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Potential of Biochar for the Removal of Waterborne Microbial Contaminants

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1. Abstract

A *Guadua chacoensis* bamboo biochar, which has shown efficacy for heavy metal, organic and inorganic compound remediation, was tested for its capacity to remove *Escherichia coli*, heterotrophic bacteria, and total coliform bacteria to determine if the biochar is a viable option for removal of pathogenic microbes in drinking water applications. Two distinct approaches were used to perform this assessment. In the first approach, an axenic culture of *E. coli* was treated using varying amounts of biochar and *E. coli* survival was assessed. In the second approach, environmental water samples taken from surface water at the Mississippi State University campus were treated with varying amounts of biochar, and the survival of heterotrophic bacteria, *E. coli*, and total coliform bacteria was assessed. Results were limited due to various issues and time constraints. One test was successful: the total coliform bacteria test for the surface water sample, in which a numerical decrease in *E. coli* was observed with an increase in biochar treatment. This decrease was statistically insignificant but coincides with the literature that suggests that biochar can reduce but not completely remove pathogens from water. Given the high standards for drinking water, biochar alone is likely not sufficient for removing pathogenic microorganisms.

2. Introduction

2.1 Background

Pathogenic microbes are the primary source of waterborne diseases, which are responsible for 2 million deaths every year (Peranovich, 2019). Examples of pathogenic microorganisms that can cause disease and death include *Cryptosporidium*, *Cyclospora*, *Escherichia coli* O157:H7, *Legionella*, *Helicobacter pylori*, hepatitis E virus, *Toxoplasma*, and others (Paul R. Hunter et al., 2001). These microbes are often carried into surface waters from

human or animal fecal matter during storm events (Cha et al., 2010). For this reason, epidemics appear frequently after heavy rainfall (Delpla et al., 2009). Even for those with access to an improved water source, these supplies are frequently contaminated with fecal coliform (Heitzinger et al., 2015).

One potential treatment option for pathogenic microbes is biochar. In previous studies a biochar was developed from discarded *Guadua chacoensis* culms to remove arsenic V from aqueous solutions (Alchouron, Navarathna, Chludil, et al., 2020; Alchouron, Navarathna, Rodrigo, et al., 2020). The goal of these studies was to develop an affordable, locally resourced, environmentally friendly product that could be utilized by households in rural regions of places like Argentina, where many people rely on well water that is naturally contaminated by arsenic. Chronic exposure to inorganic arsenic can lead to a myriad of health complications including skin, lung and bladder cancer, neurological effects, hypertension, cardiovascular and respiratory diseases, and Mellitus diabetes (Astolfi et al., 1981; Tseng, 1977; Yoshida et al., 2004).

Another prevalent source of waterborne disease and death are the pathogenic microorganisms that are found in surface waters. About 2 billion people lack microbiologically safe drinking water (Cohen & Colford, 2017). Of the 159 million people who drink directly from surface water, 147 million live in rural areas (WHO, 2017c). This study aims to determine the viability of *Guadua chacoensis* biochar as treatment for pathogenic microorganisms.

2.2 Issues associated with microbial contaminants

The consumption of pathogens via drinking water can lead to a myriad of negative outcomes. Cholera, typhoid fever, amoebiasis, and dysentery are just a few life-threatening waterborne illnesses that are spread by pathogens (Malik et al., 2012). In Argentina, diarrhea and gastrointestinal infections shorten life expectancy by over eight years (Peranovich, 2019). These

diseases threaten vulnerable age groups — those under 5 and over 50 years of age — the most severely (Peranovich, 2019). Furthermore, those who suffer from diarrhea show an increase in risk for other diseases (Malik et al., 2012). However, improved drinking water has been shown to reduce the morbidity and mortality of diarrheal diseases by an average of 22 percent and 65 percent, respectively (Esrey et al., 1991).

Water contamination and waterborne diseases are exacerbated by climate change. Where climate change causes an increase in precipitation, an increase in fecal coliform concentrations in surface water is expected (St Laurent & Mazumder, 2014). In fact, one model demonstrated that an increase in precipitation of 53.3% led to an increase of 96.0%–115.5% in fecal coliform bacteria loads entering a surface water body (Jeon et al., 2019). Seasonal and interannual variability in local rainfall explain 70% of the variability in the coliform (Delpla et al., 2009). As climate change causes temperatures to rise, increases in cyanobacteria blooms and cyanotoxins have been observed in lakes (Delpla et al., 2009). As permafrost melts and releases methane into the atmosphere, the ozone layer is depleted, allowing for increased UV radiation and more rapid decomposition of natural organic matter, which stimulates bacterial activity (Soh et al., 2008). Furthermore, higher water temperatures will likely increase pathogen survival, and there are concerns about how this will affect waterborne diseases, particularly cholera disease in Asia and South America (P.R. Hunter, 2003).

