5-2022

Investigating the effect of polymer hydrogels on the antimicrobial properties of activated carbon through the utilization of bacterial, viral, and parasitic microorganisms

Zoe Gunter

Investigating the effect of polymer hydrogels on the antimicrobial properties of activated carbon through the utilization of bacterial, viral, and parasitic microorganisms

by

Zoe Gunter

A Thesis
Submitted to the Honors College of
The University of Southern Mississippi
in Partial Fulfillment
of Honors Requirements

May 2022

Approved by:

Heather Broadhead, Ph.D., Thesis Advisor,
School of Polymer Science and Engineering

Derek Patton, Ph.D., Director,
School of Polymer Science and Engineering

Sabine Heinhorst, Ph.D., Dean
Honors College

ABSTRACT

Activated carbon (AC) has long been utilized in biomedical applications due to its innate antimicrobial characteristics. However, little is understood about the mechanism of action that allows AC to prohibit the propagation of different pathogen species. To further ascertain these characteristics and how they affect the propagation of pathogens, procedures were designed with bacterial, viral, and parasitic agents which were utilized in conjunction with varying concentrations of polymer hydrogels to examine the antimicrobial efficacy of AC. The studies performed for this thesis were conducted employing coconut-based AC and Noveon® AA-1 Polycarbophil USP or Carbopol® 974P NF hydrogels which, when introduced to bacterial, viral, and parasitic agents, limited their activity. Experiments evaluated a series of bacterial dilutions with *E. coli* and *S. aureus*, bacteriophage (viral) dilutions with T1 and Φ11 bacteriophages, and *Euglena gracilis* (parasite) dilutions using the AC listed above. However, polymer hydrogels were mainly utilized in the bacterial portion of this study due to time constraints. Results of all experimentation were observed and compared through visual examination or optical microscopy. When treated with AC, it was observed that all studied pathogens had a significant reduction of activity overall and that the presence of Polycarbophil did not impede the antimicrobial characteristics of AC at the lowest concentration. However, experimental optimization must be achieved to understand the efficacy of polymer hydrogels as a potential delivery system for AC.

Keywords: activated carbon, mechanism of action, pathogen, polymer hydrogel, Polycarbophil AA-1 USP, Carbopol 974P NF, *E. coli*, *S. aureus*, bacteriophage, *Euglena gracilis*

DEDICATION

To my friends, famil and boyfriend. Thank you all for everything, I love you.

ACKNOWLEDGMENTS

I would like to take this opportunity to thank Dr. Heather Broadhead for her guidance and reassurance through my thesis writing process. Without her aid, my thesis simply would not have come to fruition. She has been a wonderful research advisor throughout my undergraduate career and I will always be extremely grateful towards her. I would also like to thank my fellow undergraduate research peer and partner, Chloe Klusman, as the progression of our project would not have been possible without her help.

In addition, I would also like to thank Dr. Monica Tisack, Caitlyne Shirley, and Cecelia Butts whom have provided guidance and resources. Without their help, the completion of this study would have otherwise been impossible to achieve. I will continue to move through my future with the confidence, skills, and lessons I have learned while working in the Broadhead Research Group and at the Mississippi Polymer Institute.

I would also like to thank the Honors College for accepting me as a Keystone Scholar and pushing me to achieve more than I thought was possible. Also, I would like to thank the DCUR grant for funding this study, as this funding made the development of this research possible.

Finally, I would like to extend my upmost gratitude to my friends Alyssa Necaise and Zachary Ahmad whom have made my undergraduate career the most fulfilling experience of my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

AC	Activated Carbon
BPO	Benzoyl peroxide
BSL-1	Biosafety level 1
CFUs	Colony forming units
CHP	Charcoal hemoperfusion
DI	Deionized
E. coli	*Escherichia coli*
FT-IR	Fourier transform infrared spectroscopy
GRAS	Generally recognized as safe
MRSA	Methicillin-resistant *S. aureus*
PAC	Powdered activated carbon
PCB	Poly-carboxybetaine
PFUs	Phage plaque forming units
S. aureus	*Staphylococcus aureus*
XPS	X-ray photoelectron spectroscopy

CHAPTER I: Introduction and Objectives

1.1 Statement of purpose

Activated Carbon (AC) has long been recognized as an adsorbent due to its large surface area and highly porous structure upon activation.[1] As a result, it is utilized across different practices, including the water purification and the biomedical industries, due to its ability to remove potentially harmful compounds from solution.[2,3] For example, in the biomedical industry, AC is often employed for charcoal hemoperfusion (CHP), a filtration process by which contaminated blood is passed through an AC-containing capsule through which harmful toxins are removed from the blood, and the blood is returned to the patient.[2] However, while much is understood regarding AC's ability to adsorb foreign particles from solution, little has been established in terms of its potential as an antimicrobial agent for personal care applications. One study noted the efficacy of AC in removing *E. coli* from solution after one hour of incubation; however, it failed to recognize or suggest possible applications that could utilize AC as an antimicrobial agent.[4] In addition, it lacked the inclusion of a variety of pathogens, such as viral and parasitic microorganisms, of which the application of AC as an antimicrobial agent could possibly be employed. As such, this study was initiated to understand how AC may interact with and limit the propagation of bacterial, viral, and parasitic microorganisms to understand the efficacy of activated carbon as an antimicrobial agent.

Moreover, the employment of polymer hydrogels as a drug delivery system has been established through many years of research, and many polymeric hydrogels are now commercially produced – with FDA approval – for buccal, intestinal, vaginal, ophthalmic, and several other applications.[5] These hydrogels have been utilized in drug

delivery applications largely due to their ability to collapse from their swelled state upon being placed in certain biological environments.[6] The collapse of the hydrogel, in turn, allows for the release of the encapsulated drugs in a zero-order fashion.[6] While the use of polymeric hydrogels as a drug delivery system has been recognized, little is understood in terms of the efficacy of hydrogels in delivering activated carbon as an antimicrobial agent via a drug-delivering mechanism. One study examined the ability of polymeric hydrogel coatings to enhance the hemocompatibility of AC through encapsulation without inhibiting its ability to remove contaminants.[7] However, the study neglected to include particulates other than methylene blue to understand the removal of foreign compounds or microbes.[7] As a result, this study was conducted using polymeric hydrogels to understand how they may benefit or detract from the antimicrobial properties of activated carbon and to propose the utilization of hydrogels as a delivery method for activated carbon.

1.2 Bacteria

The bacteria that were utilized in this study were *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), as they are prominent bacteria that are often used in antimicrobial studies. *E. coli* is a common bacterial microorganism that exists within the gastrointestinal tract of humans without causing harm. However, through evolution, *E. coli* has mutated and produced several strains which are highly pathogenic if introduced to a human host.[8] These strains include enterohaemorrhagic, enteroinvasive, enteroaggregative, enterotoxigenic, enteropathogenic, and diffusely adherent *E. coli*, all of which interact with the human body and cause diarrhoeal disease through different

interaction mechanisms (and of which lead to the exhibition of different symptoms upon infection).[9]

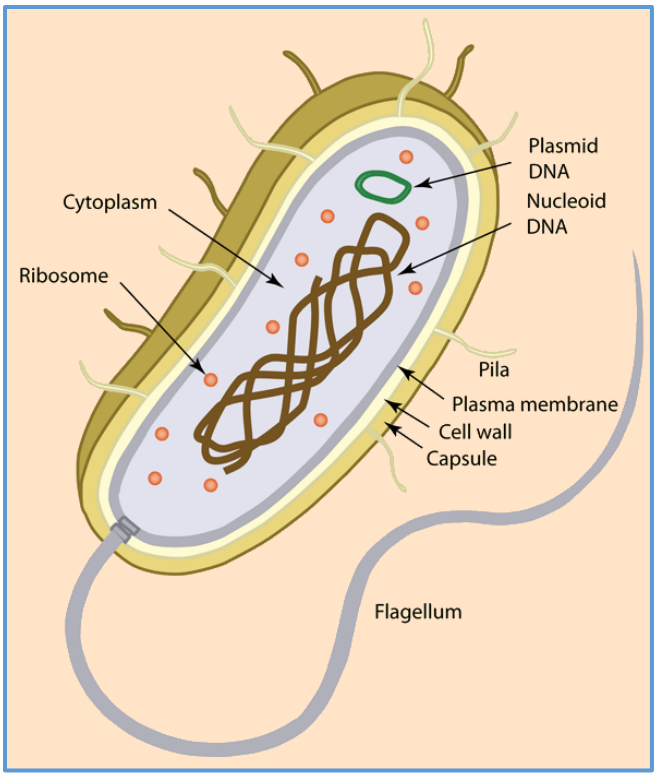

Figure 1. Example of a prokaryotic cell[10]

Moreover, *E. coli* is classified as a prokaryotic cell, as its nucleus is not membrane bound (**Fig. 1**).[10] In addition to *E. coli* being a prokaryotic cell, it is also classified as a gram-negative bacteria, meaning that its cell envelop contains an inner membrane and an outer membrane that are separated by the periplasm.[11] These components of the cell serve to provide structure, strength, and rigidity to the cell's surface which allow it to withstand mechanical loads, and in turn, resist degradation through external interference.[11] As such, the development of antimicrobial applications for the elimination of *E. coli* is rather difficult, and an understanding of how this bacterial cell interacts with an antimicrobial agent is critical for its success.

S. aureus is another common bacterial microorganism, one which is known to cause many ailments in the human body. Some instances of afflictions caused by *S. aureus* include, but are not limited to, skin lesions, osteomyelitis, furunculosis, food poisoning, and toxic shock syndrome.[12] Like *E. coli*, *S. aureus* is a bacteria that possesses many strains which infect their hosts through various probable virulence factors, including the colonization of host tissues by proteins on the cell's surface, the inhibition of phagocytosis, and the creation of toxins that lead to disease to name a few.[12]

Table 1. Modified Table Displaying the Antimicrobial Resistance of *S. aureus*[12]

Antimicrobial	Resistance Mechanism	Genetic Basis
Penicillin	β-lactamase. Enzymatic inactivation of penicillin	Plasmid
Methicillin	Expression of new penicillin-resistant penicillin-binding protein. Bypass	Novel chromosomal locus acquired from unknown source
Tetracycline	Efflux from cell Modification of ribosome	Plasmid Novel chromosomal locus acquired from unknown source
Chloramphenicol	Enzymatic inactivation	Plasmid
Erythromycin	Enzymatic modification of ribosomal RNA. Prevents drug binding to ribosome	Plasmid Transposon in chromosome
Streptomycin	Mutation in ribosomal protein. Prevents drug binding to ribosome Enzymatic inactivation	Mutation in chromosomal gene encoding drug target Plasmid
Kanamycin	Enzymatic inactivation	Plasmid Transposon in chromosome
Gentamicin	Enzymatic inactivation	Plasmid Transposon in chromosome
Trimethoprim	Alternative dihydofolate reductase. Bypass	Plasmid
Mupirocin	Alternative isoleucyl tRNA synthase. Bypass	Plasmid
Fluoroquinolones	1. Altered DNA gyrase 2. Efflux	Mutation in chromosomal gene encoding drug target Mutation increases expression of natural afflux mechanism
Antiseptics	Efflux	Plasmid

Furthermore, *S. aureus* is another example of a prokaryotic cell; however, it differs from *E. coli* as *S. aureus* is a bacteria which usually propagates and aggregates to form clusters of cells (a trait which is indicative of a gram-positive bacteria).[10,12] The

propensity to aggregate, along with some structural differences in the cell envelope (an inner membrane and an outer cell wall comprised of peptidoglycan, teichoic acids, and polysaccharides), differentiate gram-positive bacteria, such as *S. aureus*, from gram-negative bacteria, such as *E. coli*.[13] What feature *S. aureus* does share with *E. coli*, on the other hand, is its resistivity towards antimicrobial agents (**Table 1**).[12,14] Through several genetic manipulations, *S. aureus* has become heavily resistant towards most antimicrobial agents (displayed most commonly by methicillin-resistant *S. aureus* (MRSA)).[12,14] Its resistance to these antimicrobial agents ultimately arises due to the following genetic processes: mutations in chromosomal genes and accession of extrachromosomal plasmids or other genetic information in the chromosome through transposons or other methods of DNA insertion.[12] As such, developing an antimicrobial compound that is successful in eliminating or limiting the propagation of *S. aureus* has been extremely difficult in clinical studies. Furthermore, the specific strain that was chosen for this study was *S. aureus* RN4220, as it possesses a lower resistivity to antimicrobial agents than many other strains, making it a safer choice when selecting a specimen for preliminary studies involving the development of an antimicrobial agent against *S. aureus*.[14] However, this does result in limitations in regards to the accuracy of the results obtained when trying to understand the efficacy of the antimicrobial agent being developed. As a result, the limitations of this strain in providing an accurate assessment of efficacy should be kept in mind during clinical studies.

Finally, it is important to understand how *E. coli* and *S. aureus* differ in terms of what symptoms they result in when infecting the host's body. For example, Günther et al. noted that *E.coli* resulted in instantaneous inflammation upon infection whereas *S. aureus*

caused a slower inflammatory response upon infection.[15] This difference in inflammation rates had a direct correlation to the immune response of the host. It was found that the rapid inflammation rates caused by *E.coli* infection resulted in a completely activated immune response which eliminated the bacteria.[15] However, the opposite was seen with *S. aureus*, as a slower inflammation rate resulted in a lessened immune response compared to that seen by *E.coli*.[15] Therefore, the elimination of *S. aureus* is much more difficult upon infection and infections caused by this bacteria are usually chronic.[15] This difference in the infection rates of *E.coli* and *S. aureus* must be kept in mind when developing antimicrobial agents, as the physiology of the host plays an important role in the efficacy of these agents.

