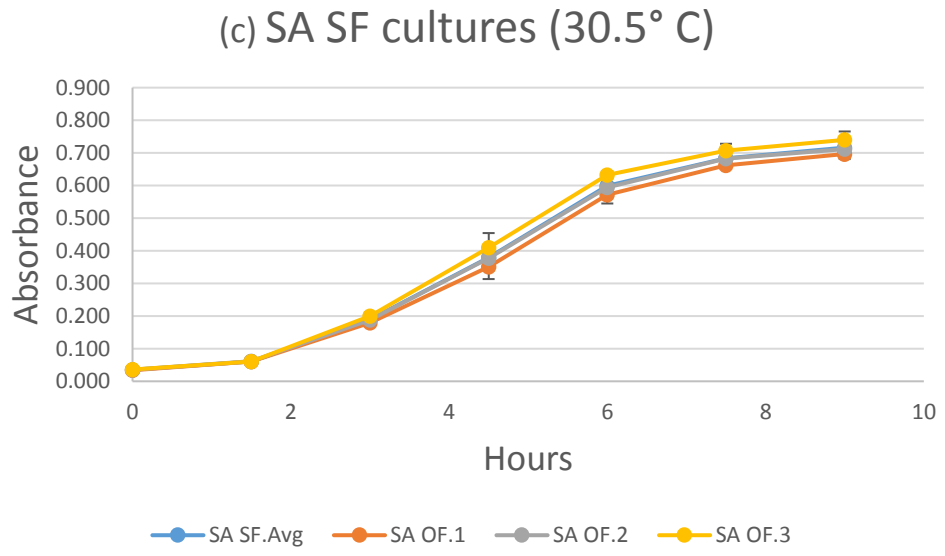


Figure 4.10: S. aureus Cultures at 30.5° C (a) control (b) S. aureus exposed to oscillating field (c) S. aureus exposed to static field. Three samples of each conditions are used and then the corresponding average value is given in blue, just for comparison.

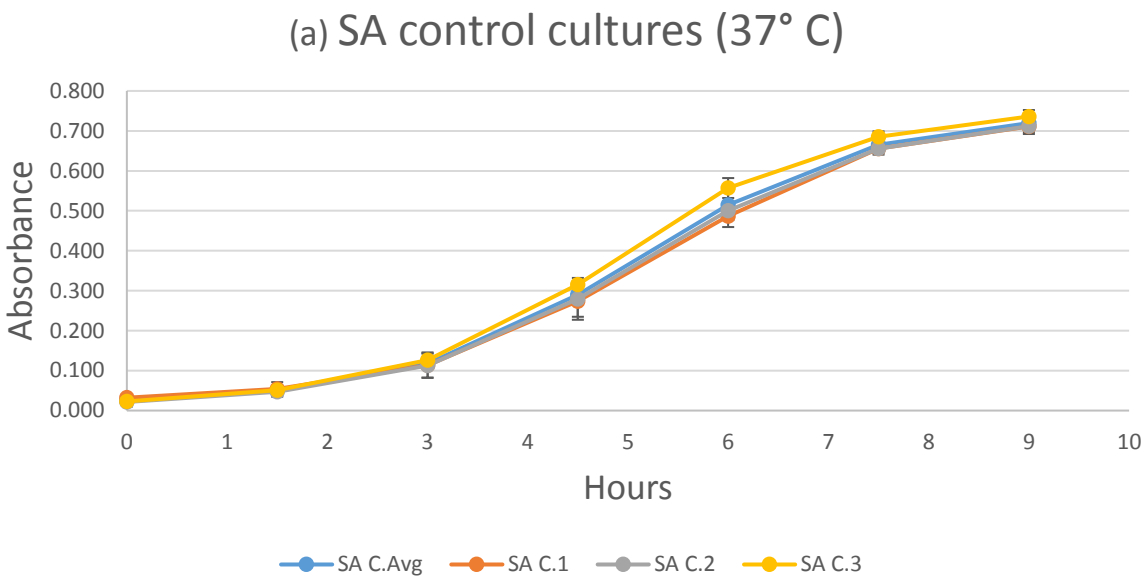
Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
SA C.Avg	0.028	SA OF.Avg	0.031	SA SF.Avg	0.035
SA C.1	0.030	SA OF.1	0.030	SA OF.1	0.034
SA C.2	0.026	SA OF.2	0.029	SA OF.2	0.036
SA C.3	0.028	SA OF.3	0.033	SA OF.3	0.036