2.3 Conventional treatment methods

Although boiling water is the most prominent household water treatment method to destroy pathogens, it has its limitations. Numerous studies have demonstrated that boiling provides quantifiable health benefits against waterborne pathogens (Cohen & Colford, 2017). However, studies also show that while boiling water significantly improves microbiological

quality, it does not completely remove the potential for waterborne pathogens, especially after the water cools and is stored (Brown & Sobsey, 2012; T. Clasen et al., 2008; T. F. Clasen et al., 2008; Fagerli et al., 2017; Luby et al., 2000; Rosa et al., 2010; Wibowo & Tisdell, 1993). Boiling is also an energy-intensive process. It is estimated that 1 kg of wood is needed to boil 1-3 L of water (Pichel et al., 2019). In peri-urban Zambia, the estimated cost of boiling was 5% of income for fuel and 7% of income for electricity (Psutka et al., 2011). In rural Vietnam, the estimated cost of wood used to boil water was $1.68 (USD) per month for wood purchasers, representing approximately 1.04% of the average monthly income (T. F. Clasen et al., 2008). Gathering wood for boiling also directly contributes to deforestation (Bolaji, 2005). Furthermore, boiling causes water losses to evaporation, which contributes to water security concerns that are growing as climate change increases water scarcity. For the hundreds of millions of families who must gather or purchase the wood, boiling water is a burdensome, expensive, and environmentally unsustainable method for disinfecting water daily (Pichel et al., 2019).

Chemical disinfection is another common treatment method for pathogens, and it is likewise limited in its feasibility in low-income rural communities. The most common form of chemical disinfection is chlorination, in which chlorine or chlorine byproducts are added to water to form hypochlorous acid (HOCl) and hypochlorite ion (OCl^-), both of which can inactivate pathogenic microbes (Pichel et al., 2019). It requires investments in chemicals, electricity, trained operators, infrastructure, and maintenance, which make it more suitable for large treatment systems than households (Pichel et al., 2019). Furthermore, chlorination is often communally rejected because the treated water has an unpleasant taste and smell (Pichel et al., 2019). There are also concerns about the effectiveness and risks of chlorination. While chlorination can reduce the residual risk due to the presence of pathogens in the water, it cannot

sufficiently kill pathogenic particles, which explains why water-mediated disease outbreaks continue to occur where chlorination is used (Schoenen, 2002). Chlorination is also ineffective against parasitic disease-carrying cysts and eggs of protozoa and helminths (WHO, 2017a) and can cause the formation of more than 40 different carcinogenic disinfection byproducts such as trihalomethanes and haloacetic acids (Zhai et al., 2017). Because of the limitations of these conventional treatment methods for rural communities, there has been a surge in efforts to develop alternative household treatment methods, such as biochar.

2.4 Benefits of biochar

Biochar is considered a low-cost, environmentally friendly water purification technology. Sometimes agricultural waste biomass is burned in place and releases particulate matter into the air, adversely impacting visibility, human health, and regional air quality (Ryu et al., 2007). Agricultural waste disposal can be minimized by using the waste as raw material for biochar, energy, and value-added products (Qambrani et al., 2017). Thus, conversion to biochar could reduce air pollution and provide a material to clean water. Several industrial processes produce biochar as a by-product that is often landfilled (Cataldo et al., 2018; Yang et al., 2019). As the biofuel industry expands, more biochar is likely to be produced as a byproduct (Zhang et al., 2018).

While numerous studies have indicated the successes of biochar in removing heavy metals (Tan et al., 2011; Wang et al., 2010), harmful gases (Asada et al., 2002), colorants (Hameed et al., 2007), inorganic arsenic (Alchouron, Navarathna, Chludil, et al., 2020; Alchouron, Navarathna, Rodrigo, et al., 2020), and organic contaminants (Wendimu et al., 2017; Zhu et al., 2018), there are limited studies on its capacity to remove microbial contaminants in drinking water applications. However, numerous stormwater studies have noted a reduction in

