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Antibacterial Potential of Activated Carbon Impregnated with Garlic Extract

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Abstract: Contamination of water resources by pathogenic microorganisms is a major concern worldwide. As an example, hospitals generate effluents with a wide range of chemical and microbiological contaminants. These effluents are generally not treated beforehand due to the high costs and are, therefore, mixed with domestic effluents in regional treatment systems. Thus, actions to maintain water quality include the development of appropriate materials for its sustainable treatment. In this context, this study aims to develop natural antibacterial materials by impregnating aqueous and alcoholic extracts of garlic in activated babassu charcoal to reduce the microbial load of effluents. This material has been tested in a standard saline solution, which simulates the composition of hospital wastewater and allows bacteria to develop. The biomaterials were characterized by Scanning Electron Microscopy, pH_{PZC} assays, Boehm's method, and microbiological assays. Significant antibacterial activities were verified for the garlic extract-impregnated biomaterials; the activated carbon functionalized with HNO_3 and impregnated with aqueous garlic extract inhibited 100% of *E. coli* growth. This result pointed to garlic extract associated with babassu activated carbon as a green alternative for the pre-treatment of complex effluents, such as hospital effluents.

Keywords: garlic; allicin; babassu; antibacterial potential; impregnated activated carbon

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1. Introduction

The high level of contamination of hospital wastewater (HWW) has caused great concern in regional treatment systems. Active ingredients from medicines and human excreta, such as surfactants, cleaning agents, disinfectants, and moisturizing solutions, as well as pathogens and fecal coliforms, are commonly found in such wastewater [1–3]. Due to the complexity of such wastewater, its proper disposal is not an easy task, and on-site treatment is still challenging. Unfortunately, the most commonly used alternative to solve the problem is liquid discharge in the municipal sewer system without any pretreatment [4].

Mixing hospital wastewater with domestic wastewater has serious environmental consequences. The sewage treatment plants are not designed to receive such effluent [5], and the toxic compounds and pathogens remain in the treated effluent [6], which may contribute to the spread of antibiotic-resistant bacteria into the environment. To solve this problem, HWW should be treated before mixing with other effluents in a particular plant with different technologies involving primary, secondary, and tertiary treatments [7,8]. Particularly, the low-cost disinfection treatment with chlorine is the most commonly used technique to mitigate the pathogen population. Spite has some huge advantages, including effectiveness against a wide spectrum of pathogenic organisms. Its use is controversial because, even at low concentrations, it is toxic to aquatic life and corrosive to pipes. Moreover, chlorine may react with organic matter in wastewater, producing even more hazardous compounds such as *trihalomethanes* [9–11]. Then, chlorine should not be considered an environmentally friendly technique. The alternative is to treat HWW with well-known natural biocides, such as garlic extracts. Such extracts contain over 200 biologically active substances, including allicin and many other biologically active substances [12].

Allicin and other sulfur-containing phytoconstituents are present in cloves of garlic (*Allium sativum* L.) and may be easily extracted. The well-known garlic antibiofilm and antibacterial activities are frequently related to human healthcare [12]. Unfortunately, these phytoconstituents are highly unstable and reactive [13], which precludes the use of garlic extract directly in the HWW treatment. Thus, to prevent the biodegradability of the biocide constituents, solid support should be impregnated with the garlic extract. Unfortunately, results related to its extract in wastewater treatment, mainly supported by solid materials, are scarcely reported.

One of the solid materials mostly used in wastewater treatment plants is activated carbon. Activated carbons have been exhaustively investigated as adsorbents, with successful results in the removal of pharmaceutical compounds commonly found in HWW [14,15]. These solids themselves do not have antimicrobial properties; however, some biocide agents may be impregnated [16,17]. Then, activated carbon impregnated with biocide properties has already been investigated with successful results, mainly related to the commonly found *E. coli* strains. Inorganic substances such as iodine-doped activated carbon or cooper hydroxide-coated carbons resulted in high *E. coli* inhibition efficiency for sanitization, disinfection, sterilization, and even health purposes [18,19]. Adsorbed antibiotics such as sulfamethoxazole and gentamycin also showed huge potential [17]. These already-reported data indicate an important scientific advance. The association of activated carbon and biocide agents creates a synergism with huge advantages in hospital wastewater treatment. In other words, the use of impregnated activated carbon minimizes the direct use of biocide agents and combines environmental and economic benefits.

Antibacterial characteristics occur when activated carbons have well-impregnated biocide substances, which is a consequence of the close attachment of the biocide molecules with the required carbon superficial groups. Changes in the superficial groups may occur through functionalization, a post-activation treatment carried out with acid or basic substances [20]. Allicin and other garlic sulfur-containing phytoconstituent molecules contain highly electronically charged regions due to the sulfide groups [21]. Then, activated carbons with low electrically charged superficial groups are preferable and are more efficient in impregnation with garlic extracts. That is the reason why commercially activated carbons may be previously functionalized with acids to increase the acid-active site concentration [22] and carbon hydrophilic properties [23].