1.3 Bacteriophages

The bacteriophages that were employed by this study were T1 and Φ11 phages. These microorganisms are intracellular viruses that infect and eliminate bacteria through different modes of propagation (**Fig. 2.**).[16]

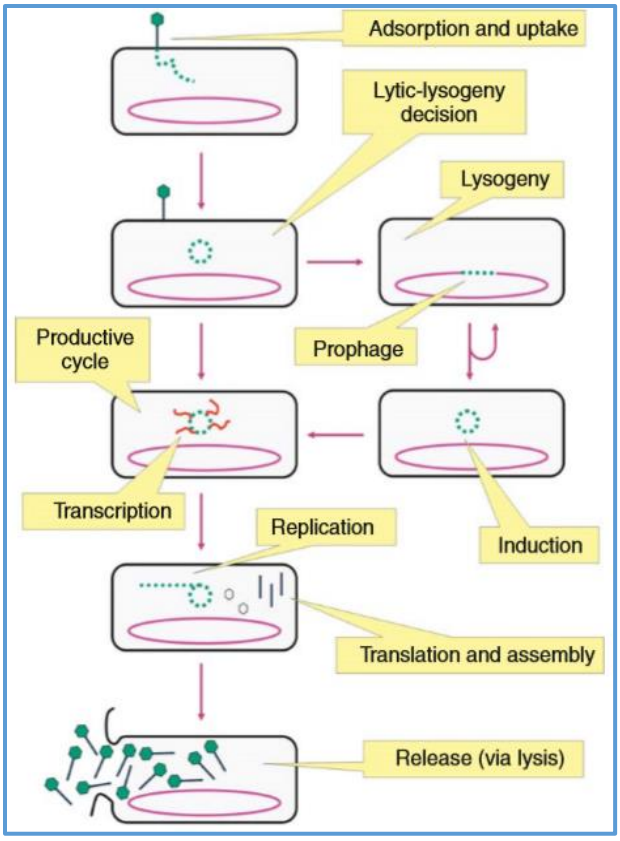

Figure 2. Illustration of the lytic and lysogenic life cycles of bacteriophages[17]

The T1 phage, of the *Siphoviridae* family, is a lytic virus of *E. coli* which means it eliminates the host cell through lysis, or the release of phage replicas from phage-infected cells.[17] The lytic cycle can be further explained through the following sequence: the attachment of the phage to the host cell and injection of the phage genome into the cell, the degradation of the host cell's genome, the propagation of the phage genome and synthesis of phage proteins, the assembly of the phages and their release from the host cell, and the death of the host cell along with new phages for furthering the cycle (as shown in **Fig. 2.**).[16,18] Through this cycle, T1 phages are able to quickly infect and eliminate bacteria through an amplified effect (i.e. the creation of a multitude of progenies through lysis). While the elimination of bacteria is usually perceived as

beneficial for antibacterial studies, the rapid propagation of the phage may create a disadvantage for other applications, such as the study of a potential antimicrobial agent's efficacy against viruses, as T1 phages are known to be notoriously persistent.[17] Therefore, it is important to keep the implications of utilizing the T1 phage in consideration when studying a potential antimicrobial agent against its propagation and determining its efficacy.

The Φ11 phage is a lysogenic virus of *S. aureus*, meaning its genome is stored as a prophage in the host cell's chromosome after injection and remains in the structures of its sister cells upon replication.[19] The lysogenic cycle can be further explained through the following sequence: the attachment of the phage to the host cell, the insertion of the prophage into the host cell, the integration of the prophage into the host cell's chromosome, and the replication of the prophage upon bacterial DNA replication.[16,17,19] As such, the viral genome is usually nondestructive and continues to lie dormant unless DNA damage of the host cell occurs, after which, induction is able to occur and lysis may be achieved (**Fig.2.**).[17] Understanding the processes by which bacteriophages eliminate bacteria is crucial in developing antimicrobial applications against viral propagation, as the efficacy of an antimicrobial agent could very easily be misconstrued as better than in reality, especially if a bacteriophage participated in the lysogenic cycle, such as the Φ11 phage. Therefore, the fact that the Φ11 phage participates in the lysogenic cycle must be considered when analyzing the results of an antimicrobial study employing both bacteria and bacteriophages, as an antimicrobial agent would likely eliminate both pathogens in the process of the study.

1.4 Parasitic microorganism: *Euglena gracilis*

Euglena gracilis was utilized for the parasitic portion of this study. *Euglena gracilis* is a single-celled eukaryotic microorganism that resides in nature as a non-parasitic green alga.[20] The basic structure of *Euglena gracilis* illustrates that it is a protist with a singular flagellum (**Fig. 3**.), a characteristic that is shared by many other microorganisms within its phylum, Euglenozoa.[21,22]

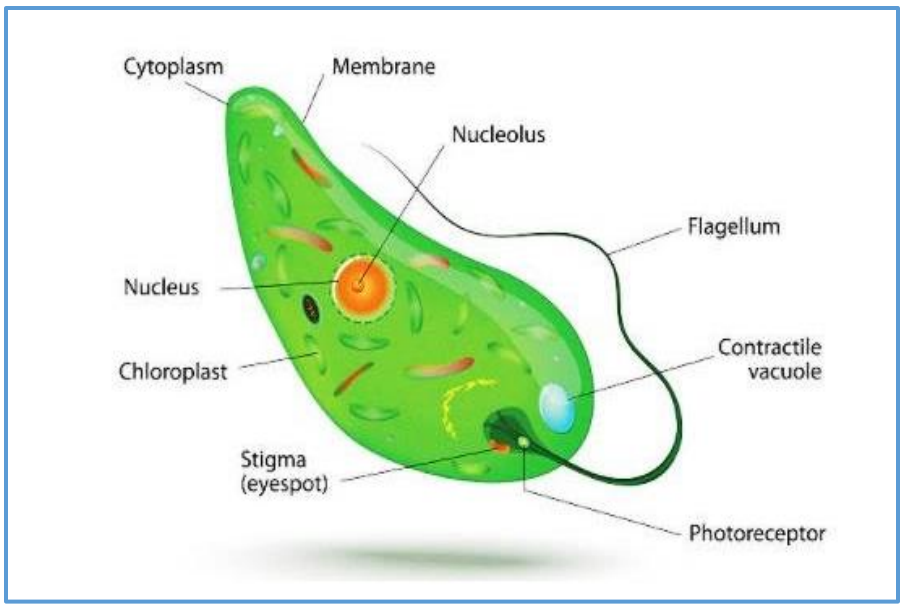

Figure 3. Structure and components of Euglena gracilis[21]

Furthermore, within the Euglenozoa phylum, there are other, parasitic microorganisms of the genus *Trypanosoma* which are referred to broadly as *trypanosomatidae* and include parasites such as *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania major*.[22] These protists have been established as a public health concern, as they can invade and negatively affect their host (commonly causing sleeping sickness, Chagas' disease, and leishmaniasis, respectively).[20,23] As such, understanding how to eliminate these microorganisms through the use of an antimicrobial agent would

be beneficial in aiding those ailed by their invasion. However, utilizing such dangerous parasitic microorganisms in a study would pose unwarranted risk to the researcher. Fortunately, *Euglena gracilis* is known to share structural and functional similarities with these *tyrpanosomatidae* which makes substituting it for the purpose of studying antimicrobial agents for the removal of parasitic microorganisms possible. The structural similarities of these microorganisms include their existence as a protist, their possession of a flagellum, their possession of mitochondrial cristae, and their shared DNA base "J."; however, their functional similarities are still being studied.[21,22,23] As of now, researchers understand that *Euglena gracilis* and the *tyrpanosomatidae* share mitochondrial targeting presequences, but the exact reason for this shared process is unknown.[22] Regardless of the reasoning, the fact that these microorganisms share many structural and functional similarities makes *Euglena gracilis* a perfect substitution candidate for the study of an antimicrobial agent's efficacy against common parasitic microorganisms.

1.5 Activated Carbon

Activated carbon (AC) was utilized in this study to understand its capabilities as an antimicrobial agent. AC is a compound that is commonly used as an industrial adsorbent due to its increased surface area and highly porous structure upon activation.[24] Moreover, the activation of AC is generally achieved through a two-step process. First, the carbonization of the raw material, which is often biomass derived, is completed at high temperatures (through either pyrolysis or hydrothermal carbonization).[25] Then, activation of the carbonized material, which results in a higher surface area and microporous structures, is finalized at high temperatures with the addition of a physical or chemical activation agent.[25]

For the physical activation of carbon material, steam or carbon dioxide are commonly used. In addition, the proposed chemical reactions that result from the usage of these activation methods (and thus, cause the incorporation of enlarged, porous structures) include the Boudouard reaction, water-steam gasification, and water-gas shift reaction (**Fig. 4.**).[25]

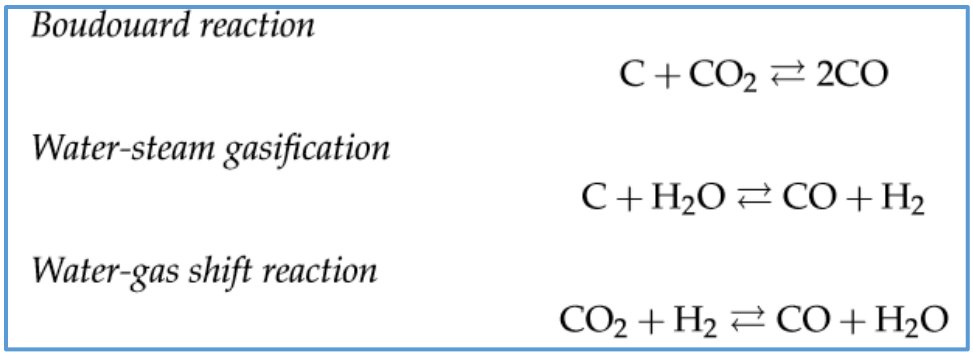

Figure 4. Physical activation reactions[25]

One study by Ghouma et al. described the physical activation of olive stones for pollutant removal applications.[26] This activation was completed through the use of water vapor at 750 °C, and the final product was found to be successful in the removal of NO_2 pollutants through the adsorption of the gas molecules to the porous structure of the created AC.[26]

Moreover, for chemical activation, several chemicals may be used, including KOH, H_3PO_4, K_2CO_3, NaOH, $ZnCl_2$, etc.[25] However, the preferred compound utilized for chemical activation of AC is KOH, as it results in more defined and voluminous micro-pores.[26] Proposed reaction mechanisms upon activation of AC with KOH are shown in Fig. 5.

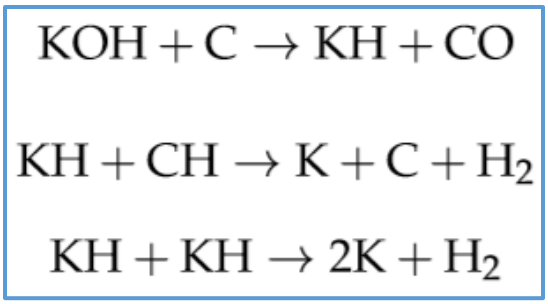

Figure 5. Proposed mechanisms of KOH chemical activation of AC[25]

For example, a study conducted by Borghei et al. utilized KOH as the chemical agent for the activation of AC derived from oak seeds to understand methylene blue adsorption.[27] It was concluded that optimal surface area (2896 m^2/g) and pore volume (1.554 cm^3/g) were achieved at 650 °C.[27] However, while chemical activation of AC results in higher, more defined pore volumes, it is important to note that the removal of excess chemical agent is required after activation which can ultimately be a disadvantage when choosing an activation route.[27] Unfortunately, the activation route of the AC utilized by this study is unknown, as it was obtained from an external source; however, characterization of its surface functionality could be completed through the use of X-ray photoelectron spectroscopy (XPS) or Fourier transform infrared spectroscopy (FT-IR) to understand this characteristic further (as the presence of organic or inorganic compounds at its surface would indicate either chemical or physical activation).[28,29]

Furthermore, efforts are being made to increase the amount of biomass utilized for AC creation, as it is renewable and cost-effective.[24] These efforts have resulted in a variety of materials being carbonized as a precursor to AC compounds, such as *camellia oleifera* shell, bamboo, cherry stones, acorns, sludge and sugarcane bagasse, tea waste, and many more.[24] The specific AC chosen for this study was coconut-shell derived AC,

as it is a renewable biomass-based compound, it possesses well-defined structures, it is considered "generally recognized as safe" (GRAS) by the FDA for pharmaceutical applications, and has been utilized previously in commercial applications.[25,30]

In addition, while AC is commonly understood to be an industrial adsorbent, its role in the biomedical industry is limited. The current biomedical applications that utilize AC are as follows: the use of AC as an electrode for biosensor development, the employment of AC's adsorption capacity for use in magnetically targeted drug delivery, the direct use of AC as a pharmaceutical to adsorb toxins upon administration to the patient, and the use of the adsorption properties of AC to remove toxins from blood in CHP.[2,3,31] In fact, the only study that was found which explored AC's role as a potential antimicrobial agent was that of Naka et al. as they examined the capability of AC to adsorb *E. coli* as a removal mechanism.[4] While this study found that *E. coli* was successfully removed through adsorption of the cells to *E. coli*'s surface, it failed to suggest the application of AC as an antimicrobial agent, and instead focused on its previously established role as an adsorbent.[4] In addition, the study also excluded other microorganisms, such as viral or parasitic pathogens, from its experiments, therefore leaving AC's efficacy against other microorganisms unexplored. As a result, this study was established in an effort to better understand and define the antimicrobial properties of AC and its efficacy as an antimicrobial agent against bacterial, viral, and parasitic microorganisms.

1.6 Polymeric Hydrogels

The polymeric hydrogels that were utilized in this study were Noveon® AA-1 Polycarbophil USP and Carbopol® 974P NF. Polymeric hydrogels are lightly – physically

or chemically – cross-linked hydrophilic polymer networks that, when placed in an aqueous solution, swell and expand in volume.[6,33] This swelling occurs due to external forces acting on the polymer structure, including osmotic pressure, electrostatic interactions, polymer-solvent interactions, and elastic forces (**Fig. 6.**).[6]

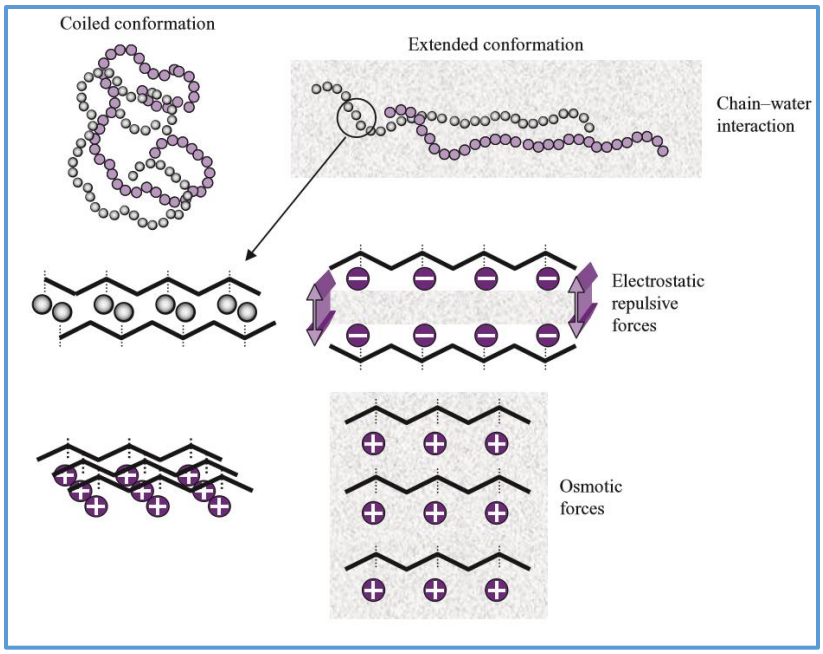

Figure 6. Forces that result in the swelling of polymeric hydrogels[6]

In addition, polymeric hydrogels are classified based on several factors, such as their material source, their polymeric structure, their physical characteristics, the cross-linking agents utilized in their creation, the presence of charged atoms, and the methods employed for the controlled release of substances.[32] These factors are crucial and must be considered when examining the possible applications of a particular polymeric hydrogel.

