Activated carbon from termite feces impregnated with silver: Preparation and evaluation of antimicrobial activity and ecotoxicity

Carvão activado obtidos de excrementos de cupim e impregnado com prata: Preparação e avaliação da actividade antimicrobiana e ecotoxicidade

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Abstract

Silver has a potent antimicrobial action, but its use is still restricted due to toxicity and aggregation problems. Impregnation with active carbon (AC) has been suggested as an alternative to solve this problem. In this study, termite feces were used to prepare composites containing silver nanoparticles and AC. Reducing agents and different amounts of H₂SO₄ and silver were used. Antimicrobial activity was assessed using the minimum inhibitory concentration (MIC) and the kinetics of microbial growth of S. aureus, E. coli and C. albicans. Phytotoxicity and acute toxicity were assessed for Cucumis sativus and Artemia salina. The composites that presented MIC 500 μg/mL for S. aureus and E. coli were AC25Ag (6 μg/mL Ag) and AC25AgB (78 μg/mL Ag), respectively. AC6AgS showed antimicrobial activity in C. albicans with MIC of 500 μg/mL (23 μg/mL Ag). AC25AgB inhibited the growth of S. aureus and E. coli for 48 and 32 h, respectively. At a concentration of 10 g/L, the compounds showed deleterious effects on seedling germination and elongation and on the chlorophyll synthesis of C. sativus. The composites showed no toxicity to A. salina. Based on these results, AC25AgB has the potential to be used in water disinfection.

Keywords: Activated carbon, silver nanoparticles, antimicrobial activity, toxicity, Cryptotermes brevis

Resumo: Activated carbon, silver nanoparticles, antimicrobial activity, toxicity, Cryptotermes brevis

A prata tem uma acção antimicrobiana potente, mas seu uso ainda é restrito devido a problemas de toxicidade e agregação. A impregnação no carvão activo (CA) tem sido aparentada como alternativa para resolver este problema. Neste estudo, fezes de cupins foram usadas para preparar compósitos contendo nanopartículas de prata e CA. Redutores e diferentes quantidades de H₂SO₄ e prata foram usados. A actividade antimicrobiana foi avaliada utilizando a concentração inibitória mínima (CIM) e a cinética do crescimento microbiano de S. aureus, E. coli e C. albicans. A fitotoxicidade e a toxicidade aguda foram avaliadas para Cucumis sativus e Artemia salina. A fitotoxicidade e a toxicidade aguda foram avaliadas para Cucumis sativus e Artemia salina. Os compósitos que apresentaram CIM 500 μg/mL para S. aureus e E. coli foram CA25Ag (6 μg/mL Ag) e CA25AgB (78 μg/mL Ag), respectivamente. O CA6AgS mostrou actividade antimicrobiana em C. albicans com CIM de 500 μg/mL (23 μg/mL Ag). O CA25AgB inibiu o crescimento de S. aureus e E. coli por 48 e 32 h, respectivamente. Na concentração de 10 g/L os compostos mostraram efeitos deletérios sobre germinação e alongamento de plântulas e na síntese de clorofila da C. sativus. Os compósitos não apresentaram toxicidade para A. salina. Com bases nos resultados podemos dizer que, o CA25AgB apresenta potencial para ser utilizado na desinfecção de água.

Palavras-chave: Carvão activo, nanopartículas de prata, actividade antimicrobiana, toxicidade, Cryptotermes brevis

Received / Recebido: 29/04/2020
Accepted / Aceite: 27/06/2020
Electronic Edition: www.alies.pt
Introduction

Recent years have seen a breakthrough in the development and research of nanotechnology and nanomaterials. Metal nanoparticles have wide functionality in a range of application areas, such as electronics, biological sensors, drugs, and water treatments. One of the recent advances is the development of silver nanoparticles (AgNPs), which have attracted much attention in scientific research due to their physical, chemical, and biological characteristics (1,2). In recent years, some nanomaterials impregnated with AgNPs have been reported to have superior bactericidal properties, and are widely used to water disinfectants (3-8).

The antibacterial effect of silver has been explored since ancient civilizations. The exact mechanism of silver compounds against microorganisms is not yet fully understood, but the most likely mechanism is the direct interaction with the cell membrane and DNA, and the triggering of biochemical cascades that lead to the production of free radicals, changing the permeability of the cell membrane (9,10).

An important issue in the antibacterial activity of AgNPs is the release of Ag+ ions due to their oxidation in the presence of water and aggregation in their dispersion due to the attractive forces between the particles and the surface. To prevent aggregation and maintain an effective antibacterial effect, the incorporation of the AgNPs into stabilizing materials has been extensively investigated (11,12). AgNPs can be synthesized by several approaches, including physical, chemical, and biological. Chemical synthesis involves the reduction of the silver ion from silver salts, such as silver nitrate, by means of reducing agents. The most commonly used reducing agents are borohydride, sodium citrate, ascorbic acid, alcohol and hydrazine compounds. The reduction of silver ions (Ag+) in aqueous solution leads to the formation of silver atoms (Ag0), followed by the formation of oligomeric clusters (13-15).

Considerable attention has been paid in recent years to impregnation of AgNPs in activated carbon (AC), a widely used material for water treatment worldwide. AC can be prepared from various organic and inorganic materials. The most commonly used are coal, rice husks, coconut, and wood, or any lignocellulosic material (16). AC is known to be an adsorbent material capable of removing a wide variety of organic and inorganic matter due to its main characteristics, which include a large surface area, a high degree of microporosity, and the presence of a broad spectrum of functional groups (17). It can come from different materials, provided the material has high carbon content. In this process, vari-

Introdução

Nos últimos anos, houve um avanço no desenvolvimento e pesquisa de nanotecnologia e nanomateriais. Nanopartículas de metal têm ampla funcionalidade em diversas áreas de aplicação, como eletrônicos, sensores biológicos, medicamentos e tratamentos de água. Um dos recentes avanços é o desenvolvimento de nanopartículas de prata (NPAg), que têm atraído muita atenção em pesquisas científicas devido às suas características físicas, químicas e biológicas (1,2). Nos últimos anos, tem sido relatado na literatura que alguns nanomateriais impregnados com NPAg têm propriedades bactericidas superiores, são amplamente utilizados para desinfetantes de água (3-8).

O efeito antibioticiano da prata tem sido explorado desde civilizações antigas. O mecanismo exacto dos compostos de prata contra os microrganismos ainda não está totalmente esclarecido, mas o mecanismo mais provável é interacção directa com a membrana celular e o ADN e o desencadeamento de cascatas bioquímicas que levam á produção de radicais livres, alterando a permeabilidade da membrana celular (9,10).

Uma questão importante na actividade antibacteriana dos NPAg é a libertação de ião Ag+ devido á sua oxidação na presença de água e agregação na sua dispersão devido às forças atractivas entre as partículas e a superfície. Para evitar a agregação e manter um efeito antibioticiano eficaz, a incorporação dos NPAg nos materiais estabilizadores tem sido extensivamente investigada (11,12).