Overall, this study aimed to evaluate the acid-functionalized activated carbon impregnated with garlic extracts and its use as a potential environmentally friendly material to reduce microbial contamination in HWW. In this work, a synthetic HWW was treated with activated carbons obtained from industrial waste, such as Babassu Activated Carbon [24,25]. The biocidal action of natural components, combined with the use of carbon, can enable the reduction of microorganisms present in effluent models without impacting the environment.

2. Materials and Methods

2.1. Materials

Garlic (*Allium sativum*) was purchased, and the extract containing allicin and its derivatives was obtained by aqueous and alcoholic extraction from garlic cloves. A commercial sample of babassu-activated carbon (BAC) was donated by Tobasa Bioindustrial de Babaçu S.A. Acid functionalization of the babassu-activated carbon was carried out with reagent-grade nitric acid. The bactericide analysis was carried out using *Escherichia coli* (*E. coli*) ATCC 25922, gently donated by the Applied Microbiology Laboratory of the State University of Maringá.

2.2. Methods

2.2.1. Garlic Extract

Fresh raw garlic cloves were disinfected in a 1% sodium hypochlorite solution for 30 min before being washed thoroughly with distilled water. The cleaned garlic cloves were crushed, and extraction was carried out with two different solvents: distilled water and ethanol. It should be noted that garlic cloves do not have a pronounced odor. However, after crushing, a typical sulfuric odor is produced due to a high concentration of sulfur compounds derived from the decomposition of allicin [26]. Moreover, the extraction was conducted using 1 g of crushed garlic gloves and 3 mL of solvent. Each suspension was kept at 5 °C for 24 h [27] and filtered through a 3 μm filter. Extract samples were frozen until immediately used in the impregnation step.

Allicin is a colorless to pale yellow liquid due to traces of elemental sulfur. The molecule and its derivatives make their accurate quantitative analysis difficult [28]. Then, to estimate the concentration of sulfurous compounds in the garlic extract, the allicin concentration was measured. Allicin in the aqueous or alcoholic extracts was quantified by measuring the enzymatic activity by determining the pyruvic acid (PA) concentration after incubation [29]. As allicin is a volatile and unstable compound [30], its concentration was immediately obtained after extraction.

2.2.2. Acid Functionalization of the Babassu-Activated Carbon (BAC)

Firstly, the babassu-activated carbon was washed with deionized water at 323.15 K and dried for 24 h at 333 K. Then, it was grounded and sieved with an average of 0.180 mm (70–100 mesh ASTM) of particle size. The pH of the solution of the suspended babassu-activated carbon was 8.3.

Functionalization with nitric acid occurred for 24 h after adding 20 g of the activated carbon in 100 mL of each 1 mol L^{-1} acid solution at 200 rpm and 25 °C. Then, the activated carbon was filtered, washed with 2 L of distilled water, and dried at 48 °C for 48 h. The activated carbon treated with nitric acid is now denominated Nitric Functionalized Carbon (NFC).

2.2.3. Impregnation of Garlic Extract in the BAC and NFC Samples

Impregnation means that chemicals are finely distributed on the internal surface of the activated carbons. Such a process promotes a synergism between these substances and the solid. In the research presented herein, 10 g of BAC or NFC samples were added to 100 mL of the aqueous or alcoholic garlic extract under 180 rpm for 24 h at room temperature. Then, the suspension was filtered, and the carbon samples were kept in a desiccator for 48 h and denominated generically as biomaterials. More specifically, BACW stands for Babassu Activated Carbon impregnated with Water extract, and NFCW stands for Nitric Functionalized Carbon impregnated with Water extract. BACE is the Babassu Activated Carbon impregnated with Ethanol extract and NFCE is the Nitric Functionalized Carbon impregnated with Ethanol extract.

The allicin composition of the impregnated samples was estimated immediately after the impregnation procedure by measuring the allicin remaining in the solution. The amount of allicin impregnated in the activated carbon samples was obtained by mass balance. The release of allicin from the biomaterials was also investigated to be sure about the success of the impregnation process. In the essays, 1 g of each biomaterial was added to 100 mL of the *E. coli*-free synthetic hospital wastewater (see Section 2.2.5). The suspension was stirred for 30 min, filtered, and allicin was again measured in the remaining solution. If allicin was detected, the impregnation step was ineffective, and the biomaterial sample was not investigated in the antibacterial runs.

2.2.4. Characterization of the Activated Carbons

Samples BAC, NFC, NFCW, and NFCE were characterized through scanning electron microscopy (SEM), (FEI, Quanta 250, Hillsboro, OH, USA), N_2 adsorption and desorption isotherms, and Fourier-transform infrared spectroscopy (FTIR), (Bruker Optik GmbH, Ettlingen, Germany). Point of Zero Charge (PZC) [31] and Boehm Titration Method [32] were techniques applied in the reference activated carbon (BAC) and the functionalized samples, and impregnation was well succeeded.