Polymer hydrogels have also been studied and employed by the biomedical and pharmaceutical industries for years due to their ability to serve in drug delivery applications. Their ability to serve in this capacity is largely due to their propensity

towards collapsing – the conversion of the polymer from its swelled state into its original, non-swelled state – when introduced to certain environments (i.e. the human body), which allows for the controlled release of drugs over time.[6,32] However, other factors that encourage the utilization of polymeric hydrogels for drug delivery applications include the tunable nature of their chemical, biological, and physical characteristics, their great biocompatibility, and their versatility in regard to the synthesis methods that may be utilized to create them.[32,33,34]

An important result of these factors relates to the mechanisms of release of hydrogels which controls how quickly and effectively these compounds release drugs. The three main release mechanisms of polymeric hydrogels include diffusion-controlled release, swelling-controlled release, and chemically-controlled release.[32] In diffusion-controlled release systems, drugs are either encapsulated by hydrogels and released (reservoir drug delivery) or dispersed in a homogenous fashion throughout the polymer matrix and released (matrix drug delivery).[32] In swelling-controlled release systems, the rate of drug release is directly proportional to the rate of the swelling of the hydrogel (as the rate of swelling of the hydrogel increases, so does the rate of drug release from the network).[32] Finally, in chemically controlled release systems, reactions occur as a result of an external stimuli which result in the hydrolytic or enzymatic cleavage of polymer chains within the hydrogel matrix and the release of encapsulated drugs.[32] One specific example of a chemically-controlled release system is that of pH responsive hydrogels which release drugs through polymer degradation once a certain pH is obtained.[35] Consequently, understanding the release mechanisms of polymer hydrogels is crucial

when studying their potential as a drug delivery vessel, as their mechanisms of release will ultimately control the rate of dispersion of the drugs upon administration.

Furthermore, several studies have been conducted in an effort to display the capabilities of polymeric hydrogels regarding their potential as a drug-delivering vessel. For example, Fu et al. described the synthesis of polyacrylic acid-polyethylene glycol hydrogels to understand how pH variations and the addition of Ca^{2+} would affect the swelling ratio of the polymer matrix.[35] Ultimately, the swelling ratio and storage modulus of the polymer were optimized at Ca^{2+} concentrations of 10 mM, and applications of this compound for the treatment of osteoarthritis were hypothesized (due to compatibility of Ca^{2+} present along the polymer matrix with that of articular cartilage present in the knee).[35] Another study by Mishra et al. describes the synthesis of poly[2-(methacryloyloxyethyl) trimethylammonium chloride-co-methacrylic acid] hydrogels for the delivery of the common cancer drug 5-fluoro-2'-deoxyuridine phosphate.[36] It was observed that drug efficacy increased while toxicity decreased upon the use of the synthesized polymer, meaning that it was affective as a controlled drug delivery agent.[36] Finally, a study by Xu et al. denoted the successful reduction of *Gardnerella vaginalis* after benzoyl peroxide (BPO) was administered through diffusion of the compound through a prepared Polycarbophil/Carbopol 934P hydrogel.[37] This research, as a result, presented a promising treatment of bacterial vaginosis through antimicrobial drug delivery of BPO.[37] The listed examples, while few in number, exhibit the vast array of applications that utilize polymeric hydrogels for drug delivery. A further understanding of the scope of this field may be ascertained by reviewing the attached references.

Additionally, while the use of polymeric hydrogels for drug delivery is widely accepted, little research has been done to combine polymeric hydrogels and AC for biomedical applications. One study by Céline et al. discusses the encapsulation of AC in a composite chitosan/AC hydrogel for potential use in wound-dressings.[38] They examined the ability of the chitosan hydrogel, the AC compound, and the chitosan/AC composite hydrogel material to adsorb diethylamine, a common compound discharged from wounds.[38] Moreover, they ultimately discovered that sorption capabilities of the hydrogel increased only marginally after the incorporation of AC.[38] However, they also observed that the sorption kinetics of the composite material greatly increased compared to the hydrogel without AC which alludes to a promising application of chitosan/AC composite hydrogels for the use of rapid wound treatment.[38] Another study, performed by Cai et al. discusses the formulation of a zwitterionic poly-carboxybetaine (PCB) hydrogel for the encapsulation of powdered activated carbon (PAC) as a method of increasing hemocompatibility.[7] Overall, it was found that the PCB-PAC hydrogel sustained the adsorption efficacy seen by PAC with a drastic reduction in the hemolysis ratio seen by the same compound.[7] This reduction of hemolysis, along with the comparable adsorption capacity of the PCB-PAC hydrogel, shows promising results for the application of this hydrogel in hemoperfusion optimization, or possibly, the creation of a new drug-delivery system for toxin removal. While the research listed above exhibits promising results for the combination of AC and polymeric hydrogels for adsorption applications, little is known regarding AC contained within polymeric hydrogels for potential antimicrobial applications, an issue that will be addressed further when discussing the research objectives of this project. In addition, the specific polymeric hydrogels utilized for this

study were Noveon® AA-1 Polycarbophil USP and Carbopol® 974P NF. These polymers are both acrylic acid-derived carbomers that have been approved for use in bioadhesive and oral and mucosal applications, respectively.[5]

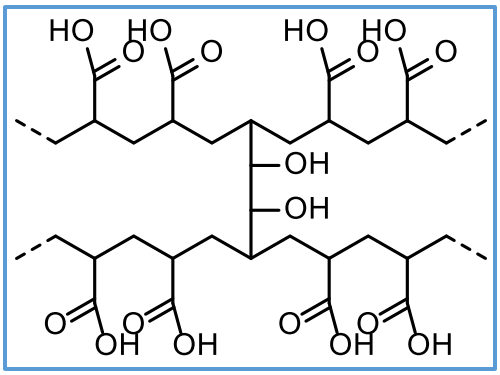

Figure 7. Structure of Polycarbophil

The main differentiating factor between the structures of these hydrogels is the compound employed for the crosslinking of the polymer chains. For Noveon® AA-1 Polycarbophil USP, divinyl glycol was used.[5] For Carbopol® 974P NF, allyl sucrose or allylpentaerythritol was utilized.[5] Experimental results ascertaining the ability of polymer hydrogels to serve as a delivery method for an antimicrobial agent, like AC, is crucial for the development of future personal care applications and were examined throughout this study.

1.7 Research Objectives

As stated previously, many research studies have attempted to explore the utilization of polymeric hydrogels in drug delivery as well as the inclusion of AC in hydrogel networks for biomedical applications. However, no study has attempted to research the application of AC encapsulated in a polymeric hydrogel network for use as an antimicrobial agent. As such, this study was conducted to understand the efficacy of AC,

combined with polymeric hydrogels, as an antimicrobial agent, to study the effect of employing polymeric hydrogels with AC, to observe the effect of hydrogel concentration on antimicrobial efficacy, and to postulate the mechanism by which activated carbon removes or adsorbs microorganisms.

CHAPTER II: Materials and Methods

2.1 Materials

2.1.1 Activated Carbon and Polymeric Hydrogels

The type of AC utilized for the experimental procedures was coconut-shell based AC and was obtained from Chemviron Carbon Service. The specific polymeric hydrogels utilized for this study were Noveon® AA-1 Polycarbophil USP or Carbopol® 974P NF, and they were obtained from Lubrizol.

2.1.2 Pathogens and Growth Media

The pathogens employed for these studies include *E. coli* (a Gram-negative bacteria), *S. aureus* (a Gram-positive bacteria), T1 bacteriophage, Φ11 bacteriophage, and *Euglena gracilis*. The *E. coli* and *S. aureus* were sourced from the Elasri Research Group at The University of Southern Mississippi. Tryptic soy broth and agar for the cultivation of *S. aureus*, Luria-Bertani broth and agar for the cultivation of *E. coli*, and Petri dishes for the growth of bacterial and phage assays were all purchased from Fisher Scientific. The T1 and Φ11 phages were provided by the Elasri Research Group of The University of Southern Mississippi. Finally, *Euglena gracilis* was purchased from the Carolina Biological Supply Company.

2.2 Experimental Methods

All bacterial, viral, and parasitic experiments were performed in a Bioflow Chamber from the GermFree Laboratories, Inc. This biosafety chamber allowed for the growth of microorganisms listed as a biosafety level 1 rating (BSL-1) which limits experiments to the use of microbes that are nonpathogenic (**Fig. 8.**). In addition, personal protection

equipment, such as gloves, safety glasses, and laboratory coats were also utilized and hands were washed before and after each experiment to avoid contamination.

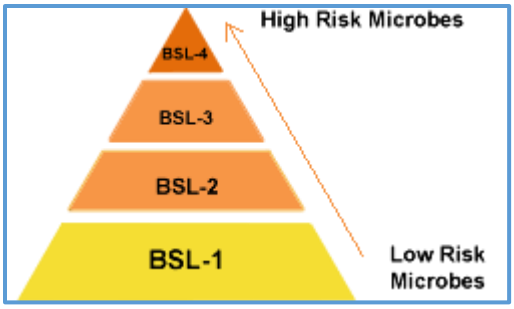

Figure 8. Biosafety level pyramid[39]

2.1.1. Bacterial ExperimentalDesign

E. coli and *S. aureus* stocks were prepared and stored at -70 °C in between experiments. Serial dilutions were performed utilizing either *S. aureus* or *E. coli* as the bacterial control. Cultures were plated and incubated for 16 hours prior to the experiment. After incubation, 30 mg of the chosen bacteria was collected into two micro-centrifuge tubes. After which, 1 mL of the chosen hydrogel – at a specific concentration – with two varying pH values of ~4.5 and ~7 were pipetted into separate tubes. The tubes were then vortexed to ensure complete suspension of the microbe. Separately, 40 micro-centrifuge tubes were labeled to indicate hydrogel pH and the inclusion of AC, as 20 of the tubes contained 30 mg of AC for each experiment. Next, the control dilution tubes, those without AC, were pipetted with 900 µL of the chosen bacteria's growth medium (Tryptic soy broth or Luria-Bertani broth). After the preparation of the dilution was complete, the control dilutions were accomplished by diluting from 10^{-1} concentrate to 10^{-10} concentrate by pipetting 100 µL of the previous dilution concentration. After the control dilutions were finished, 100 µL of each dilution concentration were pipetted into their respective

tube containing AC. The basic procedure for the serial dilution mentioned above can be visualized by **Fig. 9.**

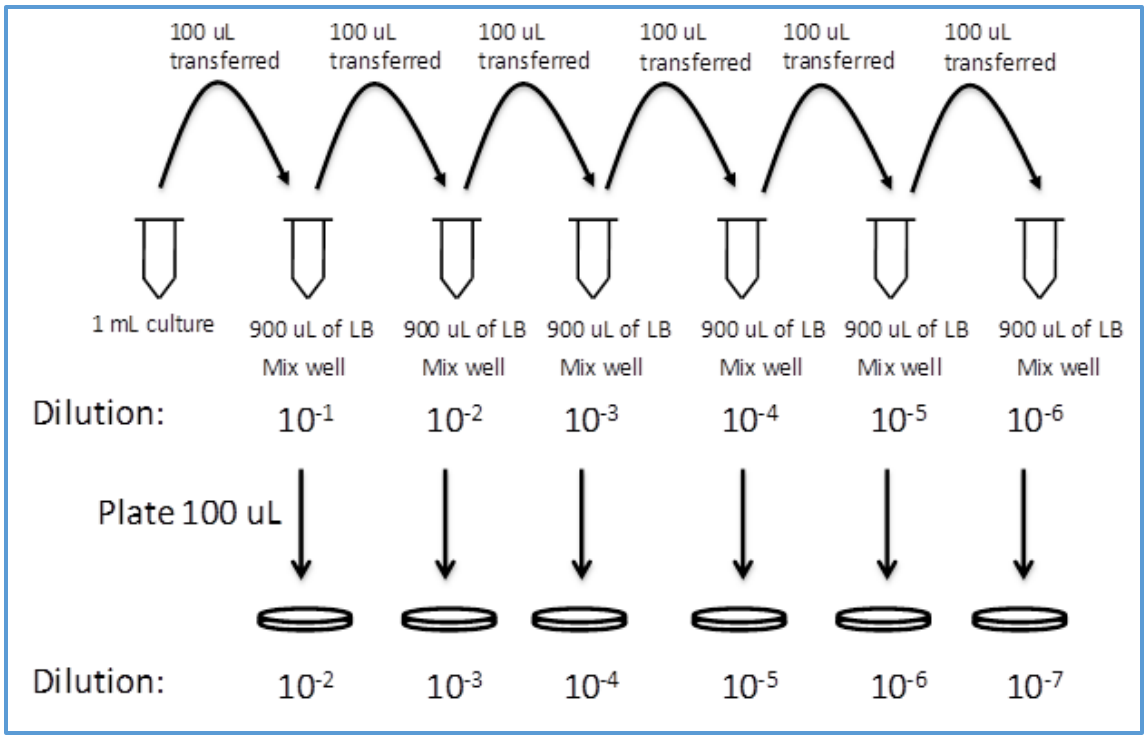

Figure 9. Basic serial dilution procedure[40]

After which, the tubes were vortexed and allowed to incubate for 2-4 hours before plating. After incubation, all concentrations were centrifuged to separate the supernatant. Then, 50 µL of each concentration were plated dropwise into an agar dish divided into four quadrants (**Fig. 10.**).

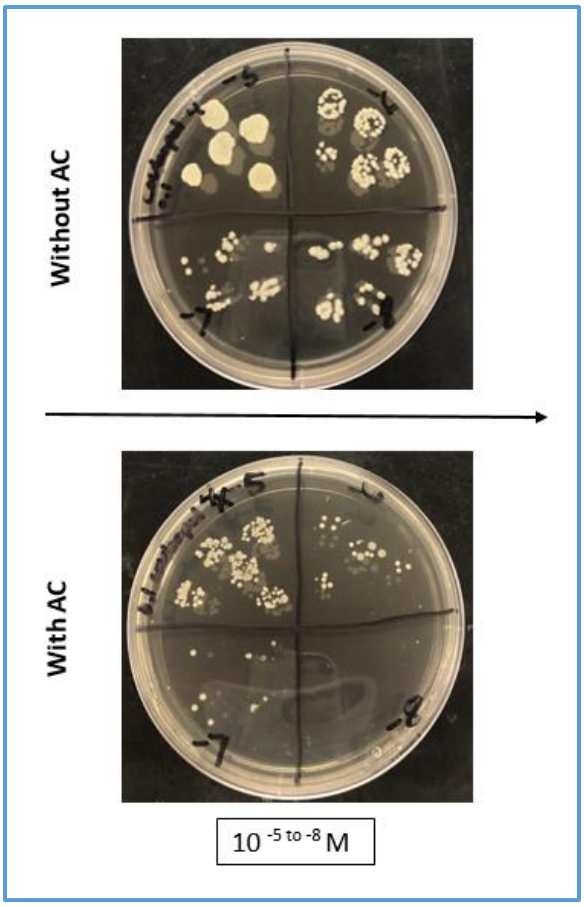

Figure 10. Example of dropwise plating technique utilized for bacterial dilutions

The Petri dish assays were then incubated for 16 hours and results and differences in AC treated dilution vs the untreated dilution were observed, compared, and recorded through the counting of bacterial colony forming units (CFUs).