Os NPAg podem ser sintetizados por várias abordagens, incluindo física, química e biológica. A síntese química envolve a redução do ião prata dos sais de prata, como o nitrato de prata, por meio de agentes redutores. Os agentes redutores mais usados são compostos de boro, hidreto, citrato de sódio, ácido ascórbico, álcool e hidrazina. A redução de íons de prata (Ag+) em solução aquosa leva á formação de átomos de prata (Ag0), seguida pela formação de aglomerados oligoméricos (13-15).

Nos últimos anos, foi prestada atenção considerável à impregnação de NPAg em carvão activado (CA), um material amplamente utilizado para tratamento de água em todo o mundo. A CA pode ser preparada a partir de vários materiais orgânicos e inorgânicos. Os mais utilizados são carvão, casca de arroz, coco e madeira ou qualquer material lenho celulósico (16). Sabe-se que a CA é um material adsorvente capaz de remover uma grande variedade de matéria orgânica e inorgânica devido ás suas principais características, que incluem uma grande área superficial, um alto grau de micropo-
ous precursors may be used to incorporate silver, such as commercial AC (18,19), corncob (20), sugarcane bagasse (21), and AC containing nutshell (17), coconut shell (22), and palm shell (23).

Based on this context, this study aimed to impregnate AgNPs in activated carbon prepared from organic matter (Cryptotermes brevis feces), using different reducing agents and different amounts of silver. The antimicrobial effect of these materials on the bacteria *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) and the yeast Candida albicans were evaluated by determining the minimum inhibitory concentration (MIC) and microbial growth kinetics. In addition, the phytotoxicity in the germination and growth of *Cucumis sativus* and the toxicity of *Artemia salina* were analyzed to determine if the material would be safe to aquatic and plant life and support its potential use for water disinfection.

### Material and Methods

#### Material

Fezes of Cryptotermes brevis fed with Pinus sp. were collected at the Biopolymers Laboratory of the Universidade do Vale do Itajaí, Brazil. All chemical reagents (grade P.A) were from Vetec, Inc. (São Paulo, SP, Brazil) and were used without purification.

#### Preparation of composites

**Preparation of activated carbon**

For the synthesis of activated carbon, termite feces were treated with 98% H$_2$SO$_4$ at a ratio of 1:1, 1:2, 1:4 and 1:6 (H$_2$SO$_4$: termite feces, w/w). Briefly, the mixture was shaken gently in a fume hood and placed in an oven at 150 °C for 24 h to achieve low-temperature complete chemical carbonization of the organic material. After carbonization, the materials were mixed with deionized water and agitated for 2 h to remove the unreacted acid (until negative sulfate test, using BaCl$_2$ (1% w/w solution) as sulfate indicator reagent). Next, the dried solid was treated at a temperature of 600 °C for 4 h in muffle furnace, in the absence of oxygen. The resulting materials were named AC50, AC25, AC12 and AC6 respectively.
Incorporation of silver nanoparticles

Synthesis without reducing agent

The AC6, AC12, AC25 and AC50 (2.0 g) were dispersed in 50 mL of distilled water. The AgNO₃ (1.0 g) was solubilized separately in 100 mL water, added to the mixture, and stirred for 10 minutes. The pH was adjusted to 10 with concentrated aqueous sodium hydroxide and the mixture stirred on a hot plate with magnetic stirring at 80 °C for 60 min, protected from light (24). Finally, the mixture was washed with distilled water and dried under vacuum for 24 h. The resulting composites were named AC6Ag, AC12Ag, AC25Ag and AC50Ag.

Synthesis with NaBH₄

The AC6, AC12, AC25 and AC50 (2.0 g) was dispersed in 50 mL of distilled water. The AgNO₃ (1.0 g) was solubilized separately in 100 mL water, added to the mixture, and stirred for 10 minutes. After the addition of NaBH₄ (0.2 g), the mixture stirred with magnetic stirring for 60 min, protected from light. Finally, the mixture was washed with distilled water and dried under vacuum for 24 h (25). The resulting composites were named AC6AgB, AC12AgB, AC25AgB, AC50AgB.

Synthesis with sucrose

The AC6, AC12, AC25, and AC50, (2.0 g) preparation were dispersed in 50 mL of distilled water. The AgNO₃ (1.0 g) was solubilized separately was solubilized separately in 100 mL water, added to the mixture and, stirred for 10 minutes. Sucrose (10 g solubilized in a sufficient amount of distilled water) was then added. The pH was adjusted to 10 with concentrated aqueous sodium hydroxide. The mixture stirred on a hot plate with magnetic stirring at 80 °C for 60 min, protected from light. Finally, the mixture was washed with distilled water and dried under vacuum for 24 h (26). The resulting composites were named AC6AgS, AC12AgS, AC25AgS, AC50AgS.

Characterization

pH on Point of zero charge (pH_{pzc})

A suspension of 100 mg AC in 20 mL of distilled water in the presence of 50 mM of NaCl was shaken for 24 h at 25 °C. The pH of the suspension was then adjusted in the range 4 to 12 with either diluted HCl or NaOH solution, using a pH meter, and was considered the initial pH of the suspensions. The final pH of the suspension was measured after the 24 h equilibration. The PZC of the adsorbents were determined from the plot of pH_{final}−pH_{initial} versus pH_{initial} of adsorbent suspensions. The point at which pH_{final}−pH_{initial} was equal to zero was considered the PZC.

Incorporação das nanopartículas de prata

Preparação sem agente redutor

CA6, CA12, CA25 e CA50 (2,0 g) foram dispersos em 50 mL de água destilada. O AgNO₃ (1,0 g) foi solublizado separadamente, adicionado à mistura e agitado durante 10 minutos. O pH foi ajustado para 10 com hidróxido de sódio aquoso concentrado e a mistura foi agitada magneticamente em placa quente à 80 °C e protegida da luz durante 60 min (24). Finalmente, a mistura foi lavada com água destilada e seca sob vácuo por 24 h. Os compósitos resultantes foram denominados como CA12Ag, CA25Ag e CA50Ag.

Preparação com NaBH₄

CA6, CA12, CA25 e CA50 (2,0 g) foram dispersos em 50 mL de água destilada. O AgNO₃ (1,0 g) foi solublizado separadamente, adicionado à mistura e agitado durante 10 minutos. Foi adicionado NaBH₄ (0,2 g) e a mistura foi agitada foi agitada magneticamente e protegida da luz durante 60 min. Finalmente, a mistura foi lavada com água destilada e seca sob vácuo por 24 h (25). Os compósitos resultantes foram denominados como CA6AgB, CA12AgB, CA25AgB e CA50AgB.