Scanning electron microscopy (SEM) was used to evaluate the surface morphology of the samples. All activated carbon samples were previously fixed to aluminum stubs and gold coated. Analyses were carried out on an FEI Quanta 250 device (Hillsboro, OH, USA). The N_2 adsorption and desorption isotherms were obtained at −195.5 °C in Accelerated Surface Area and Porosimetry System (ASAP) 2020 Micrometrics equipment (USA) with liquid N_2. Samples were outgassed for 12 h at 300 °C under vacuum (10 µm Torr). Fourier transform infrared spectroscopy (FTIR) (Bruker Optik GmbH, Ettilingen, Germany) was used to identify the main functional groups. Spectra were obtained on the equipment with a resolution of 4 cm^{-1}, in the range between 4000 and 400 cm^{-1}. KBr pellets with 0.5% of activated carbon were used.

Point of zero charges (PZC) was obtained in triplicate using batch equilibrium, where activated carbon samples were put in contact with a KNO_3 solution with different initial pH values, as detailed in [31]. The surface functional groups were determined by the Boehm titration method, also in triplicate, as reported elsewhere [33]. This technique can quantitatively detect some oxygenated superficial groups—carboxylic (COOH-), lactonic (RCOOCOR-), phenolic (PhOH-), and basic groups [32,34,35].

2.2.5. Synthetic Hospital Wastewater

The composition of the synthetic hospital wastewater was based on sewage wastewater with glucose as a carbon source to provide COD with closer similarity to real HWW [36]. Quantitative values are shown in Table 1. The synthetic hospital wastewater was immediately sterilized in an autoclave at 121 °C for 15 min before contamination with the pathogen.

Table 1. Composition of the synthetic sewage wastewater with COD similar to hospital wastewater.

Compound	Concentration (mg L^{-1})
Na_2CO_3	428.60
$CaCl_2 \cdot 2H_2O$	22.10
$ZnCl_2$	0.23
$FeCl_3$	11.63
KH_2PO_4	92.19
$C_6H_{12}O_6$	938.35
$Na_2MoO_4 \cdot 2H_2O$	0.10
$(NH_4)_2SO_4$	353.57
$CuSO_4$	0.07
$MnSO_4 \cdot H_2O$	0.12
$CoSO_4 \cdot 7H_2O$	0.48
$MgSO_4$	34.68

Source: adapted from [36].

2.2.6. Bactericide Analysis

Escherichia coli ATCC 25922 were grown in Mueller-Hilton liquid culture medium at 37 °C for 24 h. The final broth was diluted using a previously prepared water turbidity calibration curve based on the McFarland scale, turbidity versus CFU/mL. To analyze the bactericidal action in liquid media, 1 g of activated charcoal impregnated with garlic extract, under different conditions, was added to hospital wastewater solution previously contaminated with 1×10^5 CFU/mL of *E. coli*. The solution was kept at room temperature (25 °C) for thirty minutes on an orbital shaker at 150 rpm. A sample of each solution was diluted using serial dilution in saline solution, plated on Mueller Hilton agar medium, and kept at 37 °C for 24 h for the final colony count [37].

2.2.7. Disk Diffusion Antimicrobial Susceptibility Testing

Culture broth containing *Escherichia coli* ATCC 25922, as detailed in item 2.26, was diluted to a concentration of 1×10^5 CFU/mL and spread on Mueller Hilton agar plates with the aid of an inoculation loop to obtain a uniform layer of microorganisms. With the plates already inoculated with *E. coli*, sterile filter paper discs with a diameter of 3 mm were placed on the plates, and 0.010 mL of garlic extract was applied to them individually. Ten plates of each extract were incubated at 37 °C for 24 h to measure the inhibition halos formed [38].

3. Results and Discussion

3.1. Allicin Concentration in Garlic Extract

As described in Section 2.2.1, the aqueous and alcoholic extracts were obtained, and the allicin concentration was analyzed through the pyruvic acid method. The concentration of allicin in the aqueous extract ranged from 0.057 to 0.061 mmol L^{-1}, with an average concentration of 0.057 ± 0.0014 mmol L^{-1}. On the other hand, values ranging from 0.046 to 0.049 mmol L^{-1} with an average concentration of 0.047 ± 0.0007 mmol L^{-1} were obtained for the alcoholic extract. Differences in extract concentration may be related to the dipole moments of allicin (4.33) and the solvents used. The dipole moment of water (2.9) and ethanol (2.37) creates a difference in electronegativity between two bonded atoms of allicin. Since the dipole moment of water is more like the dipole moment of the allicin molecule, a greater solubility and a slightly higher allicin concentration in the aqueous solution were expected [12,28,39–41].

3.2. Characterization of the Activated Carbon Samples

The BAC SEM image shown in Figure 1a has a clean and smooth surface with apparent smaller voids, as also reported by [33] for the same babassu-activated carbon. The presence of smaller voids confirms its textural properties, as shown in Table 2 regarding N_2 adsorption/desorption. Indeed, Table 2 reveals the predominance of microporous in the BAC structure (high superficial area S_{BET}), with typical total volume (V_p) and average pore diameter (d_p) close to the ones already reported [33,42].