bacterial concentrations when sand filters are augmented with biochar (Afrooz et al., 2018; Lau et al., 2017; Mohanty et al., 2014). Likewise, one study found that an iron oxide modified biochar was effective in removing photosynthetic bacteria from wastewater (He et al., 2017). The high surface area, porous structure, carbon content, polarity, and surface characteristics of biochar are credited for its high capacity for *E. coli* removal (Lau et al., 2017; Mohanty et al., 2014; Valenca et al., 2021). In one study, zero-valent iron (ZVI) modified-bamboo biochar reduced *E. coli* growth by almost 50% more than blank control cultures, and the biochar also sorbed silver ions to form a biochar nanocomposite that fully prevented the growth of *E. coli* cultures (Zhou et al., 2014). Another study found that biochar slows *E. coli* growth but not as effectively as activated carbon (Hill et al., 2019). Using granular biochar filters to treat water for microbial contaminants is viable, as microbes such as *E. coli* can be retained on biochar surfaces in filtration (Inyang & Dickenson, 2015).

2.5 Design considerations

While it is impossible to directly measure the ability of biochar to remove every disease-causing pathogen, *E. coli* is a widely used indicator organism for enteric bacterial pathogens (WHO, 2017b). While the absence of *E. coli* is not a guarantee for the absence of pathogens, *E. coli* is expected to respond to treatment processes similarly to fecal pathogens. Similarly, heterotrophic plate counts and total coliforms serve as indicators for the effectiveness of the disinfection of bacteria and integrity of a water distribution system (EPA, 2013; WHO, 2017b). *E. coli* may not be an ideal indicator for enteric viruses and protozoa, which can be more resistant to treatment technologies than microbes and may be present in the absence of *E. coli* (WHO, 2017b).

In this study, the raw *Guadua chacoensis* bamboo biochar was tested for its capacity to remove *E. coli*, heterotrophic bacteria, and total coliform bacteria to indicate the viability of this drinking water treatment method. First, an axenic culture of *E. coli* was treated using various amounts of biochar, and *E. coli* survival was quantified. Then the biochar was challenged against environmental water samples taken from surface water at the Mississippi State University campus. The survival of heterotrophic bacteria, *E. coli*, and total coliform bacteria was monitored in the environmental samples.

3. Materials and Methods

3.1 Preparation of biochar

The preparation of the raw biochar is detailed in sections 2.1 and 2.3 of Alchouron, Navarathna, Chludil, et al. 2020. Briefly, biochar was prepared from discarded young culm fragments of *Guadua chacoensis* in Argentina. It was subjected to slow pyrolysis at 700°C. Then it was ground, wet sieved to 150-300µm, and oven dried overnight.

3.2 Preparation of buffer solution

A buffer solution was prepared with 42.5 mg potassium phosphate monobasic (Fisher Scientific) and 190 mg pure magnesium chloride (Acros Organics) per liter deionized water. The buffer was used in filtration to dilute treated samples and to rinse filtration equipment between filtrations.

3.3 Preparation of *E. coli* culture

A soy broth was prepared with 30 g Bacto Tryptic Soy Broth per liter deionized water. The broth was autoclaved at 121°C for 15 minutes. An *E. coli* BactoBead (Edvotek, JM109) was placed into 125 mL soy broth and shaken at 50 rpm in a New Brunswick Scientific C24 Incubator Shaker (Edison, NJ) at 35°C for 24 hours. Then 1 mL of the solution was transferred to

each of six 125 mL flasks of soy broth and shaken at 60 rpm in the incubator shaker at 35°C for an additional 44 hours.

3.4 *E. coli* culture testing procedure

To determine the appropriate volume of culture to produce a countable number of colonies, different volumes (0.5 mL, 1 mL, 2 mL, 4 mL, and 10 mL) of culture were added to 20 mL of buffer solution without biochar treatment and filtered according to the m-ColiBlue24 protocol, which stains *E. coli* colonies blue and other coliform bacteria red. The total blue and red colonies is the total coliform. In this protocol, a petri dish was prepared by pouring the broth provided by the manufacturer over an absorbent pad. The diluted cultures were passed through a sterile 0.45-micron filter, after which the filter was placed on the absorbent pad in the petri dish and placed upside down in an incubator at 35 ± 0.5 ˚C for 24 hours. After the incubation period, colonies were counted and recorded. The setup of the filtration system is in Figure 1.