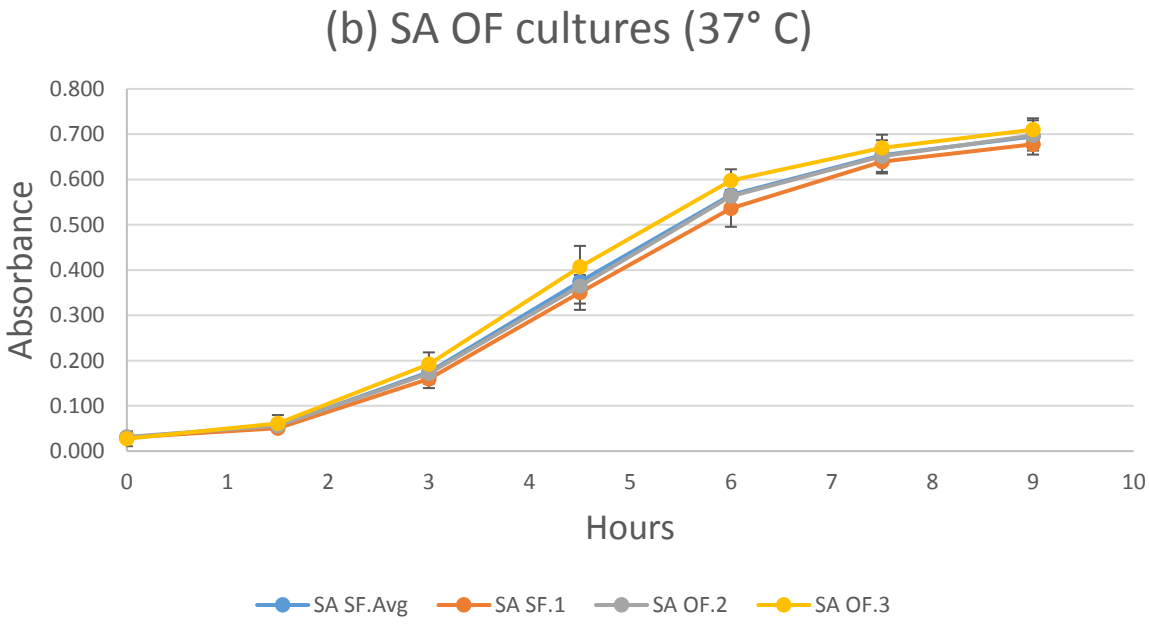
Table 4.7: Initial Values of SA (30.5°)

Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
SA C.Avg	0.728	SA OF.Avg	0.716	SA SF.Avg	0.711
SA C.1	0.710	SA OF.1	0.697	SA OF.1	0.696
SA C.2	0.723	SA OF.2	0.712	SA OF.2	0.716
SA C.3	0.750	SA OF.3	0.740	SA OF.3	0.721

Table 4.8: Final Values of SA (30.5°)

Comparing (Table 4.7) and (Table 4.8) versus (Table 4.9) and (Table 4.10), we see that the temperature difference in the growth of *S. aureus* could have played a part in the difference in population growth. Referring to the Arrhenius effect (Figure 3.1), perhaps the temperature for these bacteria found a good temperature to magnetic field ratio, whereas the bacteria on (Figure 4. 11) do have an impedance to their growth rate, influencing bacterial growth. Control and OF cultures have overlapping OD measurements with a minor bifurcation in the growth patter, then nearly coinciding again at the 9 hour mark. The SF cultures experience population difference compared to the control and OF cultures, suggesting the bacteria exposed to SF is capable of producing bacteria with notable differences, demonstrating that maybe growing the bacteria at 30.5° works better for letting the bacterium population rise quicker while higher temperatures, while not being inhospitable, continues to have less ideal climate conditions.