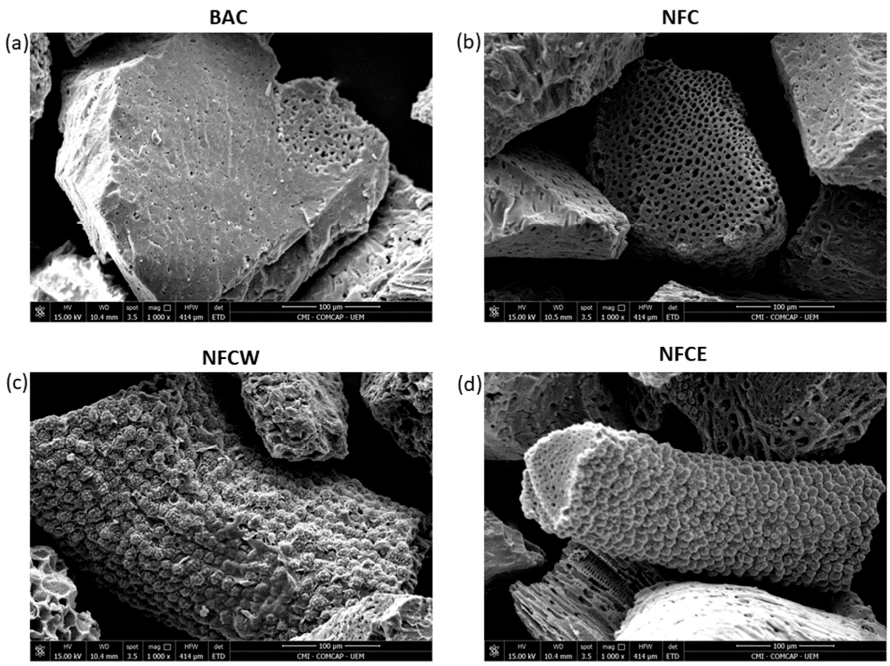

Figure 1. SEM images of activated carbon samples (**a**) BAC—Babassu Activated Carbon, (**b**) NFC—Nitric Functionalized Carbon, (**c**) NFCW—Nitric Functionalized Carbon impregnated with Water extract, (**d**) NFCE—Nitric Functionalized Carbon impregnated with Ethanol extract) at 1000× magnification.

Table 2. Textural Properties of the activated carbon samples.

Samples	S_{BET} (m^2 g^{-1})	V_p (cm^3 g^{-1})	d_p (nm)
BAC	586.08	0.3365	2.30
NFC	543.92	0.3107	2.29
NFCW	225.60	0.1333	2.36
NFCE	427.30	0.2457	2.30

According to the results shown in Figure 2, BAC presents the characteristic bands in the FTIR spectra located around 1070, 1550, 1700, and 3400 cm^{-1}. The band at 1070 cm^{-1} indicates the presence of C-O groups that are characteristic of ether groups, phenols, and alcohols. The band at 1550 cm^{-1} was associated with the vibration of the aromatic ring coupled to the carbonyl group and increased stretching of the C=O bonds. The band near 1700 cm^{-1} refers to the carbonyl absorption peaks from lactonic and carboxyl groups (C=O). After all, the band around 3400 cm^{-1}, indicates the presence of hydroxyls, usually associated with hydrogen bonding, and the presence of hydroxyls in the carboxylic groups of the activated carbons. In addition, it can be attributed to water absorbed at the surface. As a result of the charge balance of the ionization potential of the functional groups present in the BAC structure, its point of zero charges (PZC) is slightly acidic, 6.86, in agreement with [33].