Figure 1. Filtration Setup

Based on the results of the volume determination, 35 mL of culture was selected as the appropriate volume for filtration. Biochar was measured into Fenshine 3.54 x 2.75-inch Tea Filter Drawstring Tea Bags (Hengyang, China), which were then placed into sterile 150 mL

containers. The six 125 mL flasks with the *E. coli* culture were combined into one 1 L container and shaken vigorously for 20 seconds to ensure a uniform solution. 50 mL of culture was placed in each of the sterile 150 mL containers with the biochar. The biochar and culture were shaken at 150 rpm for one hour on a New Brunswick Scientific C2 Platform Shaker (Edison, NJ), per the equilibrium time determined in a previous arsenic study for this biochar (Alchouron, Navarathna, Chludil, et al., 2020). Tea bags were removed from the containers and 35 mL of this treated culture from each container was tested first for *E. coli* in a biosafety cabinet using the protocol for m-ColiBlue24 Method 10029 Membrane Filtration as described above.

3.5 Environmental sample

A large water sample was taken from Chadwick Lake at Mississippi State University (33.462470, -88.791122) using standard grab sampling procedures. All tests were performed the same day of collection.

3.6 Environmental sample testing procedure

As with the *E. coli* cultures, biochar was measured into the tea bags and placed into the sterile 150 mL containers. The large environmental water sample was shaken vigorously by hand for 5 seconds, and 100 mL of the sample was poured into each 150 mL container (Figure 2). The containers were shaken for 1 hour at 200 rpm on a New Brunswick Scientific C2 Platform Shaker (Edison, NJ). As with the *E. coli* cultures, tea bags were removed from the containers and 2 mL of this treated water from each container was tested first for *E. coli* and total coliforms using the protocol for m-ColiBlue24 Method 10029 Membrane Filtration, described in section 3.4. Pure deionized water and the buffer solution were also filtered as a negative control. An additional 2 mL of treated water was tested for heterotrophs using the Heterotrophic Plate Count

Membrane Filtration Method 8242. This method uses the same protocol as the m-ColiBlue24 protocol, except that it was incubated for 48 hours according to the manufacturer's instructions.

Figure 2. Biochar, Tea Bag, and Environmental Sample

3.7 Data Analysis

Coliform density vs. biochar treatment was analyzed using linear regression. The regression was considered significant if the F-test had a p-value of less than 0.05.

4. Results and Discussion

4.1 *E. coli* culture

Due to the global covid-19 pandemic, there were significant delays in the shipment of the BactoBeads. Because of time constraints, only one trial was run, in which solids precipitated onto the filter surface, so no colonies could be counted. No colonies were observed on top of the solids.

4.2 Environmental sample

There were also significant delays in the shipment of the materials for the environmental sample trials. After much trial and error (see Appendix), one trial yielded results for total coliform.

Figure 3 gives a visual of the total coliform colonies observed in the environmental sample with each amount of biochar treatment. “DI” stands for deionized water, and “btb” stands for “blank tea bag,” or no biochar. There were no colonies observed in the DI water, so the negative control worked as expected. There were no *E. coli* colonies observed in any of the treatments, so all colonies were non-*E. coli* coliform.