1.1.2. Bacteriophage Experimental Design

The T1 and Φ11 bacteriophage dilutions were prepared in a similar fashion to that of the bacterial dilutions. Bacteriophage solutions were stored at 4 °C in between experiments. At the beginning of the experiment, 30 mg of the chosen bacteria was collected into a micro-centrifuge tube after incubation. The bacterial dilution was performed diluting the initial concentration (30 mg of bacteria in 1 mL of the chosen

bacteria's respective broth) from 10^{-1} concentration to 10^{-10} concentration. Separately, a bacteriophage dilution was performed in another, distinct set of tubes, with 50 μL of the chosen bacteriophage being pipetted into a tube containing 900 μL of the chosen broth (Tryptic soy or Luria-Bertani broth) for the initial concentration. Additionally, 900 μL of the respective broth was pipetted into the other tubes to prepare for the dilution. The bacteriophage solution was then diluted from 10^{-1} M to 10^{-7} M. For Φ11 phage dilutions, 40 μL of calcium chloride was included in the initial concentration, as it enhanced the phages' propagation. After the bacterial and phage dilutions were performed separately, 50 μL of the phage concentrations were added to 10 mL centrifuge tubes containing 30 mg of AC (excluding the control). Then, 100 μL of their respective bacterial dilution concentrations were pipetted to the 10 mL centrifuge tubes. After these solutions were prepared, 4 mL of Tryptic soy or Luria-Bertani top agar was added to the dilution concentrations as a method to better visualize the results. After which, the solutions were vortexed and poured onto agar plates. Only the 10^{-1} M, 10^{-3} M, and 10^{-7} M concentrations were plated. Results comparing the AC versus the control dilution concentrations were observed and recorded by counting phage plaque forming units (PFUs).

2.1.3. Parasitic Experimental Design

For the *Euglena gracilis* experiments, 20 mL scintillation vials were prepared utilizing 100 drops of deionized (DI) water and varying concentrations of AC. Specifically, three vials were prepared employing 15, 30, and 90 mg of AC, respectively. A fourth vial containing the *Euglena gracilis* suspended in water was utilized as a control. 20 drops of the *Euglena gracilis* suspension was added to each vial. After which, each vial was gently mixed – manually – and allowed to rest overnight before results

were collected through optical microscopy imaging. The interaction of *Euglena gracilis* with the AC particles was observed utilizing a Keyence VHX-7000 digital microscope and compared to the control *Euglena gracilis* suspension.

2.1.4. Polymeric Hydrogel Preparation

Polymeric hydrogels were prepared uniformly for each bacterial experiment, with the only differing variables being that of the hydrogels' concentration, the hydrogels' pH, and type of hydrogel being employed. As listed previously, the polymeric hydrogels utilized in this study were Noveon® AA-1 Polycarbophil USP and Carbopol® 974P NF. These hydrogels were prepared in two distinct concentrations, either 0.1 or 0.25 w/v%. Additionally, the hydrogels were prepared at two different pH values, either ~4.5 or ~7, for each experiment to understand antimicrobial efficacy in varying bodily environments (pH values were validated utilizing a Hanna Instruments wireless pH meter). When preparing the hydrogels, 250 mL containers were filled with a specific volume of boiled DI water and allowed to cool. Then, the required amount of polymeric hydrogel powder was calculated and sieved into the container under constant stirring with a Caframo lab stirrer to ensure adequate suspension of the hydrogel in the aqueous medium. After mixing for 20 minutes, the initial pH values were collected. The polymeric hydrogels were then neutralized employing a 1.0 M KOH solution added dropwise. One container of the prepared polymeric hydrogel was neutralized to a pH value of ~4.5 and one was neutralized until a pH value of ~7 was achieved. After which, the hydrogels could be utilized for experimentation.

2.1.5 Characterization and Data Analysis

Characterization and data analysis were completed for these experiments either through graphical analysis of the CFUs, optical microscopy imaging of the *Euglena gracilis*, or general observations. To understand the bacterial results, graphical analysis was utilized, as the collection of data indicated an obvious trend that could be compared across all bacteria and polymeric hydrogels. For the viral portion of the study, general qualitative analysis was utilized to deduce the interactions between the control phage concentrations and those treated with AC through the counting PFUs. Finally, for the parasitic portion of this study, optical microscopy imaging, utilizing the digital microscope listed above, was employed to understand the interaction between the protists and the AC particles.

CHAPTER III: Results and Discussion

3.1 Bacterial Experimental Analysis

To preface the analysis of the bacterial data displayed in this section of the results, it is important to clarify that all experimental data displayed through graphical analysis was repeated in triplicate (at the very least). In addition, at the higher concentration dilution assays' CFUs were difficult, if not impossible, to quantify manually. Therefore, assays that possessed this quality were noted as too many to count (TMTC) and given an artificial colony count of 500. This was done in an effort to obtain some understanding of the data at the point where it became tangible (in the case of the dilutions that contained AC at the concentration of 10^{-5} M, as seen in **Fig. 13.**). Higher dilution concentrations (10^{-1} to 10^{-4} M) were not included, as the results across all assays at these concentrations exhibited a colony count that was described as TMTC on average, and thus, excluded from the graph to mitigate any unwarranted confusion of the obtained results. The average of all CFUs from repeated experiments was taken and graphs were made to clarify notable trends in CFU growth across all experiments which utilized the drop-plate method listed in the experimental section above (**Fig. 10.**). Finally, all pH values listed in the graphs including polymeric hydrogel employment below were rounded to the nearest integer, for simplicity (for example pH of 5 instead of ~4.5).

3.1.1 Bacterial Results without Polymeric Hydrogels

Bacterial experimentation began with control dilutions (without hydrogels) to understand the efficacy of AC as an antimicrobial agent without the presence of polymeric hydrogels, as they could possibly limit or inhibit AC from interacting with microorganisms.

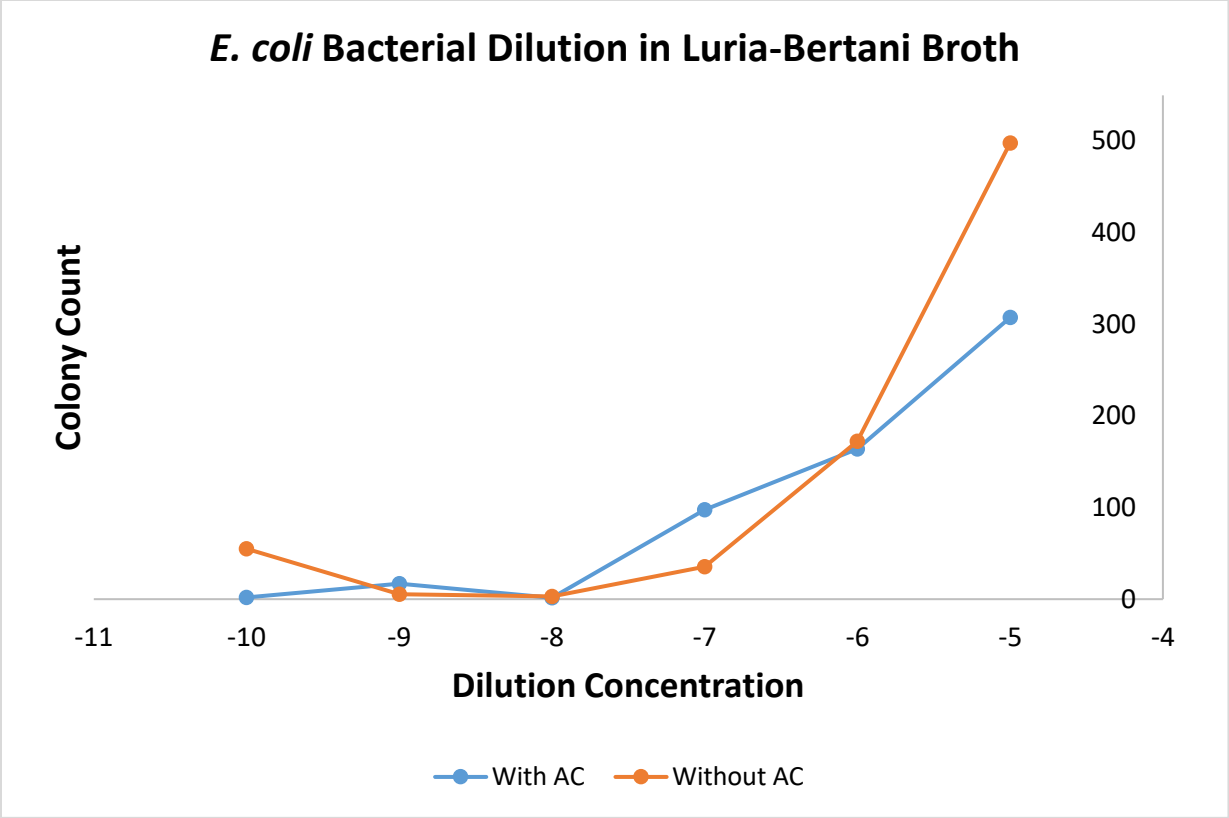

Figure 11. Graphical analysis of the bacterial dilution results utilizing the average values of all dilutions containing E. coli and Luria-Bertani broth

When analyzing the graph made to compare the average values of all *E. coli* bacterial experiments performed without the presence of polymeric hydrogels (**Fig. 11.**), discrepancies were observed in the data which challenged the efficacy of AC to act as an antimicrobial agent (specifically at the concentration of 10^{-7} M compared to the concentration of 10^{-5} M with AC). These discrepancies can ultimately be attributed to the presence of contamination and cross-contamination due mainly to storage methods and material quality (i.e. the utilization of old agar plates). Therefore, while some efficacy of AC against bacterial propagation is shown (as seen by the 10^{-5} M, 10^{-6} M, and 10^{-10} M concentrations), further experimentation incorporating updated storage and material

quality controls will be needed to delineate the efficacy of AC without the presence of polymeric hydrogels.

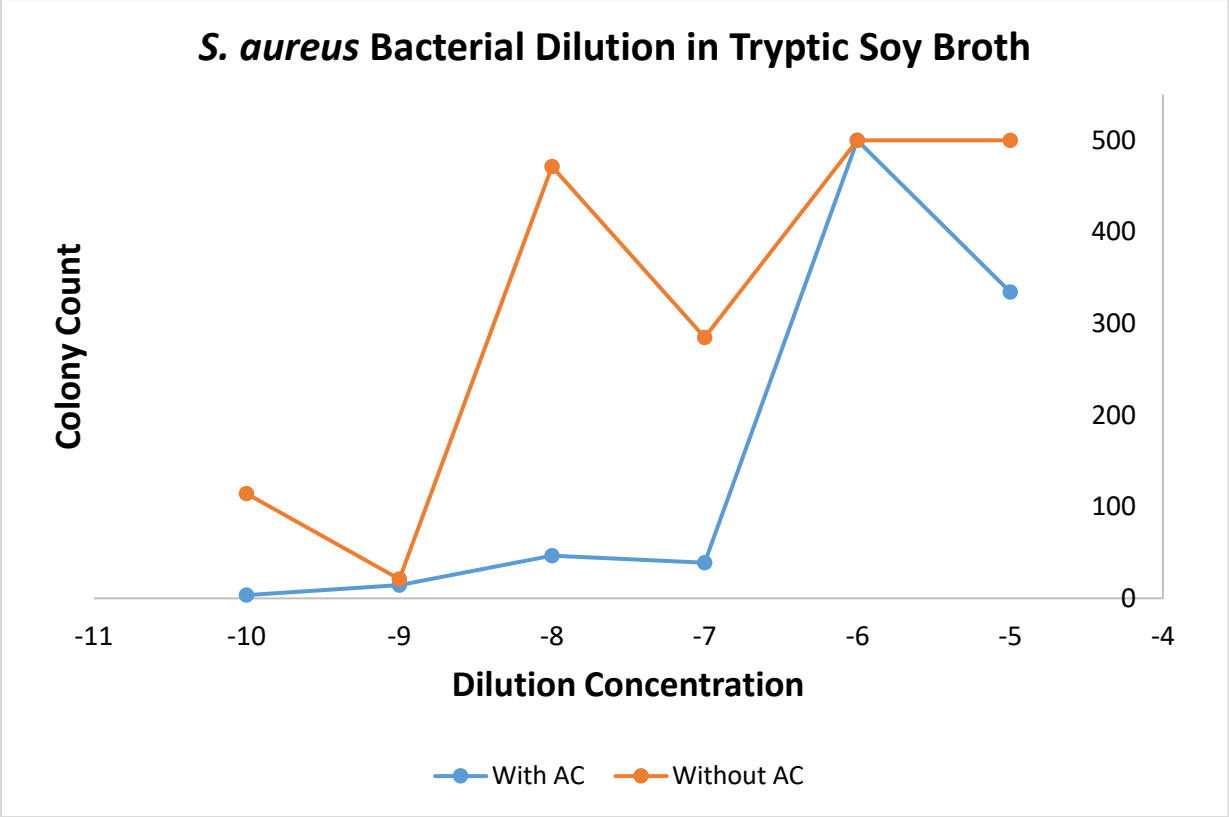

Figure 12. Graphical analysis of the bacterial dilution results utilizing the average values of all dilutions containing E. coli and Tryptic soy broth

When analyzing the graph made to compare the average values of all *S. aureus* bacterial experiments performed without the presence of polymeric hydrogels (**Fig. 12.**), a large variability in bacterial growth was observed in the control dilution (without AC). This variability may be attributed to contamination due to storage methods or cross-contamination among plates. Furthermore, the dilution containing AC exhibited a steep reduction in the propagation of bacteria as the lower dilution concentrations in comparison (10^{-7} M through 10^{-10} M). Regardless of the variability present in the control,

the antimicrobial properties of AC against *S. aureus* without the presence of polymeric hydrogels are apparent, as the dilution containing AC had far fewer CFUs overall.