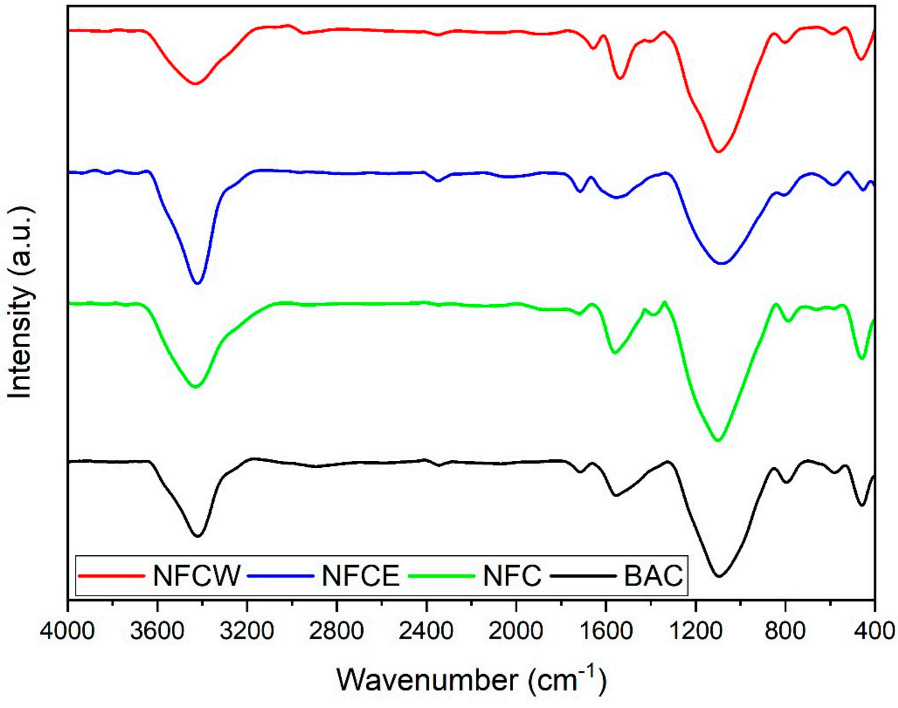

Figure 2. FTIR spectra of the activated carbon samples: NFCW (Nitric Functionalized Carbon impregnated with Water extract), NFCE (Nitric Functionalized Carbon impregnated with Ethanol extract), NFC (Nitric Functionalized Carbon), and BAC (Babassu Activated Carbon).

Through the method of Boehm (Table 3), a significant contribution of the oxygenated functional groups carboxyl, lactone, and phenol was seen, which contributed to a total of 0.55 m_{eq} g^{-1} in the babassu activated carbon. On the other hand, some basic groups are also detected (0.10 m_{eq} g^{-1}), giving an amphoteric contribution to the BAC sample. The presence of acid and some basic groups agrees with the slightly acidic point of zero charges in the BAC sample and corroborates with the respective FTIR spectrum seen in Figure 2.

Table 3. Boehm titration and PZC results.

Boehm Titration		BAC	NFC
Acid groups (m_{eq} g^{-1})	Carboxylic	0.05 ± 0.01	---
	Lactonic	0.15 ± 0.10	0.30 ± 0.06
	Phenolic	0.35 ± 0.04	0.40 ± 0.01
Basic groups (m_{eq} g^{-1})		0.10 ± 0.03	---
PZC		6.86 ± 0.00	3.07 ± 0.00

The characterization reveals the high adsorption capacity of BAC as well as a well-developed and stable surface. After the acid functionalization of BAC, the activated carbon structure oxidizes and a better surface morphology is developed, with more regular and wider cavities on the surface, as perceived in the SEM images of NFC in Figure 1b. The results found in the morphology of NFC agree with the textural analyses (Table 2). The acid functionalization slightly promoted a decrease in the surface area, decreasing the activated carbon microporosity due to the partial destruction and opening of the micropore walls. Nitric acid promoted more pronounced effects than acetic acid due to its strong acidic characteristics. Thus, NFC has a lower specific surface area than BAC, probably due to changes in the porous structure [43]. Similar characteristics were already reported [44].

Moreover, after the functionalization, a change in the intensity of the FTIR bands (Figure 1), as well as the appearance of other bands depending on the modification, were also observed. The BAC modification with nitric acid (NFC) smoothly increases the band around 1550 cm^{-1}, attributed to the exposure of the aromatic C=C bonds after the chemical leaching of the BAC surface, showing up a band around 1375 cm^{-1}, corresponding to the vibrations of $-NO_2$ bands due to the formation of various oxygen surface groups and N-O bond-containing structures (nitro groups and nitrate complexes). Electrophilic aromatic substitutions and the introduction of N-containing groups are commonly reported after treatment with nitric acid [45]. Overall, absorption spectra were rather similar, with almost identical positions and shapes suggesting lower oxidation of the carbon structure. Differences in the FTIR spectra are resultant of the change in the functional groups on the activated carbon surface and are reflected in the material acidity confirmed by the Boehm titration and PZC results presented in Table 3. After the acid treatment, the PZC became lower, going from 6.86 (BAC) to 3.07 (NFC). Since HNO_3 is a strong acid, it promoted a higher decrease in the PZC. Moreover, Table 3 shows a pronounced increase in the total acid groups of the material while the basic sites were leached. The values are expressed in m_{eq} g^{-1} of the activated carbon sample, and the results of the Boehm titration method agree with the PZC.

After the impregnation of allicin aqueous (W) and alcoholic (E) extracts in the NFC, the SEM images (Figures 1c and 1d, respectively) show the formation of a layer of a spherical nodule on the surface of the functionalized activated carbon. As a result of the formation of this additional layer, the pores are blocked, and a lower specific surface is evidenced in the textural results seen in Table 2. Impregnation decreased the superficial area and total volume of NFC samples by around 50% when samples were impregnated with an aqueous extract (NFCW), whereas only around 20% were observed for activated carbons impregnated with an alcoholic extract (NFCE). It happens because water is considered a universal solvent. Many different molecules containing sulfurous groups, besides allicin, may be extracted from the garlic cloves and strongly impregnated in the acid-functionalized activated carbons. Moreover, the pore diameter did not suffer pronounced changes in all samples.