Preparação com sacarose

CA6, CA12 , CA25 e CA50 (2,0 g) foram dispersos em 50 mL de água destilada. O AgNO₃ (1,0 g) foi solublizado separadamente, adicionado à mistura e agitado durante 10 minutos. Em seguida, foi adicionada sacarose (10 g), após ter sido solubilizada em uma quantidade suficiente de água destilada. O pH foi ajustado para 10 com hidróxido de sódio aquoso concentrado e a mistura foi agitada magneticamente em placa quente à 80 °C e protegida da luz durante 60 min. Finalmente, a mistura foi lavada com água destilada e seca sob vácuo por 24 h (26). Os compósitos resultantes foram denominados como CA6AgS, CA12AgS, CA25AgS e CA50AgS.

Caracterização

Ponto de carga zero do pH (pH_{pzc})

Uma suspensão de 100 mg de CA em 20 mL de água destilada foi agitada por 24 horas a 25 °C na presença de NaCl 50 mM. O pH da suspensão foi então ajustado na faixa de 4 a 12 com solução diluída de HCl ou NaOH, usando um medidor de pH, e foi considerado o pH inicial das suspensões. O pH final da suspensão foi medido após o equilíbrio de 24 h. O PCZ dos adsorventes foi determinado a partir do gráfico de pH_{final}−pH_{initial} versus pH_{initial} das suspensões de adsorventes. O
which pH becomes zero was named pHpcz (27). The experiments were carried out in triplicate.

Specific Surface Area (SSA)
The SSA was determined by the methylene blue (MB) adsorption method; 100 mg of AC was mixed with 20 mL of MB solution (12.5 - 150 mg/L) and the suspension was shaken for 2 h at 25 °C. Five mL of the solution was then removed, and centrifuged. The remnant concentration of MB in the fluid was determined using a UV/Vis spectrophotometer (Jasco V-630, Easton, MD, USA) at a wavelength of 655 nm. The SSA was derived from the point of complete cation replacement determined on the titration curve. When the curve deviates more than 45° from a straight line, this indicates that additional MB cannot be completely absorbed by the adsorbent. Thus, the SSA is calculated by the following equation [1], based on the amount of adsorbed MB:

\[
SSA = \frac{m_{MB}}{319.87} \times A_v \times A_{MB} \times \frac{1}{m_s} \quad [1]
\]

where \( A_v \) is Avogadro's number (6.02 x 10^{23}/mol), and AMB is the area covered by one MB molecule (130 Å²), mMB is the mass of the adsorbed MB at the point of complete cation replacement, and ms is the mass of the adsorbent (28).

Functional active groups

The functional groups on the surface of AC were characterized by the Boehm method, as follows: 50 mg of AC was dispersed in 20 mL of 0.05 M NaHCO₃, 0.025M Na₂CO₃, 0.05M NaOH, 0.05 M HCl, and shaken for 24 hours. Next, the samples were centrifuged and titrated in triplicate with 0.1 M NaOH or 0.1 M HCl, depending on the initial solution used. The number of groups on the surface of the adsorbent was analyzed as follows: NaHCO₃ (carboxylic groups), Na₂CO₃ (carboxylic groups and lactones), NaOH (carboxylic groups, lactones, and phenolic groups) and HCl (basic sites) (29).

Iodine number

The I₂ number (mg/g) for the AC was determined as described in the literature (30). Approximately 150 mg of AC was placed in a 250 mL dry Erlenmeyer flask, fully wetted by boiling for 30 s with 10 mL of dilute HCl (0.04 M) and subsequently cooled. Next, 20 mL of I₂ solution (0.05 M) was added to the mixture, which was then shaken for 30 min. The resulting solution was filtered, and 50 mL of the filtrate was titrated with sodium thiosulfate solution (0.01 M), using starch as an indicator. The remaining concentration of I₂ in the solution was then removed, and centrifuged. The remnant concentration of MB in the fluid was determined using a UV/Vis spectrophotometer (Jasco V-630, Easton, MD, USA) at a wavelength of 655 nm. The SSA was derived from the point of complete cation replacement determined on the titration curve. When the curve deviates more than 45° from a straight line, this indicates that additional MB cannot be completely adsorbed by the adsorbent. Thus, the SSA is calculated by the following equation [1], based on the amount of adsorbed MB:

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Grupos funcionais ativos

Os grupos funcionais na superfície do CA foram caracterizados pelo método de Boehm, da seguinte forma: 50 mg de CA foram dispersos em 20 mL de NaHCO₃, 0.05 M Na₂CO₃, 0.025 M NaOH, 0.05 M HCl, e agitado durante 24 horas. Em seguida, as amostras foram centrifugadas e tituladas em triplicata com NaOH 0,1 M ou HCl 0,1 M, dependendo da solução inicial utilizada. O número de grupos na superfície do adsorvente foi analisado da seguinte forma: NaHCO₃ (grupos carboxílicos), Na₂CO₃ (grupos carboxílicos e lactonas), NaOH (grupos carboxílicos, lactonas e grupos fenólicos) e HCl (íons básicos) (29).

Número de iodo

O número de I₂ (mg/g) para CA foi determinado conforme descrito na literatura (30). Cerca de 150 mg de CA foi colocado em um balão Erlenmeyer seco de 250 mL, umedecidos completamente por ebulição durante 30 s com 10 mL de HCl diluído 0,04 M e subsequentemente resfriado. Em seguida, 20 mL de solução de I₂ (0,05 M) foram adicionados à mistura, que foi agitada durante 30 min. A solução resultante foi filtrada e 50 mL do filtrado foram titulados com solução de tios-
tion was calculated from the total volume of sodium thiosulfate used.

*Amount of silver impregnated*

The amount of silver impregnated on AC was determined by atomic absorption spectrophotometry (AAS Perkin Elmer model Analyst 800). The calibration curve was prepared by serial dilutions of the 1000 mg/L stock solution (Assurance Grade Interference Check Standard/SPEX CertiPrep®, Metuchen, NJ, USA).

*Antimicrobial assays*

**Determination of minimum inhibitory concentration (MIC)**

The minimum inhibitory concentrations (MIC) of the materials for *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) were determined in 96-well microtiter plates using a standard two-fold broth microdilution of the antibacterial agents, following the guidelines of the Clinical and Laboratory Standards Institute (31). Serial dilutions of the antibacterial material were performed in Mueller-Hinton (bacteria) and Sabouraund (yeast) broths, which were inoculated with a standardized number of organisms (1.5 x 10^5 UFC/mL) and incubated overnight at 37 °C. The concentrations tested ranged from 1000 to 1.95 µg/mL. Cell growth was determined by observing the turbidity of the culture. The lowest concentration of the materials at which no visual turbidity could be observed was considered to be the MIC of the antimicrobial materials.

