

The Utilization of Activated Carbon as an Antimicrobial

Against Various Pathogens

By Chloe Klusman, Zoe Gunter, and Dr. Heather Broadhead

Activated carbon (AC) has been utilized in several biomedical applications such as wound care and hemoperfusion, and in this work, experimental procedures were designed and carried out to better understand the effect of AC on bacteria, viruses, and parasites.

Antimicrobial properties of AC are relevant to the coatings industry because they can be incorporated into gel, liquid, or other coatings to impart antipathogenic properties. Possible biomedical applications include gels used for catheter tubing, drug capsule shells, and medical implants.

These experiments analyzed the ability of various sources of AC to exhibit antimicrobial properties by prohibiting the growth and propagation of pathogens. Wood, coconut, and coal-based AC were evaluated for activity against a series of bacterial dilutions with *Escherichia coli* (*E. coli*) and *Staph aureus* (*S. aureus*), a series of viral dilutions with T1 and Φ 11 bacteriophages, and a series of parasitic dilutions with *Euglena gracilis*. Results were compared and analyzed using microscopy and other standard plating techniques.

Overall, the results collected from various trials displayed a significant reduction in the activity of the pathogens. Now that the concentration requirements and responsivity for the different pathogens are known, the potential mechanisms of interaction can be investigated.

In the presence of AC, the *Euglena gracilis* were observed to become immobile upon contact with AC. Unable to remain mobile, the *Euglena* expired. The

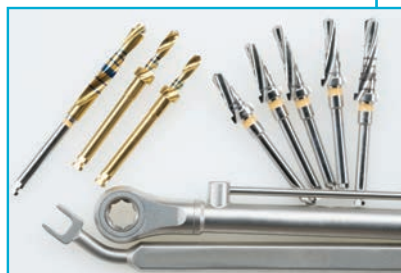
immobilization of the parasite suggests that adsorption may play a key role in antipathogenic activity, although more studies are needed to be conclusive. Further research will be conducted to use AC in new biomedical applications, which will serve not only the biomedical industry but other research areas as well.

INTRODUCTION

Activated carbon has been used in biomedical research for decades due to both its adsorptive qualities and the ease of its production. AC is most commonly generated through heterogeneous reactions that increase surface area and create highly porous structures. Many claim that the increased porosity is key to providing antimicrobial properties as their capacity for adsorption increases.¹ Accordingly, the chemical production process determines the chemical and physical characteristics, which ultimately provides the adsorptive properties of activated carbon.

AC can also be chemically tailored, as demonstrated in a study where chemical activation of olive-stone derived AC with phosphoric acid was achieved during the production process.² The phosphoric acid functionalized AC is significant because the activation of AC through chemical reactions can alter the compound's surface chemistry and thus its performance. In addition, through this activation, the micropore, mesopore, and the narrow micropore volumes were determined as well as the apparent surface area and the pore size distribution.² The extent to which the chemical activation affects these physical properties remains unknown.

The identification and utilization of the antimicrobial properties of AC is



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for Biomedical Applications

relevant to several commercial applications, as its adsorption capacity and cost-effectiveness could provide an alternative to more expensive antimicrobial products. Activated carbon is designated as GRAS (generally recognized as safe) by the FDA in some forms such as medical-grade, coconut-shell-based AC, and as such it has the potential to be quickly implemented as an antimicrobial alternative in biomedical applications.

The use of AC as an antimicrobial agent has long been of interest to researchers, as several peer-reviewed studies explore its ability to inhibit the propagation of bacteria. For example, a study performed in 2001 examined the adsorption of enterohemorrhagic *E. coli* through its exposure to AC.³ The results for the study were promising, as the propagation of the bacteria and its toxins were limited or inhibited by the presence of the AC (with better results shown at higher concentrations of AC exposure⁴).

The ease of manufacturing of AC and the breadth of raw materials makes it an effective and accessible product, as evidenced by the successful production of AC from bamboo, cherry stones, waste tea, etc., to create functional AC compounds.⁵ The study demonstrates the myriad possibilities for the production and application of activated carbon. In addition to its structural varieties, the antimicrobial and odor-reducing properties of AC make it a versatile product that can be incorporated in implant tubes, pharmaceutical capsule shells, and surface coatings. A potential commercial application is drug delivery. One study investigated the ability of AC-coated magnetic biomedical

coatings to successfully deliver drugs to their designated site. It was chosen specifically due to its apparent ability to adsorb other molecules or compounds that would be crucial in terms of drug delivery. While this recent technology is still being developed, it serves as an insight into the capabilities of AC in biomedical applications.