The impregnation of allicin in the acid-functionalized activated carbon is also confirmed by the changes in the functional groups in the FTIR spectra (Figure 2). After the NFC impregnation with allicin aqueous solution (NFCW), the band around 1550 and 3400 cm^{-1} decreased in intensity, suggesting that the impregnation occurs in the phenolic and hydroxyl active sites of the NFC. In contrast, in the NFC impregnation with allicin alcoholic solution (NFCE), the presence of alcohol increased the 3400 cm^{-1} band, while the bands around 1050 and 1550 cm^{-1} decreased. Thus, the impregnation may occur in the active sites containing C-O, C=O, and −OH linkages due to the presence of phenols, alcohols, and aromatic rings coupled to the carbonyl group. Similar results are perceived in the allicin extract impregnation into the AFC.

Thus, the results confirm that acid functionalization promotes the widening of micropores and the development of new pores, which diminishes the diffusion restriction and facilitates the diffusion and retention of large organic molecules [46], facilitating the impregnation of allicin in the carbonaceous structure. Moreover, the acid treatment also oxidizes the activated carbon structure and increases the oxygenated acid groups in the NFC sample, as also reported by [45,47], which may improve the adsorption of *E. coli*. According to Burchacka et al. [17], the adsorption of Gram-negative bacteria, such as *Escherichia coli*, onto commercially activated carbon increased with increasing macroporosity, hydrophobicity, and the presence of more oxygen species on their surface. Therefore, the pH_{PZC} diminished from 6.86 to 3.07, indicating a more negatively charged surface. Consequently, this fact may not facilitate the electrostatic attraction between the gram-negative bacteria and NFC since *E. coli* also has phospholipids and lipopolysaccharides in its outer covering layer that intensify its surface negative charge [17].

Hence, the wider pores, more homogeneous surface, and oxidized chemical structure of the NFC turn it into an easy-to-modify surface. Thus, it may be expected that NFC better impregnates the garlic extracts in the carbonaceous structures due to their favorable textural and chemical properties. After the impregnation of allicin, the oxygen-related functional groups increased. This fact can be justified by the decrease of the −OH groups (around 3400 cm^{-1}) in the FTIR spectra and the increase in the presence of C-O groups (around 1070 cm^{-1}) associated with ether, phenols, and alcohols and the aromatic ring coupled to the carbonyl group (around 1550 cm^{-1}). Therefore, the allicin aqueous extract is expected to be more favorable to the adsorption of *E. coli* than the alcoholic extract due to the lower intensity of the −OH band, which may decrease its hydrophilicity, and the greater intensity of the C-O and aromatic bands, which increase their oxygen content and hydrophobic interactions between the *E. coli* and the impregnated NFC. Burchacka et al. [17] also state that the oxygen content is crucial for the adsorption of *E. coli*.

3.3. Study of the Impregnation of Allicin in the Activated Carbon Samples

Table 4 shows the impregnation of aqueous (W) and alcoholic (E) allicin solutions onto BAC and NFC. The allicin release results from the impregnated samples are also shown in Table 4 for BACW, BACE, NFCW, and NFCE.

Table 4. Estimated amount of allicin in the impregnated samples.

Impregnated Sample	Estimated Amount of Allicin (μmol g^{-1})	% Releasing Allicin
BACW	0.553	~100%
BACE	0.465	~100%
NFCW	0.408	<20%
NFCE	0.300	<20%

It can be seen that allicin was highly impregnated in the babassu samples. Despite higher concentrations, this compound was weakly impregnated in the BAC and released almost 100% from BACW and BACE. This was due to the significant presence of basic surface groups and higher pH_{PZC} (Table 3), which repel sulfur compounds from the extract. Therefore, the impregnated BAC samples were not used in the antimicrobial runs.

Nevertheless, the wider pores, more homogeneous, and available oxidized acid surface of the NFC promoted an efficient impregnation with much lower allicin released (<20%). It means a strong impregnation due to the presence of acid and oxidized functional groups that attract the sulfurous groups of allicin and its sulfurous derivative compounds.

3.4. Microbiological Analysis

The antimicrobial potential of the impregnated activated carbon was assessed using an in vitro microbiological assay and evidence of *E. coli* growth inhibition. Antimicrobial susceptibility testing by disc diffusion showed inhibition halos of 16 ± 1.3 mm for extracts obtained in water and 14 ± 1.0 mm for alcohol-based extracts, as can be seen in the images of the diffusion discs in Figure 3. According to Moreira et al. [48], this inhibition was significant, demonstrating the potential of the extracts in static media.