(a) SA control cultures (37° C)
Absorbance
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
1
2
3
4
5
6
7
8
9
10
Hours
SA C.Avg
SA C.1
SA C.2
SA C.3


(b) SA OF cultures (37° C)
Absorbance
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
1
2
3
4
5
6
7
8
9
10
Hours
SA SF.Avg
SA SF.1
SA OF.2
SA OF.3

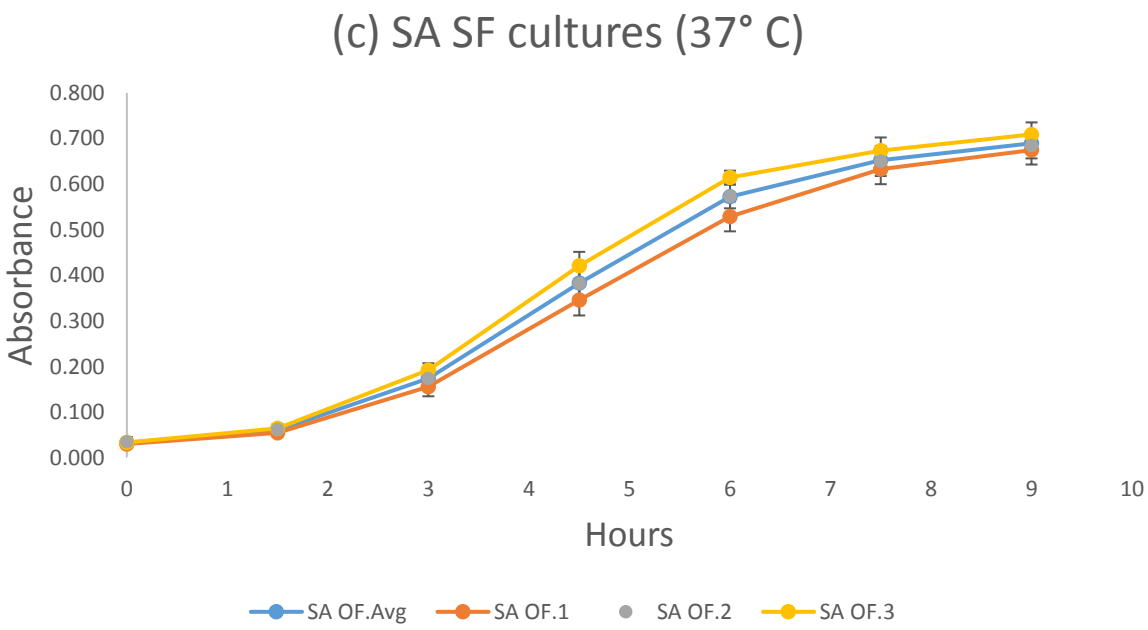


Figure 4.11: S. aureus Cultures at 37° C (a) control (b) S. aureus exposed to oscillating field (c) S. aureus exposed to static field. Three samples of each conditions are used and then the corresponding average value is given in blue, just for comparison.

Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
SA C.Avg	0.026	SA OF.Avg	0.030	SA SF.Avg	0.033
SA C.1	0.032	SA OF.1	0.030	SA SF.1	0.031
SA C.2	0.022	SA OF.2	0.031	SA SF.2	0.035
SA C.3	0.023	SA OF.3	0.027	SA SF.3	0.034

Table 4.9: Initial Values of SA (37°)

Iteration	Absorbance	Iteration	Absorbance	Iteration	Absorbance
SA C.Avg	0.720	SA OF.Avg	0.690	SA SF.Avg	0.695
SA C.1	0.711	SA OF.1	0.675	SA SF.1	0.678
SA C.2	0.712	SA OF.2	0.685	SA SF.2	0.697
SA C.3	0.736	SA OF.3	0.709	SA SF.3	0.710

Table 4.10: Final Values of SA (37°)