Bacterial testing was conducted using *E. coli* and *S. aureus* in the presence of AC. After the initial mixing and incubation period, the plated solutions exposed to AC had visibly fewer bacterial colonies of *E. coli* (Figure 1). Conversely, *S. aureus* can be seen to possess fewer colonies in the control group (Figure 2). The dilution concentrations selected for the graphs in Figure 1 and Figure 2 were concentrations that quantitatively displayed the antimicrobial qualities of AC. To understand the variations and complexities of the trends characteristic to each bacterium, further research must be conducted; however, the initial results found in this study are promising. In the future, we are interested in techniques that allow direct observation of the surface of AC to conclusively prove the theory that adsorption is the mechanism of action for AC.

Viral testing was completed using T1 and Φ 11 bacteriophages, which are types of viruses that effectively kill bacteria on surfaces. T1 bacteriophages are effective against gram-negative bacteria, *E. coli*, while Φ 11 bacteriophages are effective against gram-positive bacteria, *S. aureus*. T1 and Φ 11 are not broadly effective against gram-negative and gram-positive bacteria and were chosen because they are selective against certain strains of *E. coli* and *S. aureus*



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The Utilization of Activated Carbon as an Antimicrobial Agent

FIGURE 1—Serial dilution of *E. coli* with various AC at the 10^{-5} dilution

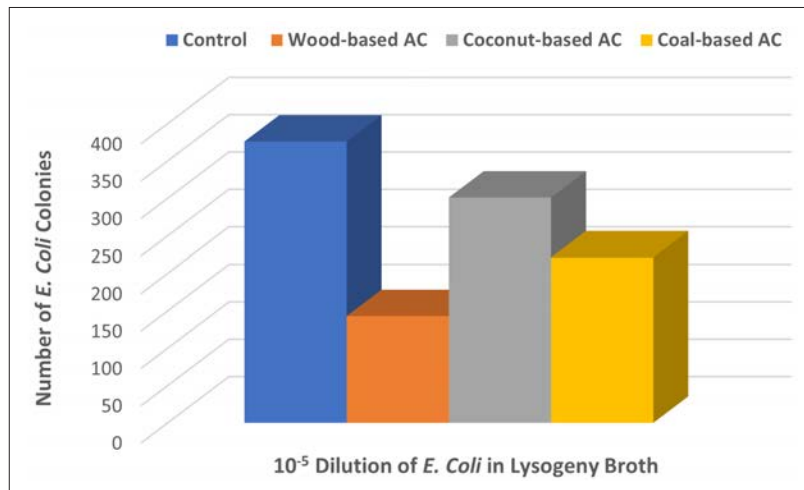


FIGURE 2—Serial dilution of *S. aureus* with various AC at the 10^{-7} dilution

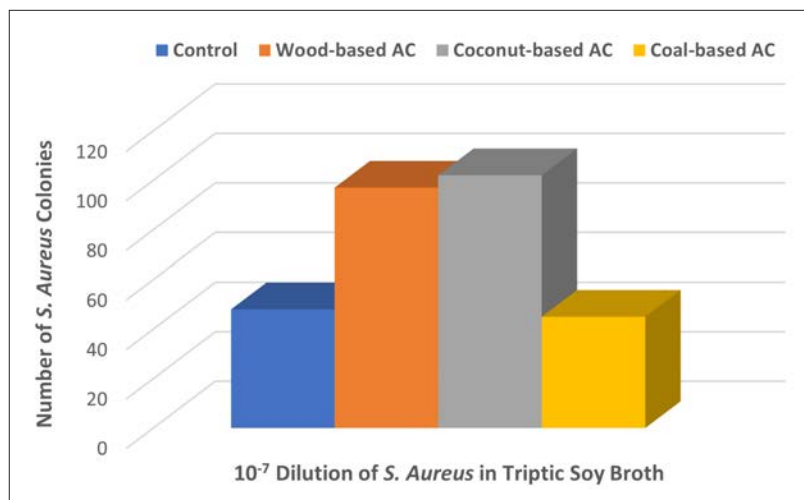


FIGURE 3—*Euglena*—as indicated by the red arrows—solution with 30 mg AC in deionized water at 400X magnification