3.1.2 Bacterial Results with Polymeric Hydrogels

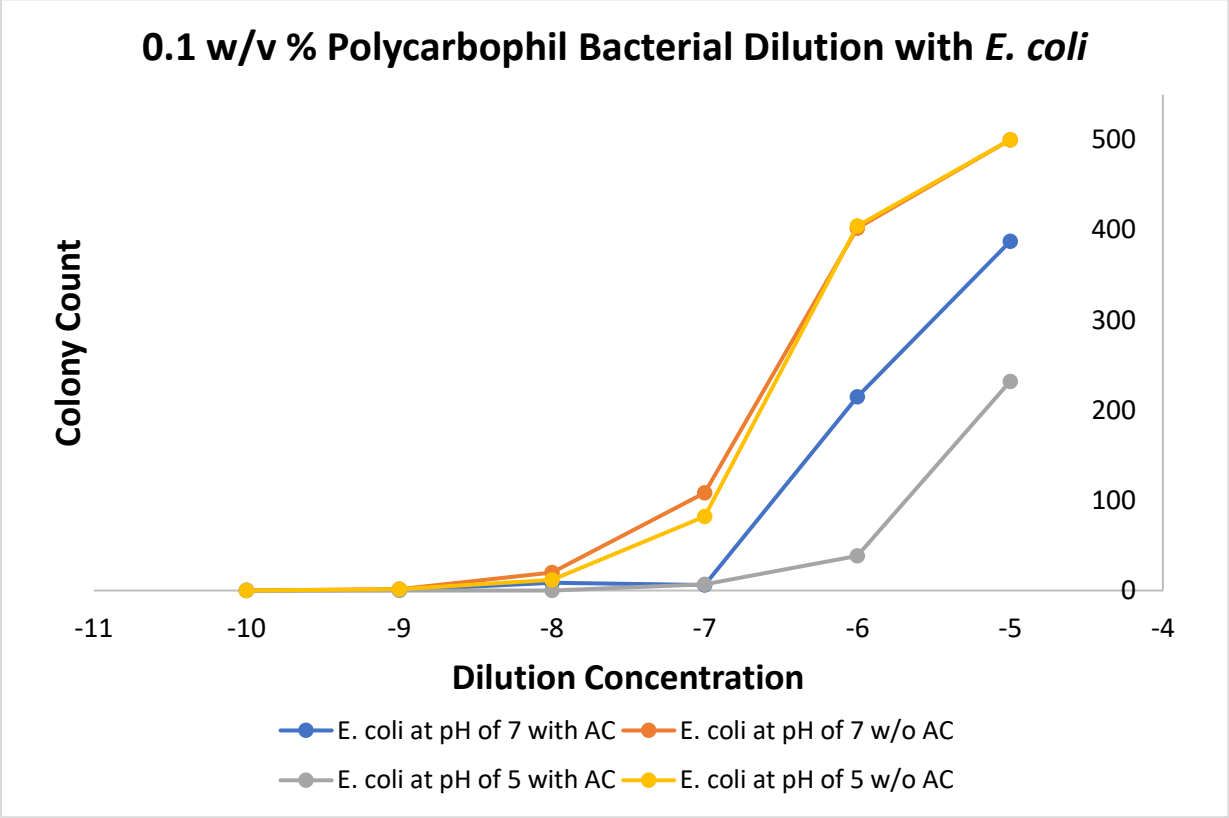

Figure 13. Graphical analysis of the bacterial dilution results utilizing the average values of all dilutions containing E. coli and 0.1 w/v % Polycarbophil

When analyzing the graph made to compare the average values of all bacterial experiments performed utilizing 0.1 w/v % Polycarbophil and *E. coli* (**Fig. 13.**), it was observed that the dilutions containing a combination of the AC particles with Polycarbophil resulted in the overall reduction of bacterial propagation at both pH values. At lower concentrations (10^{-9} to 10^{-10} M), both the control dilutions and those which contained AC exhibited similar results due to the absence of bacteria present in solution.

However, at higher concentrations of bacteria, the dilutions containing AC exhibited fewer CFUs when compared to the dilutions without AC. This disparity in CFUs indicates a clear efficacy possessed by AC to inhibit bacterial interactions in solution (although the mechanism by which this is achieved is still unknown). As a result, AC could be understood to obtain substantial antibacterial properties. In addition, the continued efficacy of AC despite the presence of the hydrogel indicates the efficacy of the 0.1 w/v % Polycarbophil as a potential carrier for the purpose of drug delivery. However, this would need to be confirmed through further experimentation (though preliminary results are promising).

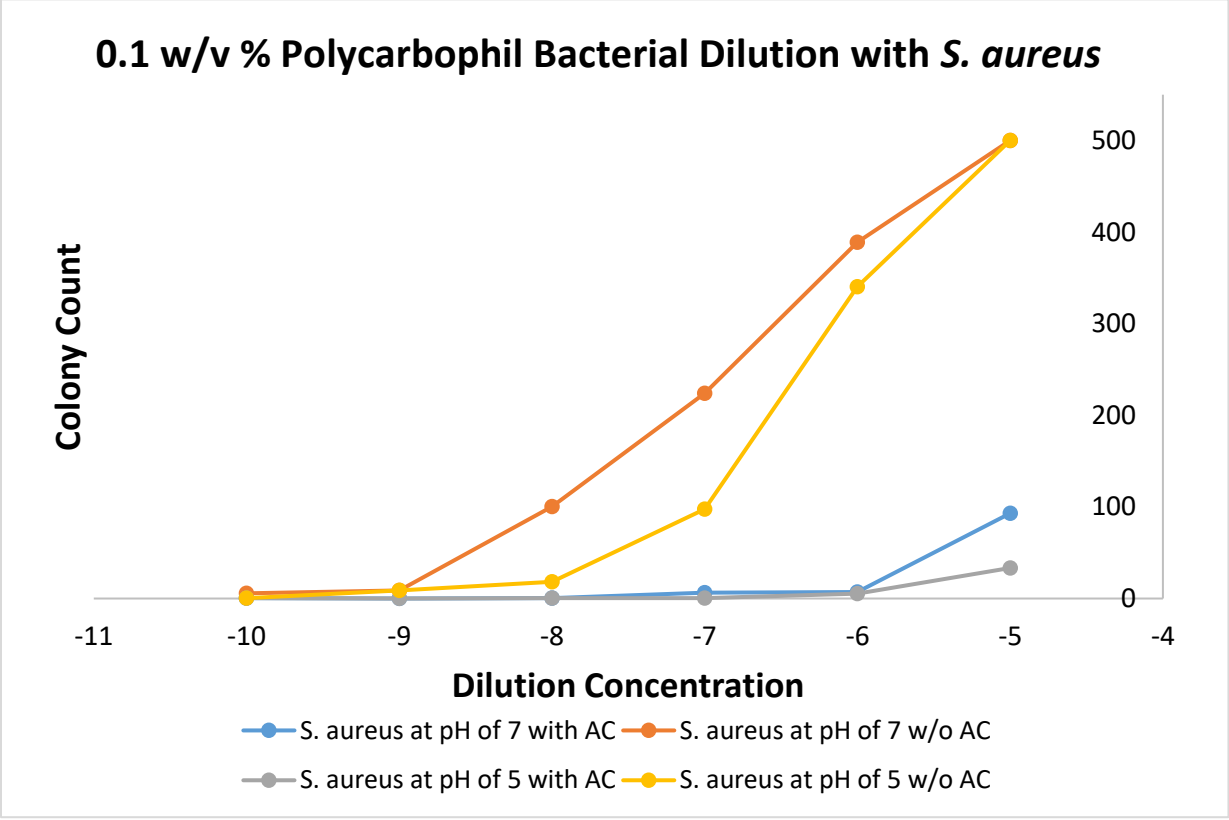

Figure 14. Graphical analysis of the bacterial dilution results utilizing the average values of all dilutions containing E. coli and 0.1 w/v % Polycarbophil

Furthermore, when analyzing the graph comparing average values of all bacterial experiments performed utilizing 0.1 w/v % Polycarbophil and *S. aureus* (**Fig. 14.**), it was observed that the dilutions containing both the AC particles and Polycarbophil resulted in an overall reduction of bacterial propagation at both pH values. In fact, the reduction of *S. aureus* in the dilutions containing AC, on average, was much higher than that seen by a similar method performed above with the employment of *E. coli* (**Fig. 13.**). This large difference in the initial colony count across the two averages indicates that the efficacy of AC as an antibacterial is higher with *S. aureus* than with *E. coli*. However, when comparing these two experiments, it can be understood that AC displays a notable antibacterial activity against both gram-positive and gram-negative bacteria. In addition, the continued efficacy of AC despite the presence of the hydrogel displayed by the results above further indicate the efficacy of the 0.1 w/v % Polycarbophil as a potential carrier for the purpose of drug delivery.

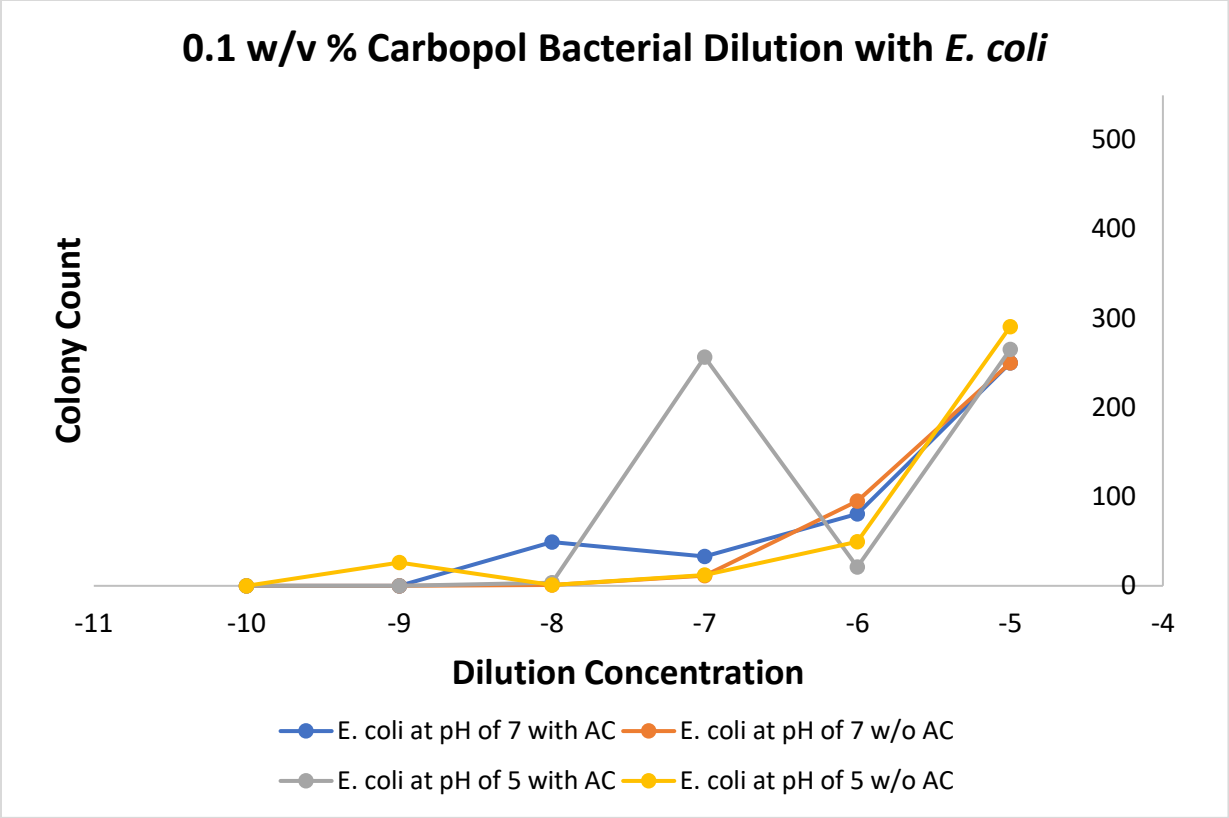

Figure 15. Graphical analysis of the bacterial dilution results utilizing the average values of all dilutions containing E. coli and 0.1 w/v % Carbopol

When analyzing the graph made to compare the average values of all bacterial experiments performed utilizing 0.1 w/v % Carbopol and *E. coli* (**Fig. 15.**), a large variability in bacterial growth was observed in the dilutions containing AC (especially at the 10^{-7} concentration in the dilution containing AC and Carbopol at a pH of 5). However, this variability could be attributed to a cross-contamination from other concentrations present in the same agar plate. In addition, all dilutions exhibited similar CFUs throughout the experimental data. As a result, no conclusion can be drawn regarding the efficacy of AC as an antibacterial agent within Carbopol hydrogels. The stark difference between the results displayed by the 0.1 w/v % Polycarbophil experiments compared to that of the 0.1 w/v % Carbopol experiments suggests some

form of inhibition against the AC particles' efficacy by the presence of the polymeric hydrogel; however, this will need to be confirmed through further experimentation.