The *S. aureus* bacteria grown under 37° C has a higher OD quantity overall than the OD of *E. coli* populations, but still do not grow population size or an absorbance as high as the 30.5° C populations, suggesting that the bacteria may reach more desirable conditions at slightly less warm conditions. The density of the SA population at this temperature also had more varied growth rates on the three orientations in respect to one another, only giving a small perturbation to each field, whereas the perturbations on the 30.5° C were more prominent and affected the growth rates more clearly. While the initial optical density (Table 4.9) was still higher based upon the higher temperature activating the bacterial response in enzymatic processes, the temperature and magnetic field interaction was not as ideal as compared with the 30.5° C populations, suggesting the Arrhenius effect played a role in dampening the growth along with the perturbation of the magnetic field upon the bacterium.

4.3 - Summary

With the application of thermodynamics into the bacterium, we should see how the magnetic fields can affect the system and depending on the range of temperature could create a higher diffusivity rate on the fluids. The temperature change affects the growth rate and the interaction can change based off the magnetic field intensity and can create a gradient with the bacterial membrane, increasing the enzymatic processes of the bacterial cells. With different temperature gradients and magnetic field applications, several observations are noted; temperature differences allow the bacterium to grow much more effectively or can instead slow down the metabolic processes and slow the growth of bacterial populations depending on what range of the spectrum the bacterium are being incubated under.

With the utilization of the range of temperature to growth relationship, we can try to grow different strains of bacterium at more specific temperatures where bacterial growth can be

more pronounced. With the magnetic field present, bacteria grown under control conditions and then placed under a magnetic field will grow more effectively for a few hours, effectively increasing the enzymatic processes on the bacterial cells, demonstrating a correlation between the two, but the presence of the field may also slow the bacterial growth. A consistent pattern noted on the data, regardless of the magnetic field strength; once bacteria was placed under the effect of a magnetic field, the optical density rose, but nearing the end of the readings, the control bacteria would inevitably outgrow the optical density of the bacteria under a magnetic field configuration. When a second generation of magnetically treated bacteria was grown under new tubes of TSB, it was observed that the bacterial populations showed less growth under the optical density measurements versus the control quantities. The maximum difference in the behavior of the interaction of the two is noted in the log phase of the bacterium before reaching the saturation point, where the cultures would inevitably intersect.

Under the assumption that the bacteria have morphological changes after exposure to a magnetic field as referred in (Figure 2.2), the surface area of the magnetic field exposed bacterium could be using the cellular membrane to have a larger intake of nutrients compared to the normal counterpart. If this holds true, then these elongated bacteria could be running out of nutrients and filling its environment with waste by-products, causing the populations to hit the stationary phase much quicker under a magnetic field that is forcing its growth, which would explain the magnetic field exposed bacteria set under a control orientation to have a greater OD as it has more time to reach the stationary phase and allowed to grow naturally instead of forcefully.

Further studies could include more specific temperature points where bacterial growth of *Staphylococcus aureus* or *Escherichia coli* can grow under more ideal conditions under the Arrhenius observation, selecting temperature points which would improve upon the

bacterial growth rates, yielding improved comparison of pronounced growth via the temperature – magnetic field correlation. To boost the accuracy of the temperature influence on the bacterium, insulation onto the coils could be introduced, addressing the heat dissipation issue present in the static field coils, thus allowing the bacteria in the static field configuration to not be influenced to the additional degree of temperature which was present throughout the process. Supplementary studies on this experiment could be replicated while inverting the field strength of the coils, using 0.9 mT on the oscillating field configuration and 0.5 mT on the static field configuration and performing an inner comparison of the data, demonstrating the effect of influence of the field strength versus the type of field present in the experiment. Another modification to the experiment could include using the both magnetism and temperature simultaneously instead of the step model used in this experiment. This interaction of the two perturbations, if used at more optimal conditions, could effectively dampen the growth rate of bacterium and demonstrating what type of effect separately influencing the bacteria versus doing a simultaneous perturbative effect upon the bacterium. In order to prove my theory on the dampening of the optical density on the magnetically treated bacteria, plating the magnetic field exposed bacterium could be done as well, thereby observing the morphological effects to validating the conclusions in this experiment.

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