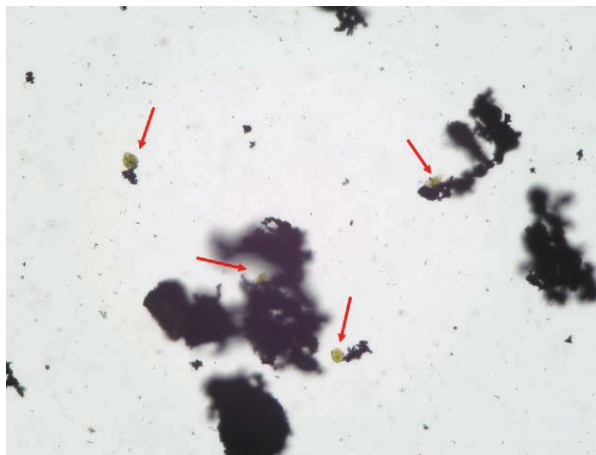
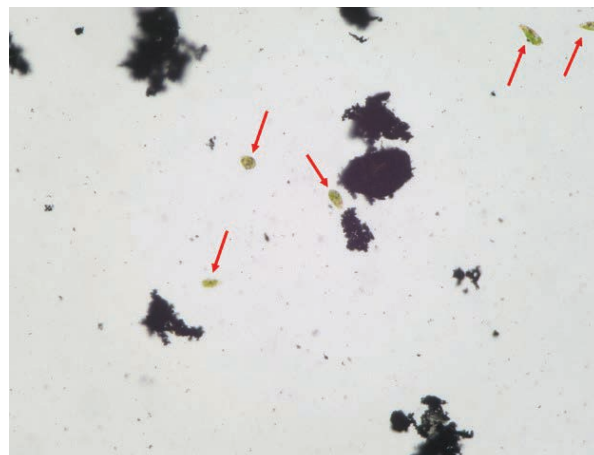


FIGURE 4—*Euglena*—as indicated by the red arrows—solution with 15 mg AC in deionized water at 400X magnification



respectively. Bacterial growth is commonly used as an indicator that the viral bacteriophages have been killed. The absence or reduction of bacterial growth indicates the viral bacteriophages have not been hindered. Experiments indicated bacterial growth in the presence of bacteriophages that had and had not been exposed to AC prior to the incubation period. When comparing the control dilution to that of the dilutions exposed to AC, the group that was exposed to AC had visibly fewer phage plaques and a larger amount of bacterial growth, thus indicating AC's activity against viral pathogens.

Parasitic testing was conducted using the flagellate protist, *Euglena gracilis*. As *Euglena* must maintain constant movement to survive, the experiment was able to test the change in activity of *Euglena* in a constant water solution with various amounts of AC by monitoring its mobility via microscope. The *Euglena* that contacted the AC particles were unable to detach from the particle, causing their movement to be limited, resulting in specimen fatality. The lower concentrations of AC in solution had less interactions with the active *Euglena*, as shown in *Figure 3* and *Figure 4*. In these images, the solution with 30 mg of AC was far better at prohibiting the movement of the *Euglena* than the solution with 15 mg of AC. This could be a

result of less AC exposed to the *Euglena*, allowing them to maneuver and swim around the particles.

The effect of AC on the selected pathogens displayed a promising outlook for using AC in varying applications and devices that require antimicrobial coatings. Further research is needed to conclusively identify the mechanism or mechanisms by which AC exhibits effectiveness, and to facilitate bringing new innovative products to market. The extensive list of raw materials to produce AC is seemingly unlimited. However, the motivation to understand how AC works continues and testing environments have been expanded to include water, tryptic soy broth, lysogeny broth, and artificial mucus to further antimicrobial applications. ❄

The study was conducted by the founding company, G.I. Jean, and has received the support of the Mississippi Defense Initiative. This study was performed in collaboration with The University of Southern Mississippi School of Polymer Science and Engineering. The authors would like to thank Angie Yonce of G.I. Jean and Dr. Monica Tisack and Caitlyne Shirley of the Mississippi Polymer Institute for their guidance and support throughout these studies.

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