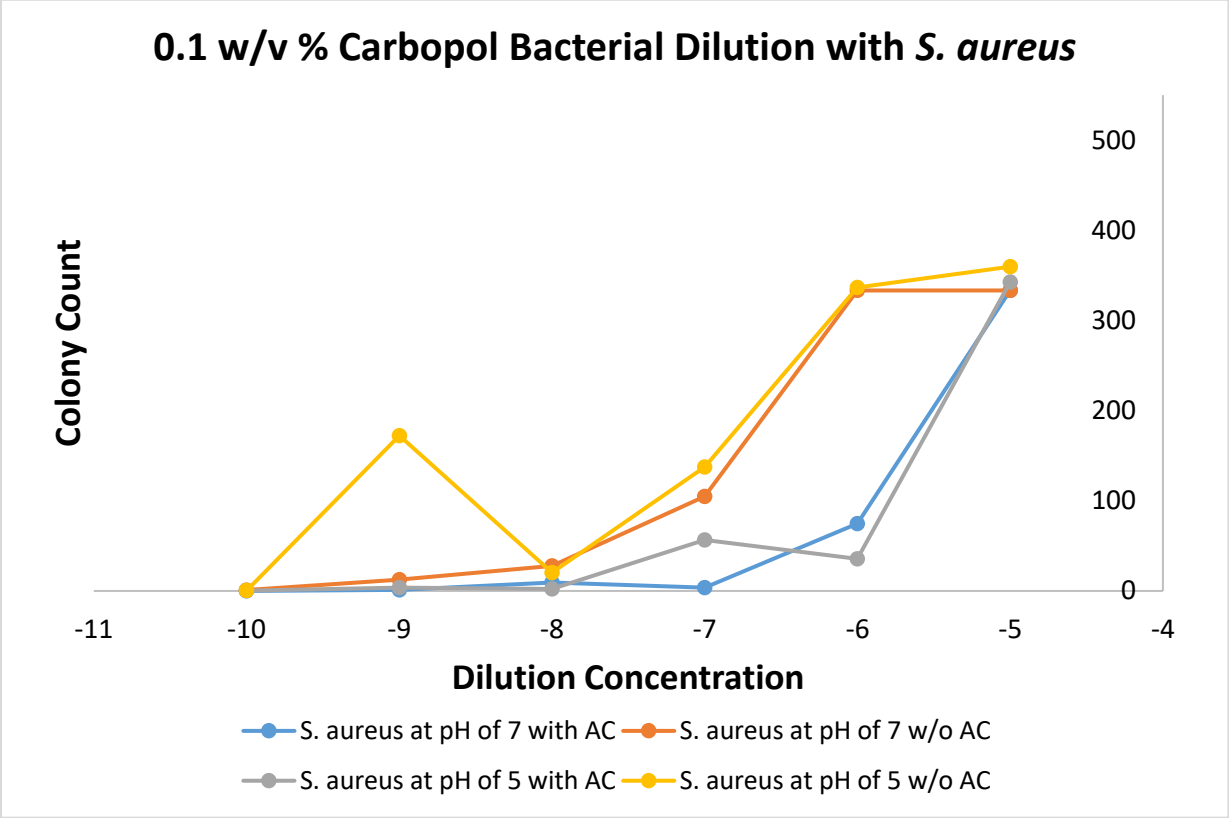

Figure 16. Graphical analysis of the bacterial dilution results utilizing the average values of all dilutions containing S. aureus and 0.1 w/v % Carbopol

When analyzing the graph made to compare the average values of all bacterial experiments performed utilizing 0.1 w/v % Carbopol and *S. aureus* (**Fig. 16.**), a general reduction in the propagation of bacteria was seen by the dilutions containing AC at both a pH of 5 and 7. Variability was still present (as seen by the 10^{-9} M concentration of the dilution without AC at a pH of 5); however, this may be attributed a number of factors, including cross-contamination from other plated dilutions. Regardless, compared to that of the dilutions containing 0.1 w/v % Carbopol and *E. coli*, the *S. aureus* experiments

were superior at exhibiting the efficacy of AC as an antibacterial agent within the hydrogel, as the reduction of CFUs upon the introduction of AC was greater for these experiments compared to experiments that utilized *E. coli* and Carbopol. This trend of resistivity – regarding *E. coli* – was also present in the experiments containing 0.1 w/v % Polycarbophil, suggesting that AC is more applicable in the removal of gram-positive bacteria, but this theory will need to be explored in later studies.

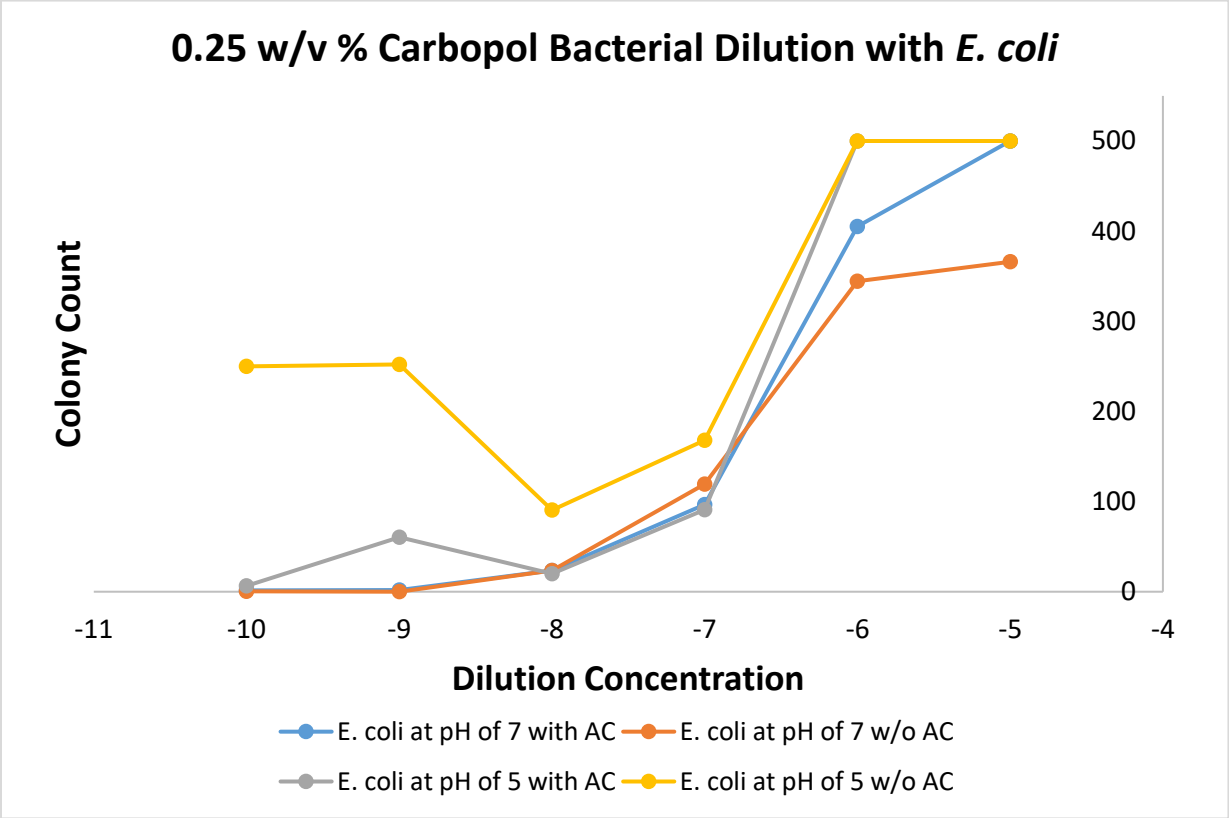

Figure 17. Graphical analysis of the bacterial dilution results utilizing the average values of all dilutions containing E. coli and 0.25 w/v % Carbopol

When analyzing the graph made to compare the average values of all bacterial experiments performed utilizing 0.25 w/v % Carbopol and *E. coli* (**Fig. 17.**), the data was not observed to exhibit any clear trend regarding the efficacy of hydrogels as a carrier for AC. When analyzing the graph above, it was noted that the dilutions containing AC had

more bacteria present than the control dilution at a pH of 7 (with the exception of the 10^{-7} concentration). As a result, the data shown in the graph was recognized as inconclusive; however, hypotheses were made to postulate why the efficacy of the hydrogels with AC varied in this round of experiments compared to those containing 0.1 w/v % Polycarbophil and will be discussed further below.

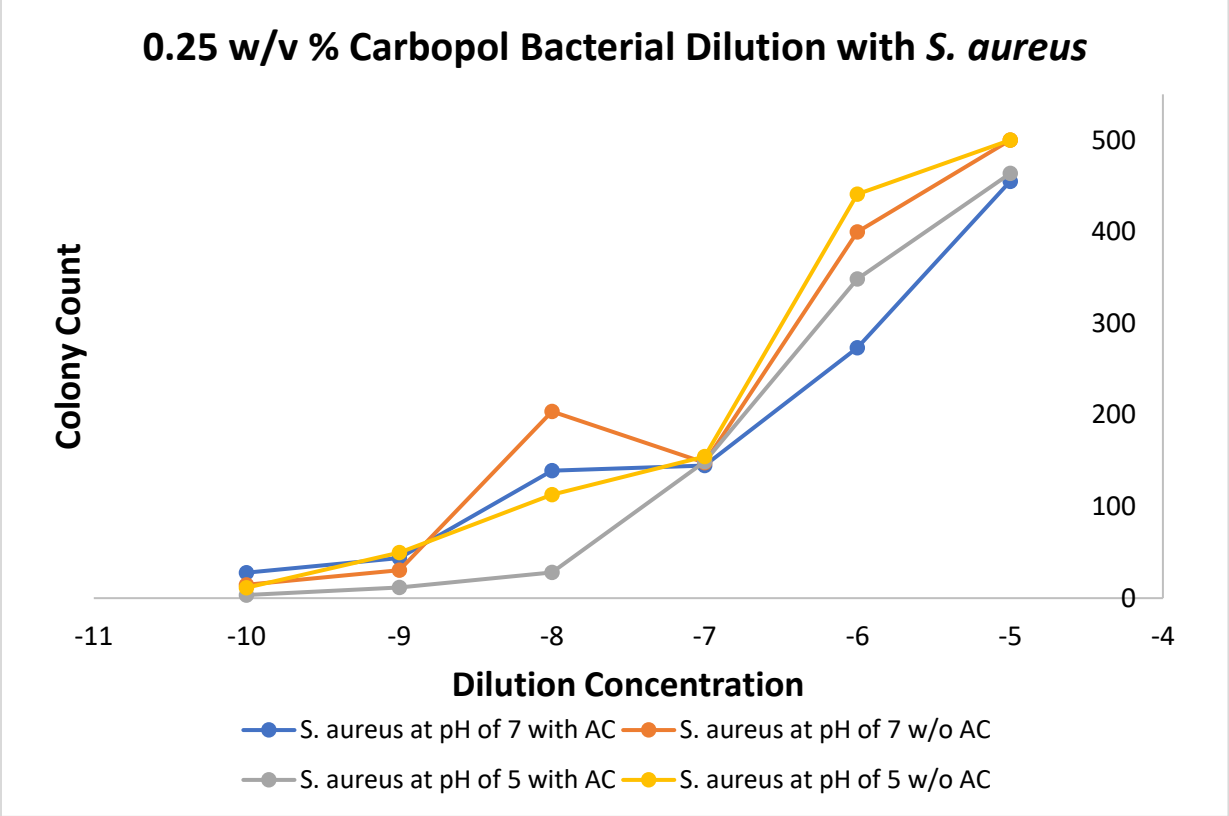

Figure 18. Graphical analysis of the bacterial dilution results utilizing the average values of all dilutions containing E. coli and 0.25 w/v % Carbopol

Finally, when analyzing the graph made to compare the average values of all bacterial experiments performed utilizing 0.25 w/v % Carbopol and *E. coli* (**Fig. 18.**), it was observed that the efficacy of the hydrogels to act as a carrier of AC was minimal if at all present. This conclusion was drawn, as the variability in bacterial growth was inconsistent throughout the experiments that employed 0.25 w/v % Carbopol which can

be seen by the dilution concentrations listed from 10^{-7} through 10^{-10}. At these concentrations, the higher CFUs alternate from the dilutions without AC to those with AC at a pH of 7. It must be noted, however, that the dilutions containing AC at a pH of 5 remained consistent, alluding to some pH dependency of AC or the polymeric hydrogel which allows for a more effective delivery and inhibition of bacterial cells. Ultimately, it has been theorized that the Carbopol hydrogel, regardless of concentration, exhibits some form of inhibition against the AC's ability to act as an antibacterial agent. Unfortunately, until this can be proven, it will remain a theory that can be explored later through further experimentation.

3.2 Viral Experimental Analysis

Bacteriophage dilutions were performed and plaque forming units (PFUs) were recorded. Graphs comparing the average number of PFUs per bacteriophage experiment were created to understand the efficacy of AC as an antiviral agent by comparing control dilutions (without AC) to dilutions with AC. It is important to note that all experimentation was repeated, at minimum, in triplicate before the graphs were created. Furthermore, assays containing PFUs that were noted as TMTC were automatically given the arbitrary value 500 for simplicity and discussion purposes.

3.2.1 T1 Bacteriophage Results and Analysis

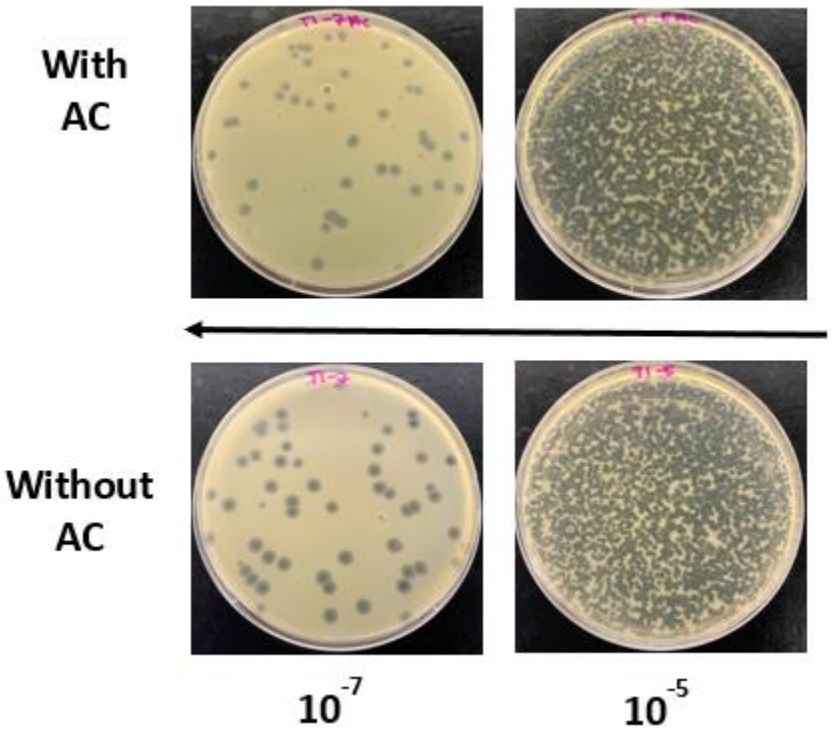

Figure 19. T1 bacteriophage experimental results comparing the control dilution (without AC) with that which includes AC (including only 10^{-5} M and 10^{-7} M for simplicity)

The results collected from the T1 bacteriophage experiments (**Fig. 19.**) indicated high antiviral activity in the dilutions with AC, as the presence of phage plaques, which qualitatively denotes the phages' ability to remove or eliminate its respective bacteria, was significantly reduced compared to the control dilutions (without AC). This was confirmed by graphical analysis of the average value of PFUs collected from all T1 phage dilutions performed (**Fig. 20.**).