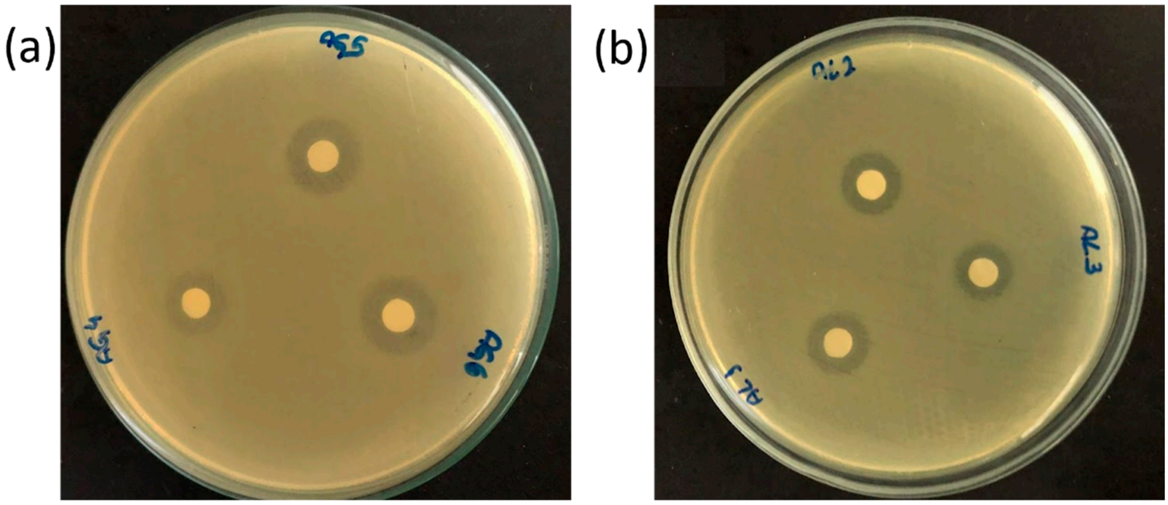

Figure 3. Antimicrobial susceptibility testing by disc diffusion for (**a**) water-based garlic extract, 16 ± 1.3 mm, and (**b**) ethanol-based garlic extract, 14 ± 1.0 mm (mean and standard deviation values obtained from 10 replicates).

As expected from the characterization and discussion presented in Section 3.2, the static tests performed for BAC and NFC did not show any inhibitory activity against the pathogen on plates [16,17]. The carbon surface itself has no antibacterial properties. On the other hand, after the modification and allicin impregnation, the biomaterials NFCW and NFCE acquired more C-O and C=C functional groups, increasing the hydrophobic interactions and showing significant inhibitory activity responses. Therefore, the synergistic effect of the aqueous garlic extract impregnated in NFCW and NFCE produced significant antibacterial activity, inhibiting around 100% of the growth of *E. coli* present in the synthetic effluent, as can be seen in Table 5.

Table 5. Antibacterial activity of coals impregnated with garlic extracts containing allicin against *E. coli*.

Sample	Raw Effluent (0.1 μL)	Post-Treatment (Mean ± sd) (Colonies)	Efficiency (%)
BAC	>200	>200	0.0%
NFC	>200	>200	0.0%
NFCW	>200	0 ± 0.67	100%
NFCE	>200	1 ± 0.51	99.5%

The images shown in Figure 4 demonstrate the inhibition of bacterial growth after the application of the extract, which is consistent with the efficacy results shown in Table 5. No colonies were detected in the effluents treated with NFCW and NFCE, a total reduction of 5 logs, which places the extract associated with activated carbon as a potential bactericidal agent, given that the average concentration of microorganisms in hospital effluents is around 10^4 to 10^7 CFU/mL [49–52].

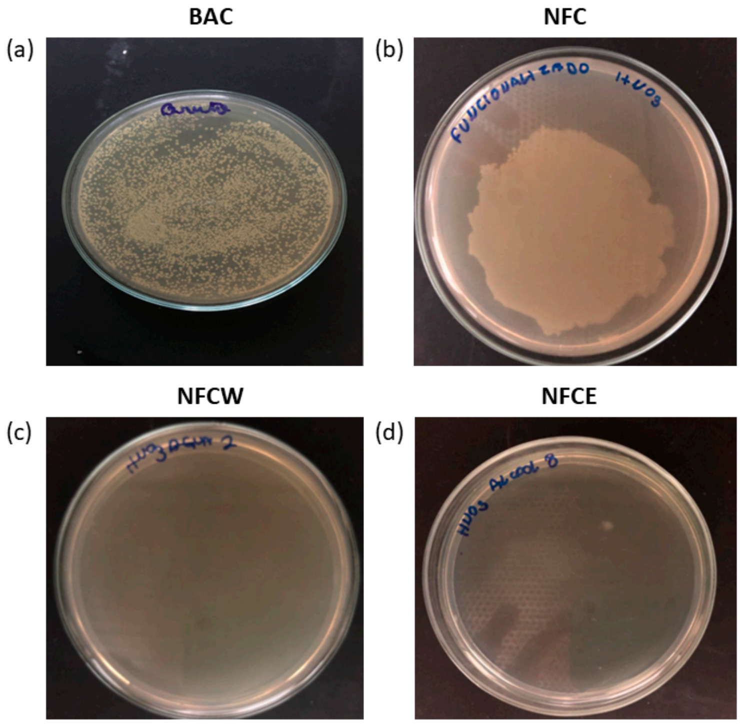

Figure 4. Microbiological test images of activated carbon samples (**a**) BAC—Babassu Activated Carbon, (**b**) NFC—Nitric Functionalized Carbon, (**c**) NFCW—Nitric Functionalized Carbon impregnated with Water extract, (**d**) NFCE—Nitric Functionalized Carbon impregnated with Ethanol extract).