**Microbial growth kinetics**

Suspensions (50 mL) of *S. aureus* (1.5 x 10^6 UFC/mL), *E. coli* (1.5 x 10^6 UFC/mL) and *C. albicans* (1.5 x 10^4 UFC/mL) were prepared in Mueller-Hinton (bacteria) and Sabouraund (yeast) broths containing different concentrations of composites, according to the MIC, and incubated at 37 °C with continuous agitation (120 rpm). After a pre-determined incubation time, the antimicrobial efficacy was determined by measuring OD at 600 nm.

sulfato de sódio (0.01 M), utilizando amido como indicador. A concentração remanescente de I₂ na solução foi calculada a partir do volume total de tiossulfato de sódio utilizado.

*Quantidade de prata impregnada*

A quantidade de prata impregnada em CA foi determinada por espectrofotometria de absorção atómica (AAS Perkin Elmer model Analyst 800). A curva de calibração foi preparada por diluições em série da solução estoque 1000 mg/L (Assurance Grade Interference Check Standard/SPEX CertiPrep®).

*Ensaios antimicrobianos*

**Determinação da concentração inibitória mínima (CIM)**

As concentrações inibitórias mínimas (CIM) dos materiais para *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) e *Candida albicans* (ATCC 10231) foram determinadas em placas de microtitulação de 96 poços usando uma micro diluição padrão de 2 vezes em caldo dos agentes antibacterianos, seguindo as diretrizes do Instituto de Padrões Clínicos e Laboratoriais (31). Diluições seriadas do material antibacteriano foram realizadas em caldos Mueller-Hinton (bactérias) e Sabouraund (levedura), que foram inoculados com um número padronizado de organismos (1,5 x 10^5 UFC/mL) e incubados durante a noite a 37°C. As concentrações testadas variaram de 1000 a 1,95 µg/mL. O crescimento celular foi determinado pela observação da turbidez da cultura. A menor concentração dos materiais nos quais não foi observada turbidez visual foi considerada a CIM dos materiais antimicrobianos.

*Cinética de crescimento microbiano*

Suspensões (50 mL) de *S. aureus* (1,5 x 10^6 UFC/mL), *E. coli* (1,5 x 10^6 UFC/mL) e *C. albicans* (1,5 x 10^4 UFC/mL) foram preparadas em Mueller-Hinton (bactérias) e caldos Sabouraund (levedura) contendo diferentes concentrações de compósitos, de acordo com a CIM, e incubados a 37 °C com agitação contínua (120 rpm). Após um tempo de incubação predeterminado, a eficácia antimicrobiana foi determinada pela medição da DO a 600 nm.
Phytotoxic assays with Cucumis sativus

Seed germination tests

*Cucumis sativus* seeds were incubated in Petri dishes containing one qualitative filter paper grade 1. Ten seeds, geometrically scaled, were incubated with 5 mL of distilled water (control) or respective AC suspension at concentrations of 1, 10, 100, 1000, and 10,000 mg/L, for 7 d at 25 °C in the dark. The experiments were performed in triplicate. The number of germinated seeds was counted, and the root length was measured. Sequentially germinated seeds were incubated at 60 °C for 48 h to determine the dry weights. The seedlings were grouped and weighed together in each replicate and the results were normalized for the number of germinated seeds.

To provide a comprehensive interpretation, seed germination, and root elongation can be combined in a germination index (GI), according to equation [2]

$$GI \, (\%) = \frac{G_sL_s}{G_cL_c} \times 100 \quad [2]$$

where Gs and Ls are seed germination (%) and root elongation (mm) for the sample; Gc and Lc the corresponding control values (32).

Seedling elongation inhibition (SEI %) provides information about the inhibition (positive values) and stimulation (negative values) of the seed germination. SEI (%) can be calculated by the equation:

$$SEI \, (\%) = \frac{L_c-L_s}{L_c} \times 100 \quad [3]$$

where Lc and Ls are root elongation (mm) for the control and sample.

Measurement of chlorophyll content

*Cucumis sativus* seeds were kept in contact with AC at a concentration of 10,000 mg/L in the dark for 7 d, until germination, and then for 48 h in the light. Next, fresh leaves (300 mg) were collected and placed in contact with 10 mL of 95% ethanol for 48 h under light. The absorption of the samples was then measured in a UV-Vis spectrophotometer at 648 and 664 nm (33). Chlorophyll A, chlorophyll B, and total chlorophyll were determined by the following equations:

$$ChlA = 13.36A_{648} - 5.19A_{664} \quad [4]$$

$$ChlB = 27.43A_{648} - 8.12A_{664} \quad [5]$$

$$Total \, chlorophyll = ChlA + ChlB \quad [6]$$

Ensaios de fitotoxicidade com Cucumis sativus

Teste de germinação das sementes

As sementes de *Cucumis sativus* foram incubadas em placas de Petri contendo um papel filtro qualitativo número 1. Dez sementes, escaladas geometricamente, foram incubadas com 5 mL de água destilada (controle) ou a respectiva suspensão CA nas concentrações de 1, 10, 100, 1000 e 10.000 mg/L, durante 7 dias a 25 °C, no escuro. As experiências foram realizadas em triplicado. O número de sementes germinadas foi e o comprimento das raízes foi medido. Sementes germinadas sequencialmente foram incubadas a 60 °C por 48 h para determinar os pesos secos.

As mudas foram agrupadas e pesadas em cada réplica e os resultados foram normalizados para o número de sementes germinadas. Para fornecer uma interpretação abrangente, a germinação das sementes e o alongamento das raízes podem ser combinadas em um índice de germinação (GI), de acordo com a equação:

$$GI \, (\%) = \frac{G_sL_s}{G_cL_c} \times 100 \quad [2]$$

onde Gs e Ls são a germinação das sementes (%) e o alongamento das raízes (mm) para a amostra; Gc e Lc os valores de controle correspondentes (32).

A inibição do alongamento de mudas (SEI%) fornece informações sobre a inibição (valores positivos) e estimulação (valores negativos) da germinação das sementes. SEI (%) pode ser calculado pela equação:

$$SEI \, (\%) = \frac{L_c-L_s}{L_c} \times 100 \quad [3]$$

onde Lc e Ls são alongamento da raiz (mm) para o controle e a amostra.

Teor de clorofila

As sementes de *Cucumis sativus* foram mantidas em contacto com CA na concentração de 10 g/L no escuro por 7 dias, até a germinação e, em seguida, por 48 horas na luz.

Em seguida, folhas frescas (300 mg) foram coletadas e colocadas em contacto com 10 mL de etanol a 95% por 48 h sob luz. A absorção das amostras foi então medida em um espectrofotômetro UV-Vis a 648 e 664 nm. A clorofila A, clorofila B e a clorofila total foram determinadas pelas seguintes equações (33):

$$ChlA = 13.36A_{664} - 5.19A_{648} \quad [4]$$

$$ChlB = 27.43A_{648} - 8.12A_{664} \quad [5]$$

$$Clorofila \, total = ChlA + ChlB \quad [6]$$
Evaluation of cell death using Evans blue dye

Loss of cell viability was studied using the Evan’s blue staining method. Four root tips with 1 cm of length from control and treated seeds were stained with 2 mL of 0.25% (w/v) aqueous solution of Evans blue for 15 min, then washed with distilled water. The samples were then incubated for 1 h at 50 °C with a sodium lauryl sulfate solution in methanol-water (50:50), and the absorbance was measured at 595 nm (34).