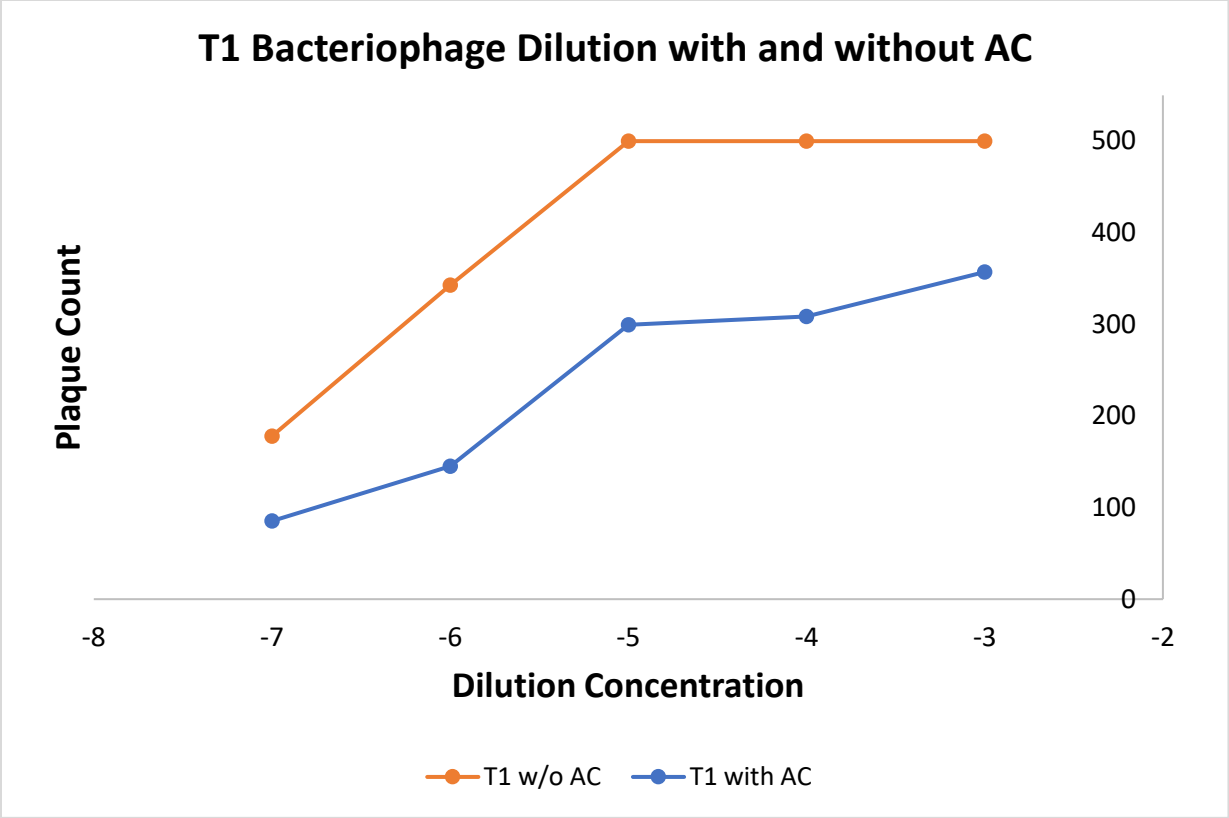

Figure 20. Graphical analysis of the T1 bacteriophage dilution results utilizing the average values of all dilution PFUs

As a result, it was observed that AC had a large effect on the propagation of T1 bacteriophages compared to the control dilution, and therefore, was determined to possess preliminary efficacy as an antiviral agent. However, to better understand the experimental results of the bacteriophage experiments, the experimental procedure should be updated, repeated, and optimized with the inclusion of polymeric hydrogels before decisive conclusions can be drawn and the efficacy of AC as an antiviral agent within hydrogels determined.

3.2.2 Φ11 Bacteriophage Results and Analysis

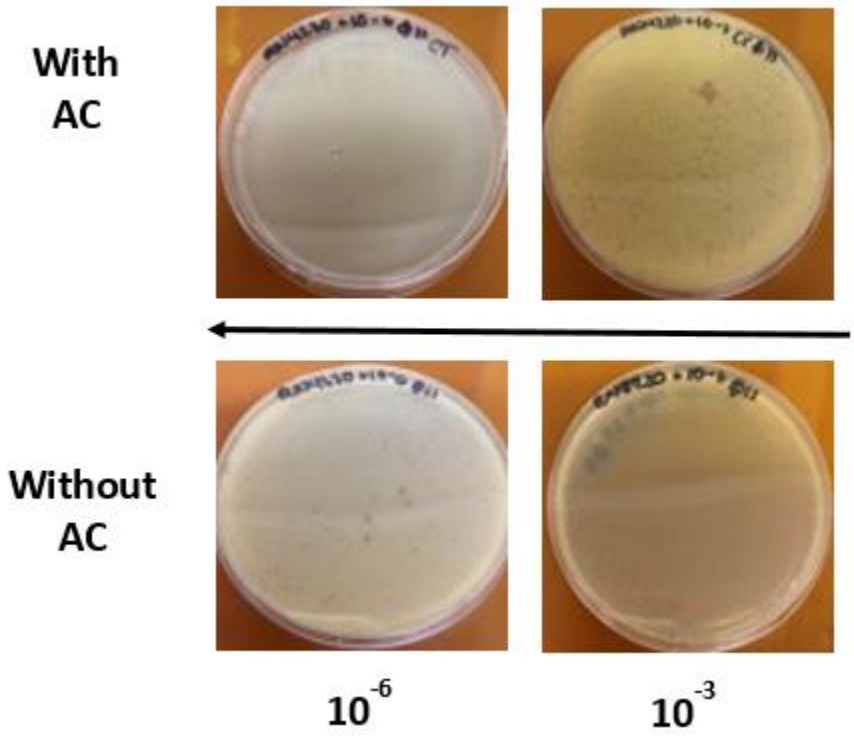

Figure 21. Φ11 bacteriophage experimental results comparing the control dilution (without AC) with the dilution containing AC (including only 10^{-3} M and 10^{-6} M for simplicity)

The results collected from the Φ11 bacteriophage experiments were observed to have similar trends in PFU formation when compared to that of the T1 bacteriophage dilutions. This similarity between the two bacteriophage results was observed through qualitative observations of the agar assays (**Fig. 21.**) and graphical analysis of the average PFUs across all Φ11 bacteriophage experiments (**Fig. 22.**)

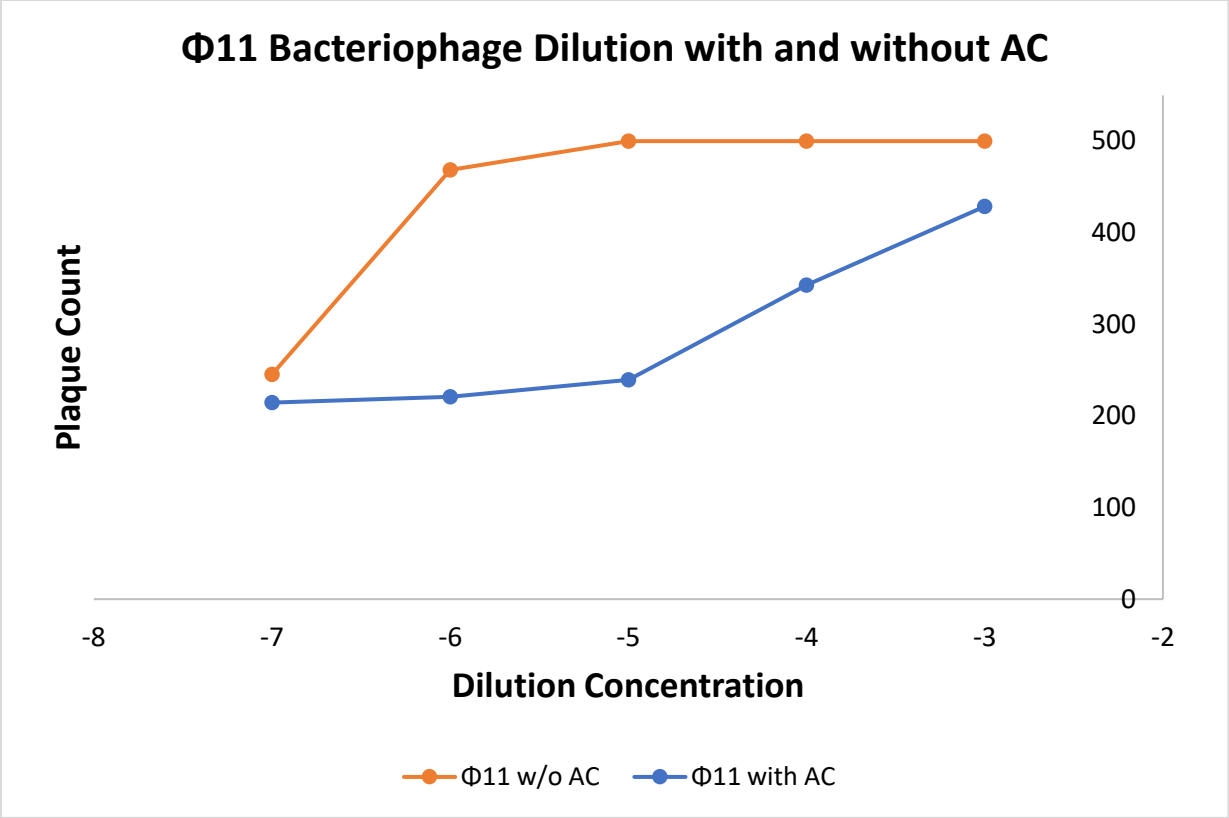

Figure 22. Graphical analysis of the Φ11 bacteriophage dilution results utilizing the average values of all dilution PFUs

The presence of PFUs was reduced significantly when comparing the dilution with AC to the dilution without. The dilutions containing AC had far fewer PFUs on average compared to the dilutions without, indicating some efficacy of AC as an antiviral agent. However, as polymeric hydrogels have not been employed in this portion of the study, it can be understood that an alteration and optimization of the experimental procedure is required to recognize AC's potential more holistically and decisively as an antiviral agent with the presence of the hydrogel as a carrying agent.

3.3 Parasitic Experimental Analysis

3.3.1 Parasitic Experimental Analysis without Polymeric Hydrogels

As mentioned previously, *Euglena gracilis* visualization was achieved through optical microscopy. To complete this, 2 to 3 drops of the mixed suspension was pipetted onto a microscope slide and images of the *Euglena gracilis* were captured at 400X magnification. When analyzing the interactions made between *Euglena gracilis* and the AC particles, it was ultimately deduced that as the concentration of AC increased, so did the interactions between the protists and the AC particles. However, with higher concentrations of AC present, it became difficult to optically visualize these interactions (such as was seen by the 90 mg suspension). As a result, optimal visualization of the protists and AC particles was achieved within the concentration range of 15 to 30 mg of AC. Within Fig. 23. below, interactions between the microorganisms and the AC particles were emphasized by circling the protists in the images as they moved throughout the suspension. As can be seen by Fig. 23., limitations of the protists' movement increased as the concentration of AC increased, as there were more protist-AC particle interactions captured in the images with higher concentrations of AC (such can be seen by the images containing 15 mg and 30 mg of AC). However, visualization of AC particle-*Euglena gracilis* interactions were limited at the highest concentration of AC (the concentration containing 90 mg of AC), making it difficult to deduce interactions through optical microscopy. As a result, the optimal concentration of AC needed for total inhibition of *Euglena gracilis* movement cannot be concluded. Furthermore, from these results, it can be generally understood that AC was effective in prohibiting the movement, and therefore, the propagation of these microorganisms. This prohibition,

ultimately, alludes to the promising capability of AC as a potential antimicrobial agent effective against parasitic protists.

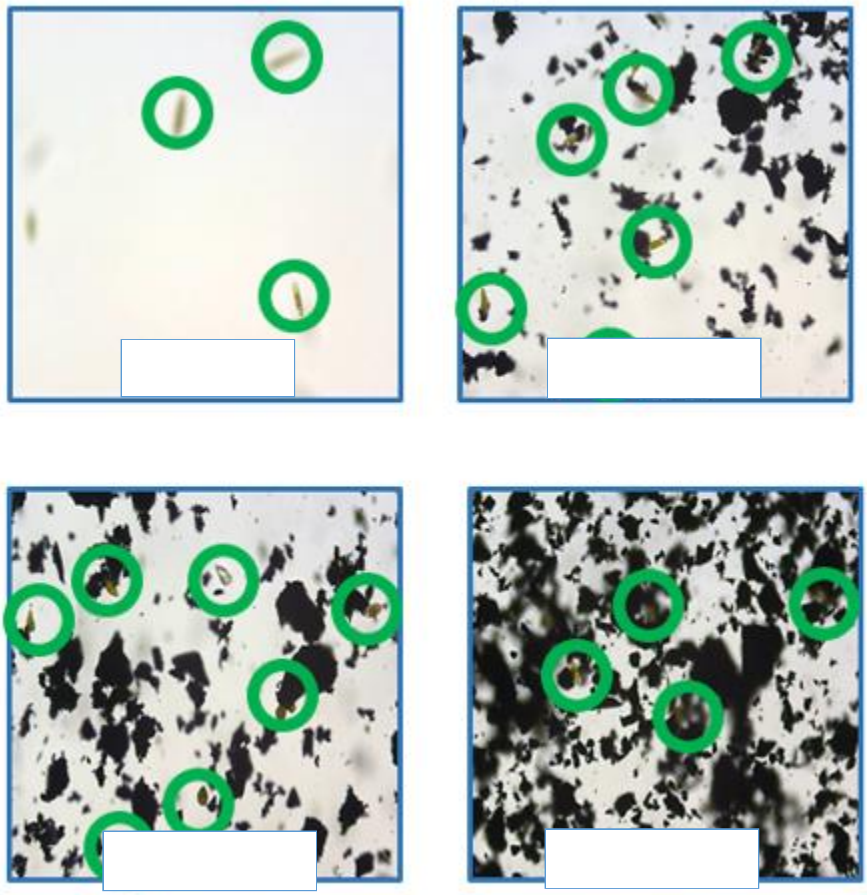

Figure 23. Euglena gracilis optical images with varying concentrations of AC present

3.3.2 Parasitic Experimental Analysis with Polymeric Hydrogels

Preliminary *Euglena gracilis* experimentation with the implementation of polymeric hydrogels was performed utilizing 0.5 w/v % Polycarbophil at pH values of ~4.5 and ~7 and 15 mg of AC to understand how or if interactions between AC particles and protists were altered in the presence of hydrogels (**Fig. 24.**).

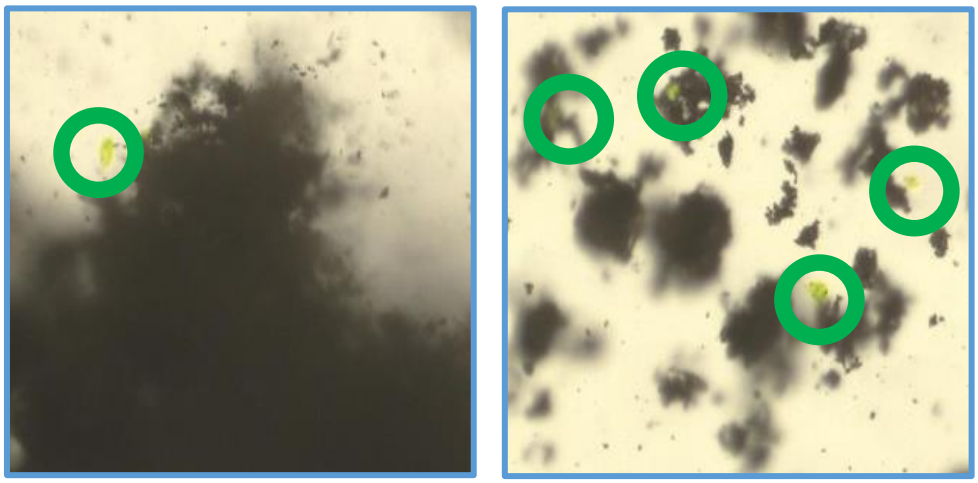

Figure 24. Euglena gracilis interactions with 15 mg of AC in 0.5 w/v % Polycarbophil at pH of ~4.5 (left) and ~7 (right)

Euglena gracilis suspensions were prepared by a similar procedure listed in the experimental section, with the only alteration being the addition of 0.5 mL of hydrogel into the vials containing DI water, *Euglena gracilis*, and AC. Since this was a preliminary study to determine the differences in interactions between AC and *Euglena gracilis* when polymeric hydrogels were introduced, vials containing varying concentrations of AC were not prepared. This development must be explored in later studies.