Garlic has substances with a wide range of biological activities, including antibacterial actions. Possibly, the antimicrobial potential presented in the aqueous and alcoholic extracts investigated in this work is related to the presence of allicin, the main active phytochemical found in this food [53]. Similar results were already indicating the antibacterial action of garlic allicin against a range of Gram-positive and Gram-negative bacteria, including multidrug-resistant *E. coli* strains [21,54–56].

Indeed, activated carbon has been used as a support for bioactive natural extracts due to their chemical stability [57]. This property can be an important ally in effluent treatment systems using activated carbon impregnated with antimicrobial actives, where the pathogen is attached to the biomaterial and subsequently inhibited by the action of extract components.

Several works have been developed on the modification of granular activated carbon and other filter media to increase the inhibition of *E. coli* growth; see Table 6. The results obtained with allicin impregnated in BAC corroborate those of Burchacka et al. [17], who reported that activated carbon with adsorbed antimicrobial agents could provide an attractive background with potential as a new base material for pathogen elimination compared to the use of antibiotics. These authors achieved a percentage reduction in contamination of 20 to 72%. Natori et al. [18], using activated carbon doped with iodine, and Li et al. [19], using copper(II) hydroxide nanoparticles associated with carbon, obtained log reduction ranges of 4 and 3 logs, respectively. Compared to these studies, allicin extracts were able to reduce logs by up to 5 logs. Nevertheless, aqueous extracts should be chosen to provide an environmentally friendly antibacterial carbon for hospital wastewater purposes. Different supports for materials of natural origin, such as iron oxide and ferric aluminum supported on sand, can promote similar reductions of up to 99% of the microbial load in rainwater, but erosion of the components and accumulation in the filtration systems can cause clogging with prolonged use of the systems [58,59]. Indeed, activated carbon has been used as a support for bioactive natural extracts due to its chemical stability [57]. This property can be an important ally in wastewater treatment systems using activated carbon impregnated with antimicrobial agents, where the pathogen is attached to the biomaterial and subsequently inhibited by the action of extract components.

Table 6. A comparison of the results obtained in this work with the ones reported in the literature concerning the inhibition of *E. coli* growth.

Support	Impregnated Agent	Inhibition of *E. coli* Growth	Reference
Pharmaceutical activated carbon	None	20.5 to 26.5%	[17]
Pharmaceutical activated carbon	Sulfamethoxazole	47 to 72%	[17]
Activated carbon	Molecular iodine (I_2)	4.0 log E coli reduction	[18]
Activated carbon	Copper(II) hydroxide nanoparticles	3.0 to 1.6 log reduction	[19]
Sand	Iron Oxide	98 to 99%	[59]
Sand	Ferric and aluminum hydroxide	>99%	[58]
Acid-treated Activated Carbon	Allicin alcoholic solution	99.5%—5 log reduction	This Work.
Acid-treated Activated Carbon	Allicin aqueous solution	100%—5 log reduction	This Work.

4. Conclusions

The results presented herein detail a promising environmentally friendly biomaterial with biocidal properties. The babassu activated carbon, when functionalized with nitric acid, provided an attractive surface for the highly electronically charged regions of the sulfur compounds in garlic extracts. It means that allicin as well as other sulfur compounds presented in the aqueous extract were efficiently retained and successfully originated as a biocidal solid material with more than 99.5% of *E. coli* antibacterial activity presented in synthetic sewage similar to hospital wastewater.

To sum up, the highly effective biomaterial synthesized from a green route has proven applicability in the removal of pathogens in water and wastewater treatment systems, opening new perspectives for the development of in situ treatment of complex effluents such as those from healthcare systems.

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Abbreviations

Abbreviation	Meaning
BAC	Babassu Activated Carbon
BACW	Babassu Activated Carbon Impregnated with Water Extract
BACE	Babassu Activated Carbon Impregnated with Ethanol Extract
NFC	Nitric Functionalized Carbon
NFCW	Nitric Functionalized Carbon Impregnated with Water Extract
NFCE	Nitric Functionalized Carbon Impregnated with Ethanol Extract
COD	Chemical Oxygen Demand
FTIR	Fourier-Transform Infrared Spectroscopy
HWW	Hospital Wastewater
PZC	Point of Zero Charge
SEM	Scanning Electron Microscopy
S_{BET}	Superficial Area According to BET Model
V_p	Total Volume of the Activated Carbon
d_p	Average Pore Diameter of the Activated Carbon

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