Hatching the cysts

The tests were performed according to the protocol developed and validated (35). Dehydrated cysts of *Artemia salina* were hatched in saline medium (38 g/L sea salt) at pH 8.0. The cysts were dispersed in the medium (100 mg of cysts per 100 mL of medium) and incubated for 24 h under light and aeration.

Mortality Rate of Artemia nauplii

The acute toxicity was determined by measuring the number of dead *Artemia*. The 24-hour-old nauplii were transferred to 24-well plates, with 10 nauplii placed in contact with the nanomaterial suspensions (2 mL) at concentrations of 25, 50, 75, 100, 125, 500, 1000, and 10000 mg/L. Ten replicates were used for each treatment. Saline medium was used as negative control and a solution of 60 mg/L *K₂Cr₂O₇* was used as positive control. The plates were incubated in the dark at 24 °C. The number of dead nauplii was evaluated after 24 and 48 h, with immobile nauplii being considered dead. The test was considered valid if less than 10% of the control nauplii were immobile.

The mortality rate was calculated by following the formula:

\[
\% \text{Mortality} = \frac{\text{Number of dead nauplii}}{\text{Initial number of nauplii}} \times 100 \quad [7]
\]

Statistical Analysis

All experiments were performed in triplicate. Statistical significance was determined by analysis of variance (ANOVA), followed by Dunnett’s method and Tukey’s test, to determine the statistical significance of each parameter among the treatments, with significant differences at \( p < 0.05 \), using the software program GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). All experimental data are presented as mean values ± standard deviations.

Avaliação da morte celular usando o corante azul de Evans

A perda de viabilidade celular foi estudada usando o método de coloração azul de Evans. Quatro pontas de raiz com 1 cm de comprimento, das sementes controle e tratadas, foram coradas com 2 mL de solução aquosa a 0,25% (m/v) de azul de Evans por 15 min e depois lavadas com água destilada. As amostras foram então incubadas por 1 h a 50 °C com uma solução de lauril sulfato de sódio em metanol-água (50:50), e a absorbância foi medida a 595 nm (34).

Incubação dos cistos

Os testes foram realizados de acordo com um protocolo desenvolvido e validado (35). Os cistos desidratados de *Artemia salina* foram incubados em meio salino (sal marinho 38 g/L) com pH 8,0. Os cistos foram dispersos no meio (100 mg de cistos por 100 mL de meio) e incubados durante 24 h sob luz e injeção de ar comprimido.

Taxa de mortalidade Artemia nauplii

A toxicidade aguda foi determinada medindo o número de Artemia mortas. Os nauplii com 24 h de idade foram transferidos para placas de 24 poços, com 10 nauplii colocados em contato com as suspensões de nanomateriais (2 mL) nas concentrações de 25, 50, 75, 100, 125, 500, 1000 e 10000 mg/L. Dez réplicas foram usadas para cada tratamento. O meio salino foi usado como controle negativo e uma solução de *K₂Cr₂O₇* a 60 mg/L foi usada como controle positivo. As placas foram incubadas no escuro a 24 °C. O número de nauplii mortos foi avaliado após 24 e 48 h, sendo considerado nauplii imóvel. O teste foi considerado válido se menos de 10% dos nauplii controle estivessem imóveis.

A taxa de mortalidade foi calculada seguindo a fórmula:

\[
\% \text{Mortalidade} = \frac{\text{Número de nauplii mortos}}{\text{Número inicial de nauplii}} \times 100 \quad [7]
\]

Análises estatísticas

A significância estatística foi determinada pela análise de variância (ANOVA), seguida pelo método de Dunnett e pelo teste de Tukey, para determinar a significância estatística de cada parâmetro entre os tratamentos, com diferença significativa em \( p < 0.01 \), utilizando o programa GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Todos os dados experimentais são apresentados como valores médios ± desvios padrão.
**Results and Discussion**

**Preparation of activated carbon**

Among the various types of chemical activation agents, H$_2$SO$_4$ is frequently used for the preparation of activated carbon from lignocellulose products. The carbonization of termite feces with H$_2$SO$_4$ resulted in yields ranging from 43.8 to 48.7%, with no direct relationship between the yield and the amount of acid added to the AC. The H$_2$SO$_4$ causes cleavage of bonds, leading to dehydration and elimination reactions that release volatile products such as water, acetic acid, methanol, and other compounds (36). A similar result was found in the synthesis of AC from flax fibers, where the amounts of H$_2$SO$_4$ added, from 10 to 18 mol/L, did not show a significant variation in material yield (37).

The characteristics of the activated carbons prepared at 600 °C using different impregnation ratios of H$_2$SO$_4$ were studied using SEM technique. As shown in Figure SI1, the surface of treated termite feces was homogenous, smooth, and flat (38). After chemical activation, SEM (Figure SI1) showed the internal pores of the prepared AC12. The pores and cavities resulted from evaporation of the activating agent (H$_2$SO$_4$) during carbonization, as indicated in literature (38, 39).

The characteristics of the activated carbons prepared at 600 °C using different impregnation ratios of H$_2$SO$_4$ are shown in Table 1. The specific surface area (SSA) determined by the amount of adsorbed methylene blue (MB) was calculated by equation [1] (28). Using H$_2$SO$_4$ as the activating reagent, the maximum surface area was obtained for the activated carbon AC6. Interestingly, the activating reagent, the maximum surface area was calculated by equation [1] (28).

**Characterization of AC**

The internal and external surface of the prepared AC was studied using SEM technique. As shown in Figure SI1a, the surface of treated termite feces was homogeneous, smooth, and flat (38). After chemical activation, SEM (Figure SI1) showed the internal pores of the prepared AC12. The pores and cavities resulted from evaporation of the activating agent (H$_2$SO$_4$) during carbonization, as indicated in literature (38, 39).

The characteristics of the activated carbons prepared at 600 °C using different impregnation ratios of H$_2$SO$_4$ are shown in Table 1. The specific surface area (SSA) determined by the amount of adsorbed methylene blue (MB) was calculated by equation [1] (28). Using H$_2$SO$_4$ as the activating reagent, the maximum surface area was obtained for the activated carbon AC6. Interestingly, the activating reagent, the maximum surface area was calculated by equation [1] (28).

**Preparação do CA**

Entre os vários tipos de agentes químicos de activação, o H$_2$SO$_4$ é frequentemente usado para a preparação de carvão activado a partir de materiais lenho celulósico. A carbonização das fezes de cupim com H$_2$SO$_4$ resultou em rendimentos variando de 43,8 a 48,7%, sem relação directa entre o rendimento e a quantidade de ácido adicionado ao CA. O H$_2$SO$_4$ causa clivagem de ligações, levando a reacções de desidratação e eliminação de produtos volátiles, como água, ácido acético, metanol e outros produtos químicos (36). Resultado semelhante foi encontrado na preparação de CA a partir de fibras de linho, onde as quantidades de H$_2$SO$_4$ adicionadas, de 10 a 18 mol/L, não apresentaram variação significativa no rendimento do material (37).