Overall, interactions between *Euglena gracilis* and AC were not inhibited by the presence of Polycarbophil at a pH of ~7; however, aggregation of AC particles was observed within the suspension containing Polycarbophil at a pH of ~4.5 which ultimately limited the number of interactions possible (**Fig. 24.**). This inhibition of AC may be attributed to improper mixing of the suspension, but this must be investigated further through repeated experiments. On the other hand, interactions made between AC particles and protists at a pH value of ~7 were comparable to suspensions without the polymeric hydrogel present, alluding to some efficacy of polymeric hydrogels as a drug-

delivery mechanism, as the presence of Polycarbophil did not interfere with AC's ability to adsorb the protists to its surface.

CHAPTER IV: Conclusion and Future Works

AC and polymeric hydrogels have been employed by the biomedical industry for years, however, little research has been done to combine the adsorption properties of AC and the drug-delivery capabilities of polymeric hydrogels to create an antimicrobial application for the removal of pathogens from certain bodily environments. This study attempted to understand that possibility by studying the antimicrobial efficacy of AC through the development of several experimental procedures utilizing bacterial, viral, and parasitic microorganisms. In this study, polymeric hydrogels were employed to understand and analyze their drug-delivering capabilities. Ultimately, it was found that AC possesses adequate antimicrobial efficacy; however, further investigation is needed to make decisive conclusions regarding its ability to serve as an antimicrobial agent within polymeric hydrogels. In addition, it was also observed that polymeric hydrogels do have the capability to perform as a drug-delivering agent (as its presence with the AC particles did not prevent AC's antibacterial activity); however, this result was limited to the 0.1 w/v % Polycarbophil experiments. As a result, more experiments must be performed utilizing the Polycarbophil at varying concentrations to understand its efficacy further.

In order to validate and expound on the hypotheses made by this research, additional efforts must be made to understand the effect of polymeric hydrogels on the antimicrobial efficacy of AC particles. To do this, one should further validate the efficacy of the polymeric hydrogels as a drug-delivery agent with the viral and parasitic portions of this study, explore the critical concentration of the polymeric hydrogels to understand the point at which they hinder the antimicrobial efficacy of AC, and optimize the experimental procedures so as to produce viable data.

REFERENCES

1. Shejale, K. P.; Shejale, K. P.; Yadav, D.; Patil, H.; Patil, H.; Saxena, S.; Saxena, S.; Shukla, S.; Shukla, S. Evaluation of Techniques for the Remediation of Antibiotic-Contaminated Water Using Activated Carbon. *Mol. Syst. Des. Eng.* **2020**, *5* (4), 743–756. https://doi.org/10.1039/C9ME00167K.
2. Shalkham, A. S.; Kirrane, B. M.; Hoffman, R. S.; Goldfarb, D. S.; Nelson, L. S. The Availability and Use of Charcoal Hemoperfusion in the Treatment of Poisoned Patients. *Am. J. Kidney Dis.* **2006**, *48* (2), 239–241. https://doi.org/10.1053/J.AJKD.2006.04.080.
3. Alkhatib, A. J.; Alzaailay, K.; Alkhatib Biomed J Sci, A. J.; Res, T. The Appropriate Use of Activated Charcoal inPharmaceutical and Toxicological Approaches. *Biomed. J. Sci. Tech. Res.* **2018**, *5* (1), 001–003. https://doi.org/10.26717/BJSTR.2018.05.001170.
4. Naka, K.; Watarai, S.; Tana; Inoue, K.; Kodama, Y.; Oguma, K.; Yasuda, T.; Kodama, H. Adsorption Effect of Activated Charcoal on Enterohemorrhagic Escherichia Coli. *J. Vet. Med. Sci.* **2001**, *63* (3), 281–285. https://doi.org/10.1292/JVMS.63.281.
5. Polymers for Pharmaceutical Applications. *Lubrizol*. October 25, 2021.
6. Omidian, H.; Park, K.; Sinko, P. Pharmaceutical Polymers. In *Martin's Physical Pharmacy and Pharmaceutical Sciences*; 2016.
7. Cai, N.; Li, Q.; Zhang, J.; Xu, T.; Zhao, W.; Yang, J.; Zhang, L. Antifouling Zwitterionic Hydrogel Coating Improves Hemocompatibility of Activated

Carbon Hemoadsorbent. *J. Colloid Interface Sci.* **2017**, *503*, 168–177. https://doi.org/10.1016/J.JCIS.2017.04.024.

8. Nataro, J. P.; Kaper, J. B. Diarrheagenic Escherichia Coli. *Clin. Microbiol. Rev.* **1998**, *11* (1), 142–201. https://doi.org/10.1128/CMR.11.1.142.

9. Hunter, P. R. Drinking Water and Diarrhoeal Disease Due to Escherichia Coli. *J Water Heal.* **2003**, *1* (2), 65–72.

10. Escherichia Coli http://hyperphysics.phy-astr.gsu.edu/hbase/Biology/ecoli.html (accessed Apr 16, 2022).

11. Mathelié-Guinlet, M.; Asmar, A. T.; Collet, J. F.; Dufrêne, Y. F. Lipoprotein Lpp Regulates the Mechanical Properties of the E. Coli Cell Envelope. *Nat. Commun. 2020 111* **2020**, *11* (1), 1–11. https://doi.org/10.1038/s41467-020-15489-1.

12. Foster, T. Staphylococcus. In *Medical Microbiology*; Galveston (TX): University of Texas Medical Branch at Galveston, 1996.

13. Huang, L.; Xiang, Y. Structures of the Tailed Bacteriophages That Infect Gram-Positive Bacteria. Current Opinion in Virology 2020, 45, 65–74.

14. Nair, D.; Memmi, G.; Hernandez, D.; Bard, J.; Beaume, M.; Gill, S.; Francois, P.; Cheung, A. L. Whole-Genome Sequencing of Staphylococcus Aureus Strain RN4220, a Key Laboratory Strain Used in Virulence Research, Identifies Mutations That Affect Not Only Virulence Factors but Also the Fitness of the Strain. Journal of Bacteriology 2011, 193 (9), 2332–2335.

15. Günther, J.; Petzl, W.; Bauer, I.; Ponsuksili, S.; Zerbe, H.; Schuberth, H. J.; Brunner, R. M.; Seyfert, H. M. Differentiating Staphylococcus Aureus from

Escherichia Coli Mastitis: S. Aureus Triggers Unbalanced Immune-Dampening and Host Cell Invasion Immediately after Udder Infection. *Sci. Reports 2017 71* **2017**, *7* (1), 1–14. https://doi.org/10.1038/s41598-017-05107-4.

16. Steward, K. Lytic vs Lysogenic – Understanding Bacteriophage Life Cycles | Technology Networks. *Immunology and Microbiology*. August 28, 2018.

17. Hyman, P.; Abedon, S. T. Bacteriophage: Overview. *Encycl. Microbiol.* **2019**, 441–457. https://doi.org/10.1016/B978-0-12-801238-3.02506-X.

18. Gontijo, M. T. P.; Vidigal, P. M. P.; Lopez, M. E. S.; Brocchi, M. Bacteriophages That Infect Gram-Negative Bacteria as Source of Signal-Arrest-Release Motif Lysins. *Res. Microbiol.* **2021**, *172* (2), 103794. https://doi.org/10.1016/J.RESMIC.2020.103794.

19. Lee, C. Y.; Iandolo, J. J. Structural Analysis of Staphylococcal Bacteriophage Phi 11 Attachment Sites. *J. Bacteriol.* **1988**, *170* (5), 2409. https://doi.org/10.1128/JB.170.5.2409-2411.1988.

20. Perez, E.; Lapaille, M.; Degand, H.; Cilibrasi, L.; Villavicencio-Queijeiro, A.; Morsomme, P.; González-Halphen, D.; Field, M. C.; Remacle, C.; Baurain, D.; Cardol, P. The Mitochondrial Respiratory Chain of the Secondary Green Alga Euglena Gracilis Shares Many Additional Subunits with Parasitic Trypanosomatidae. *Mitochondrion* **2014**, *19 Pt B* (PB), 338–349. https://doi.org/10.1016/J.MITO.2014.02.001.

21. Morotti, A. L. M. Synthesis of GPI Anchor Analogues to Support the Discovery of New Molecular Targets of Trypanosoma Cruzi Síntese de

Análogos de Âncora de GPI: Uma Contribuição Para a Descoberta de Novos Alvos Moleculares de Trypanosoma Cruzi, Ribeirão Preto-University of São Paulo-Ribeirão Preto, 2018.

22. Krnáčová, K.; Vesteg, M.; Hampl, V.; Vlček, Č.; Horváth, A. Euglena Gracilis and Trypanosomatids Possess Common Patterns in Predicted Mitochondrial Targeting Presequences. *J. Mol. Evol.* **2012**, *75* (3–4), 119–129. https://doi.org/10.1007/S00239-012-9523-2.

23. Hammond, M. J.; Nenarokova, A.; Butenko, A.; Zoltner, M.; Dobáková, E. L.; Field, M. C.; Lukeš, J. A Uniquely Complex Mitochondrial Proteome from Euglena Gracilis. *Mol. Biol. Evol.* **2020**, *37* (8), 2173–2191. https://doi.org/10.1093/MOLBEV/MSAA061.

24. Thomas, B. N.; George, S. C. Production of Activated Carbon fromNatural Sources. *Trends Green Chem.* **2015**, *1* (1), 0–0. https://doi.org/10.21767/2471-9889.100007.

25. Correa, C. R.; Kruse, A. Biobased Functional Carbon Materials: Production, Characterization, and Applications—A Review. *Mater. 2018, Vol. 11, Page 1568* **2018**, *11* (9), 1568. https://doi.org/10.3390/MA11091568.

26. Ghouma, I.; Jeguirim, M.; Dorge, S.; Limousy, L.; Matei Ghimbeu, C.; Ouederni, A. Activated Carbon Prepared by Physical Activation of Olive Stones for the Removal of NO2 at Ambient Temperature. *Comptes Rendus Chim.* **2015**, *18* (1), 63–74. https://doi.org/10.1016/J.CRCI.2014.05.006.

27. Borghei, S. A.; Zare, M. H.; Ahmadi, M.; Sadeghi, M. H.; Marjani, A.; Shirazian, S.; Ghadiri, M. Synthesis of Multi-Application Activated Carbon

from Oak Seeds by KOH Activation for Methylene Blue Adsorption and Electrochemical Supercapacitor Electrode. *Arab. J. Chem.* **2021**, *14* (2), 102958. https://doi.org/10.1016/J.ARABJC.2020.102958.

28. Ruiz-Rosas, R.; García-Mateos, F. J.; Gutiérrez, M. del C.; Rodríguez-Mirasol, J.; Cordero, T. About the Role of Porosity and Surface Chemistry of Phosphorus-Containing Activated Carbons in the Removal of Micropollutants. *Front. Mater.* **2019**, *6*, 134. https://doi.org/10.3389/FMATS.2019.00134/BIBTEX.
29. Juan, Y.; Ke-qiang, Q. Preparation of Activated Carbon by Chemical Activation under Vacuum. Environ. Sci. Technol. 2009, 43 (9), 3385–3390.
30. *Activated Carbon Processing - Executive Summary*; 2002.
31. Thambiliyagodage, C.; Mirihana, S.; Gunathilaka, H. Porous Carbon Materials in Biomedical Applications. *Biomed. J. Sci. Tech. Res.* **2019**, *22* (4). https://doi.org/10.26717/BJSTR.2019.22.003798.
32. Ghasemiyeh, P.; Mohammadi-Samani, S. Hydrogels as Drug Delivery Systems; Pros and Cons. *Trends Pharm. Sci.* **2019**, *5* (1), 7–24. https://doi.org/10.30476/TIPS.2019.81604.1002.
33. Sharma, G.; Thakur, B.; Naushad, M.; Kumar, A.; Stadler, F. J.; Alfadul, S. M.; Mola, G. T. Applications of Nanocomposite Hydrogels for Biomedical Engineering and Environmental Protection. *Environ. Chem. Lett.* **2018**, *16* (1), 113–146. https://doi.org/10.1007/S10311-017-0671-X.

34. Hoare, T. R.; Kohane, D. S. Hydrogels in Drug Delivery: Progress and Challenges. *Polymer (Guildf).* **2008**, *49* (8), 1993–2007. https://doi.org/10.1016/J.POLYMER.2008.01.027.

35. Fu, X.; Hosta-Rigau, L.; Chandrawati, R.; Cui, J. Multi-Stimuli-Responsive Polymer Particles, Films, and Hydrogels for Drug Delivery. *Chem* **2018**, *4* (9), 2084–2107. https://doi.org/10.1016/J.CHEMPR.2018.07.002.

36. Reddy, N. S.; Rao, K. S. V. K. Polymeric Hydrogels: Recent Advances in Toxic Metal Ion Removal and Anticancer Drug Delivery Applications. *Indian J. Adv. Chem. Sci.* **2016**, *4* (2), 214–234.

37. Xu, S.; Cavera, V. L.; Rogers, M. A.; Huang, Q.; Zubovskiy, K.; Chikindas, M. L. Benzoyl Peroxide Formulated Polycarbophil/Carbopol 934P Hydrogel with Selective Antimicrobial Activity, Potentially Beneficial for Treatment and Prevention of Bacterial Vaginosis. *Infect. Dis. Obstet. Gynecol.* **2013**, *2013*. https://doi.org/10.1155/2013/909354.

38. Céline, P. B.; Antoine, V.; Denis, B.; Laurent, V.; Laurent, D.; Catherine, F. Development and Characterization of Composite Chitosan/Active Carbon Hydrogels for a Medical Application. *J. Appl. Polym. Sci.* **2013**, *128* (5), 2945–2953. https://doi.org/10.1002/APP.38414.

39. *1.2: Biosafety Levels and PPE*; Biology LibreTexts, 2021.

40. Two ligations completed, one with a 3:2 ration the other a 3:3 ratio of killer red and TRP. http://2014.igem.org/Team:CSU_Fort_Collins/Notebook/KillSwitch/Sep (accessed Apr 1, 2022).