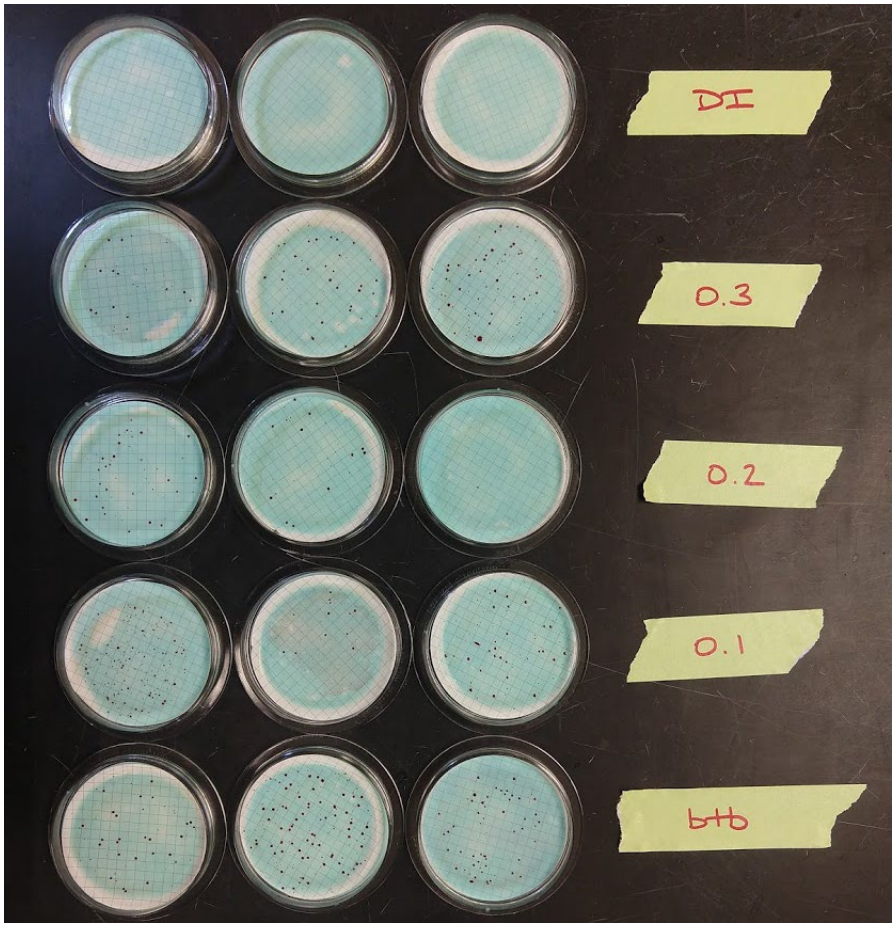

Figure 3. Total Coliform Colonies

Figure 4 is a plot of the colony counts in Figure 3 and shows that an increase in biochar led to a numerical decrease in total coliform. However, the regression line has a p-value of 0.106, so it is not significant.

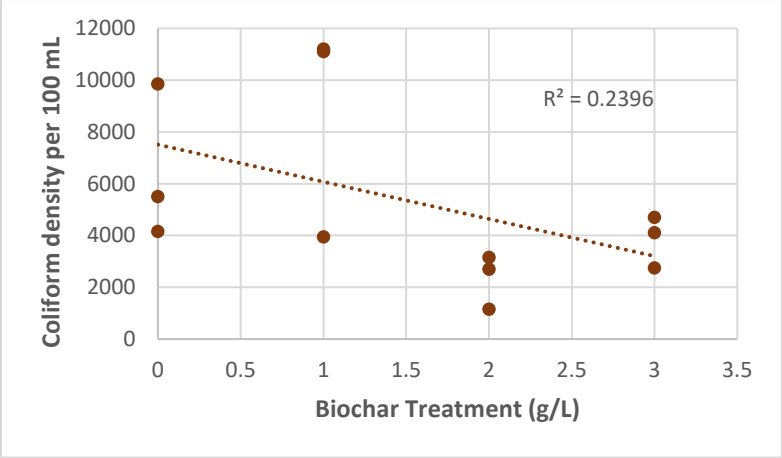

Figure 4. Coliform Density After Biochar Treatment

Even though the regression line was not significant, the reduction in coliform was approximately 50%, which is consistent with the reductions in *E. coli* growth observed in a study of ZVI modified bamboo biochar (Zhou et al., 2014). The general trend of results in this study was consistent with the trends in the literature, showing reduction but not elimination of coliform bacteria through biochar treatment (Hill et al., 2019; Lau et al., 2017; Mohanty & Boehm, 2014).

More testing with increased amounts of biochar and varied water sources is needed. Increased amounts of biochar would indicate the optimal amount of biochar and the highest capacity for removal. These tests may also validate the observed trend and explain the increase in colonies at 0.3 g. A successful trial with the *E. coli* cultures (section 4.1) and other surface water sources would help to understand the role of competition in the efficacy of the biochar.

5.0 Conclusion

Microbial contaminants in drinking water are a serious threat to public health, and the need for an affordable and sustainable treatment technology remains. Although this study's results were limited due to various issues and time constraints, the general trend observed was a decrease in total coliform as biochar treatment increased. However, the overall reduction of 50% was not significant. Further studies are needed to better understand bacteria-biochar interactions and the role of competition in biochar capacity. While biochar can be effective at reducing the concentration of microbial contaminants, it might not be practical for drinking water applications, as the public health standard is complete removal. For this reason, biochar may be better suited for wastewater and stormwater applications where water is discharged to surface waters. Pathogens in surface waters are often measured using *E. coli* as a surrogate and are a water quality indicator. The presence of pathogens can impair use for primary contact recreation, for which standards are less stringent than drinking water.

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Appendix

Trial and error process for environmental sample filtration:

Description	**Problem with Results**	**Modification**
No tea bag.	Biochar interfered with counting colonies of coliform and heterotrophs.	Place biochar in tea bag.
Used all agar provided by manufacturer for coliform and heterotrophs.	No colonies observed in coliform or heterotrophs.	Pour out excess agar from absorbent pad.
Filtered 35 mL samples.	Too many colonies to count for both coliform and heterotrophs.	Filter smaller volume.
Filtered 2 mL sample for coliform.	No problems with coliform. Heterotrophs not tested.	None needed.