As hemiceluloses são prontamente hidrolisadas por ácidos diluídos. A celulose, devido à sua alta cristalinidade e densidade coesiva é insolúvel em água e mais resistente a ácidos diluídos. No entanto, ácidos fortes podem fazer com que a celulose inche ou até se dissolva. O ácido sulfúrico concentrado dissolve rapidamente a celulose em temperaturas ambiente, formando uma solução clara que, quando deixada em repouso, escurece como resultado da carbonização da celulose. O material lenho celulósico é o principal componente das fezes de cupim, e estudos nos quais a serragem foi tratada com H$_2$SO$_4$ concentrado mostraram que os polissacarídeos são hidrolisados e podem ser removidos, enquanto a linina é deixada como um resíduo escuro/colorido não hidrolisado. Em suma, a preparação do CA por desidratação de qualquer biomassa é uma tarefa difícil, pois depende do tipo de matéria-prima, do método de tratamento e do uso pretendido do CA (37).

**Caracterização do CA**

A superfície interna e externa do CA preparado foi estudada pela técnica MEV. Como mostrado na Figura SI1a, a superfície das fezes de cupim tratadas era homogênea, lisa e plana (38). Após activação química, o MEV (Figura SI1) mostrou os poros internos do CA12 preparado. Os poros e cavidades resultaram da evaporação do agente activador (H$_2$SO$_4$) durante a carbonização, como indicado por Hamed, et al., 2016 (39) e Chahm, et al., 2019 (38).

As características dos carvões activos preparados a 600 ºC usando diferentes taxas de impregnação de H$_2$SO$_4$ são mostradas na Tabela 1. A área superficial específica (ASE) determinada pela quantidade de azul de metileno adsorvido (AM) foi calculada pela equação [1] (28).
when the impregnation ratio increased from 1:6 to 1:1 (termite feces:H$_2$SO$_4$, the surface area decreased from 303.4 to 162.5 m$^2$ g$^{-1}$). The Boehm titration method provides important information regarding the surface properties of carbon materials. These properties, in turn, may interfere with the performance of the materials in the adsorption processes. The results are shown in Table 1. The results of the titration results revealed that the total acidity of the AC surface increased with the amount of H$_2$SO$_4$ used in the activation. Functional acid groups have been broadly studied, and the results reported in the literature have demonstrated that H$_2$SO$_4$ oxidation allows the formation of carboxyl sites. Total acid sites (TAS) of the ACs increased up to 400% when the amount of H$_2$SO$_4$ used was increased, mainly reflecting an increase in phenolic groups. However, TAS decreased when a ratio of 1:1 H$_2$SO$_4$ was used for the oxidation of the wood-based activated carbon. These results are similar to those reported in literature, showing that H$_2$SO$_4$ oxidation increases the total acid groups, mainly due to the formation of carboxylic, phenolic and hydroxyl groups. In addition, the quantity of basic sites increased after chemical oxidation of the activated carbon (40-42).

Table 1 shows the pH$_{pcz}$ results of the experiments performed with the ACs, where the pH ranged from 4 to 12. According to Table 1, the pH$_{pcz}$ decreased with increasing amounts of H$_2$SO$_4$ used in the activation. Below the pH$_{pcz}$ value, the surface of AC is positively charged due to protonation. Above the pH$_{pcz}$ the AC surface has a negative charge (43).

The iodine number is a measure of micropore content. Utilizando H$_2$SO$_4$, como reagente de activação, foi obtida a área superficial máxima para o carvão activado CA6. Curiosamente, quando a taxa de impregnação aumentou de 1:6 para 1:1 (FC:H$_2$SO$_4$), a área de superfície diminuiu de 303.4 para 162.5 m$^2$ g$^{-1}$.

O método de titulação de Boehm fornece informações importantes sobre as propriedades da superfície de materiais de carbono. Essas propriedades, por sua vez, podem interferir no desempenho dos materiais nos processos de adsorção. Os resultados são mostrados na Tabela 1. Os resultados da titulação revelaram que a acidez total da superfície do CA aumentou com a quantidade de H$_2$SO$_4$ usado na activação. Grupos ácidos funcionais têm sido amplamente estudados, e os resultados relatados na literatura demonstraram que a oxidação com H$_2$SO$_4$ permite a formação de ácidos carboxílicos. Os ácidos orgânicos totais (AOT) dos CAs aumentaram até 400% quando a quantidade de H$_2$SO$_4$ usado foi aumentada, refletindo principalmente um aumento nos grupos fenólicos, Tabela 1. No entanto, o conteúdo de AOT diminuiu quando uma proporção de 1:1 de H$_2$SO$_4$ foi usada para a oxidação do carvão activado à base de madeira. Esses resultados são semelhantes aos relatados na literatura mostrando que a oxidação com H$_2$SO$_4$ aumenta o total de grupos ácidos, principalmente devido à formação de grupos carboxílico, fenólico e hidroxilo. Além disso, a quantidade de sítios básicos aumentou após a oxidação química do carvão activado (40-42).

A Tabela 1 mostra os resultados de pH$_{pcz}$ dos experimentos realizados com os CAs, onde o pH variou de 4 a 12. De acordo com a Tabela 1, o pH$_{pcz}$ diminuiu com o aumento de quantidades de H$_2$SO$_4$ usado na ativação.

Table 1 – Characterization parameters of activated carbon
Tabela 1 – Parâmetros de caracterização do carvão ativado

|         | I$_1$ (mg/g) | SSA$^2$ / ASE (m$^2$/g) | pH$_{pcz}^3$ / pH$_{ez}^3$ | Carboxylic acid groups / Phenol groups | Lactone groups / Grupos acido caboxílicos | Phenol groups / Grupos fenólicos | Total acid sites / Total de sítios ácidos | Basic sites / Sítios básicos |
|---------|--------------|--------------------------|-----------------------------|---------------------------------------|-------------------------------------------|------------------------------------|----------------------------------------|-----------------------------|
| AC6     | 171.9        | 303.4                    | 9.1                         | 9.0                                   | -                                         | 26.9                                | 35.9                                   | 2.7                         |
| AC12    | 173.7        | 235.4                    | 7.5                         | 23.4                                  | 0.4                                       | 22.9                                | 46.7                                   | 1.3                         |
| AC25    | 144.5        | 152.2                    | 5.6                         | 2.3                                   | 3.6                                       | 144.5                               | 150.4                                  | 4.9                         |
| AC50    | 146.3        | 162.5                    | 5.2                         | 2.3                                   | 5.4                                       | 104.6                               | 112.3                                  | 7.7                         |

1Iodine number; 2Specific surface area; 3pH point zero charge; 1Número de Iodo; 2Área superficial específica; 3pH ponto de carga zero
and was obtained by the standard method, the measurement of the accessibility of pores with dimensions ≥1.0 nm (17, 44). Table 1 shows the iodine number value of ACs. According to Table 1, the microporosity of the materials had no significant difference in relation to the amount of H₂SO₄ used; the iodine number ranged from 146.3 to 173.7 mg/g. The results indicate that AC has a moderate iodine number (45).

The composites containing activated carbon and silver nanoparticles were prepared in one-step. Ag⁺ ions were reduced to Ag₀ directly on the AC surface with an inorganic (NaBH₄) (46) reducing agent (equation [8]), or an organic reducing agent (sucrose) (47) (equation [9]). A series of composites was prepared without the addition of reducing agents. The chemical reaction involved in the formation of silver nanoparticles by NaBH₄ and sucrose are represented by:

$$\text{AgNO}_3 + \text{NaBH}_4 \rightarrow \text{Ag} + 1/2\text{H}_2 + 1/2\text{B}_2\text{H}_6 + \text{NaNO}_3$$  \[8\]
$$\text{CH}_2\text{OH}[(\text{CHOH})_n\text{CHO} + 2\text{Ag}^+ + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{OH}[(\text{CHOH})_n\text{COOH} + 2\text{Ag} + \text{H}^+]$$  \[9\]

Table 2 shows the amount of silver present in the synthesized ACs. The highest amount of silver was found in the composites prepared using NaBH₄ as a reducing agent. The use of sucrose resulted in composites with considerable amounts of silver. On the other hand, composites prepared without reducing agents resulted in a small amount of silver being incorporated into the AC. As shown in Table 2, the amount of acid used in the preparation of the ACs did not influence the amount of silver incorporated into the composite.

Abaixo do valor de pHₚₑₙ, a superfície do CA é carregada positivamente devido à protonação. Acima do pHₚₑₙ, a superfície do CA tem uma carga negativa (43). O número de iodo é uma medida do conteúdo de microporos e foi obtido pelo método padrão para medir a acessibilidade de poros com dimensões ≥1.0 nm (17,44). A Tabela 1 mostra o valor do número de iodo dos CA. De acordo com a Tabela 1, a microporosidade dos materiais não apresentou diferença significativa em relação à quantidade de H₂SO₄ utilizado; o número de iodo variou de 146,3 a 173,7 mg/g. Os resultados indicam que o CA tem um número moderado de iodo (45). Os compósitos contendo carvão activado e nanopartículas de prata foram preparados em uma etapa. O íon Ag⁺ foi reduzido para Ag₀ diretamente na superfície da CA com um agente redutor inorgânico (NaBH₄) (46) (equação [8]), ou um agente redutor orgânico (sacarose) (47) (equação [9]). A reação química envolvida na formação de nanopartículas de prata por NaBH₄ e sacarose é representada por:

$$\text{AgNO}_3 + \text{NaBH}_4 \rightarrow \text{Ag} + 1/2\text{H}_2 + 1/2\text{B}_2\text{H}_6 + \text{NaNO}_3$$  \[8\]
$$\text{CH}_2\text{OH}[(\text{CHOH})_n\text{CHO} + 2\text{Ag}^+ + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{OH}[(\text{CHOH})_n\text{COOH} + 2\text{Ag} + \text{H}^+]$$  \[9\]

A Tabela 2 mostra a quantidade de prata presente nos CA preparados. A maior quantidade de prata foi encontrada nos compósitos preparados usando NaBH₄ como agente redutor. O uso de sacarose resultou em compósitos com quantidades consideráveis de prata. Por outro lado, os compósitos preparados sem agentes redutores resultaram na incorporação de uma pequena quantidade de prata no CA. Como mostrado na Tabela 2, a quantidade de ácido usada na preparação dos CAs não influenciou a quantidade de prata incorporada no compósito.

| Composite / Composto | Silver load / quantidade de prata (mg/g) |
|----------------------|----------------------------------------|
| AC6Ag                | 23.4                                   |
| AC12Ag               | 19.5                                   |
| AC25Ag               | 11.7                                   |
| AC50Ag               | 58.6                                   |
| AC6AgB               | 133.7                                  |
| AC12AgB              | 109.3                                  |
| AC25AgB              | 156.2                                  |
| AC50AgB              | 136.6                                  |
| AC6AgS               | 46.9                                   |
| AC12AgS              | 50.8                                   |
| AC25AgS              | 31.2                                   |
| AC50AgS              | 35.7                                   |
Antimicrobial activity of the composites

Minimal inhibitory concentration (MIC)

The MIC is defined as the lowest concentration of a drug that will inhibit the visible growth of microorganisms after a period long (24 h), the growth of colony to a turbid bacteria culture observable to the naked eye. The MIC of the compounds to E. coli, S. aureus and C. albicans were determined using the double dilution method. The representative growth status of microbial strains in the presence of varying concentrations of disinfectants is shown in Table 3.

The results of the silver MIC were calculated in proportion to the amount of silver present in the composite (Table 2). The ACs without silver (AC6-AC50) did not present antibacterial or antifungal activity against the tested microorganisms.

Table 3 shows that the AC25Ag and AC12Ag composites prepared without reducing agents had the same MIC (500 µg/mL) for both S. aureus and E. coli. The AC25AgB composite prepared with NaBH₄ as reducing agent had a MIC of (500 µg/mL) for S. aureus, the composites AC12AgB, AC25AgB, and AC50AgB had a MIC of (500 µg/mL) for E. coli. The AC12AgS composite prepared using sucrose, had a MIC of (500 µg/mL) for both E. coli and C. albicans, and AC6AgS had a MIC of (500 µg/mL) for S. aureus. The MIC values found are well below those reported in the literature for materials carbonaceous impregnated nanoparticles: graphene oxide/Ag⁰ MIC 2000 µg/mL for S. aureus and E. coli (48); commercial activated carbon MIC 600 µg/mL and 1250 µg/mL for E. coli and S. aureus respectively (18).

The efficiency of nanomaterials is even more evident when we compare the MIC values based on the concentration of Ag. It was observed that the amount present in the ACAg compounds prepared without reducing agent (6-58 µg/mL) is lower than those found in the compounds prepared with NaBH₄ (78-136 µg/mL). One of the hypotheses for these results is the presence of Ag⁺ ions in the composite prepared without reducing agent. Higher toxicity against bacteria has been reported for the Ag⁺ ion compared to Ag⁰ (49). The decrease in antimicrobial activity with the increase of silver may be related to the increase in nanoparticle size, decreasing the potential for the release of silver ions and leading to a reduction in the antibacterial activities of the materials (50-51).

The MICs of 8.5 µg/mL and 6.0 µg/mL found for both E. coli and S. aureus for AC12Ag and AC25Ag, respectively, were very close to that reported for commercial activated carbon (10 and 5 µg/mL) (52). The MIC for E. coli to MIC 2500 µg/mL for S. aureus and E. coli (48); commercial activated carbon MIC 600 µg/mL and 1250 µg/mL for E. coli and S. aureus respectively (18).

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Atividade antimicrobiana dos compósitos

Concentração inhibitória mínima (CIM)

A CIM é definida como a menor concentração de um medicamento que inibirá o crescimento visível do microorganismo após 24 h, o crescimento da colonia em uma cultura de bactérias turva é observável a olho nu. A CIM dos compostos para E. coli, S. aureus e C. albicans foi determinada usando o método de dupla diluição. O status de crescimento representativo das cepas microbianas na presença de concentrações variadas de desinfetantes é mostrado na Tabela 3.

Os resultados da CIM de prata foram calculados proporcionalmente à quantidade de prata presente no compósito (Tabela 2). Os CAs sem prata (CA6-CA50) não apresentaram actividade antibacteriana ou antifúngica contra os microorganismos testados. A Tabela 3 mostra que os compósitos CA25Ag e CA12Ag preparados sem agentes redutores tinham a mesma CIM 500 µg/mL para S. aureus e E. coli. O compósito preparado com NaBH₄ como agente redutor CA25AgB apresentou CIM 500 µg/mL para S. aureus, os compósitos CA12AgB, CA25AgB e CA50AgB apresentaram CIM 500 µg/mL para E. coli. O compósito preparado com sacarose, CA12AgS, teve uma CIM de 500 µg/mL para E. coli e C. albicans, e CA6AgS teve uma CIM de 500 µg/mL para S. aureus. Os valores de CIM encontrados estão bem abaixo dos relatados na literatura para materiais carbonáceos impregnados com nanopartículas de prata:óxido de grafeno/Ag⁰ CIM 2000 µg/mL para S. aureus e E. coli (48); carvão ativado comercial CIM 600 µg/mL e 1250 µg/mL para E. coli e S. aureus respectivamente (18).

A eficiência dos nanomateriais é ainda mais evidente quando comparamos os valores de CIM com base na concentração de Ag. Observa-se que a quantidade presente nos compostos preparados sem o agente redutor CAAg (6-58 µg/mL) é menor que a encontrada nos compostos preparados com NaBH₄ (78-136 µg/mL). Uma das hipóteses para esses resultados é a presença de ião Ag⁺ no compósito preparado sem agente redutor. A literatura relata que o ião Ag⁺ apresenta maior toxicidade contra bactérias que o Ag⁰ (49). A diminuição da atividade antimicrobiana com o aumento da prata pode estar relacionada ao aumento do tamanho das nanopartículas, o que diminui o potencial de liberação de íons Ag⁺, levando a uma redução nas atividades antibacterianas dos materiais (50, 51).

As CIMs de 8,5 µg/mL e 6,0 µg/mL encontradas para E. coli e S. aureus para CA12Ag e CA25Ag, respectivamente, foram muito próximos do relatado para carvão ativado comercial (10 e 5 µg/mL) (52). A CIM de E. coli para MIC 2500 µg/mL para S. aureus e E. coli (48); commercial activated carbon MIC 600 µg/mL and 1250 µg/mL for E. coli and S. aureus respectively (18).

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Antimicrobial activity and toxicity of activated carbon with Ag

Actividade antimicrobiana e toxicidade de carvão activado contendo Ag

Table 3 – Minimal inhibitory concentration (MIC) of silver composites on S. aureus, E. coli, and C. albicans

| Composite / Compósito | S. aureus | E. coli | C. albicans |
|-----------------------|-----------|---------|-------------|
|                       | Total     | Ag      | Total       | Ag      | Total     | Ag      |
| AC6Ag                 | 1000      | 23      | 1000        | 23      | >1000     | >23     |
| AC12Ag                | 500       | 8.5     | 500         | 8.5     | >1000     | >19     |
| AC25Ag                | 500       | 6       | 500         | 6       | >1000     | >11     |
| AC50Ag                | 1000      | 58      | 1000        | 58      | >1000     | >58     |
| AC6AgB                | 1000      | 133     | 1000        | 133     | >1000     | >133    |
| AC12AgB               | 1000      | 109     | 500         | 54      | >1000     | >109    |
| AC25AgB               | 500       | 78      | 500         | 78      | >1000     | >156    |
| AC50AgB               | 1000      | 136     | 500         | 68      | >1000     | >136    |
| AC6AgS                | 1000      | 46      | 500         | 23      | 500       | 23      |
| AC12AgS               | 2000      | 100     | 1000        | 50      | >1000     | >50     |
| AC25AgS               | 2000      | 71      | 1000        | 31      | >1000     | >36     |
| AC50AgS               | 2000      | 75      | 1000        | 50      | >1000     | >38     |

E. coli with AC12Ag and AC25Ag was approximately half of that found for activated carbon obtained from coconut shell, 16 µg/mL (53). However, it was well above the results found for activated carbon prepared with bamboo, which had MIC values > 3.0 µg/mL against both E. coli and S. aureus (54).

Growth curves of the microorganism

Growth inhibition effects of the composites AC25AgB and AC6AgB were demonstrated with the microorganism growth curves. The bacterial growth was monitored by measuring the optical density at 600 nm (OD600) based on the turbidity of the cell suspension. As shown in Figure 1A, the lag phase of E. coli is less than 4 h for the control group. However, incubation with AC25AgB significantly prolonged the lag phase to 24 h, at dose of 250 mg/L and over 30 h for doses of 500, 750, and 1000 mg/L. For S. aureus, the lag phase was extended from 1 to 4 hours at a dose of 250 mg/L and over 48 h for doses of 500, 750, and 1000 mg/L (Figure 1B). It appears that the AC25AgB had a greater effect on Gram-positive than Gram-negative bacteria. The lag phase of C. albicans was less than 4 h for the control group, but was extended to 24 h when exposed to 500 mg/L AC6AgB composite. At doses of 1000, 1500 and 2000 mg/g, the lag phase was extended to 48 h, Figure 1C. These results are in agreement with those presented in the MIC determination, which showed antimicrobial activity at the same concentrations. Growth inhibition effects of E. coli, encontrada no CA12Ag e no CA25Ag, foi aproximadamente metade da encontrada para o carvão activado obtido da casca de coco, 16 µg/mL (53). No entanto, ficou bem acima dos resultados encontrados para o carvão activado preparado com bambu, que apresentou CIM > 3,0 µg/mL para E. coli e S. aureus (54).

Curva de crescimento dos micro-organismos

Os efeitos de inibição do crescimento dos compósitos CA25AgB e CA6AgB foram demonstrados com as curvas de crescimento de microorganismos. O crescimento bacteriano foi monitorado medindo a densidade óptica a 600 nm (DO600) com base na turbidez da suspensão celular. Como mostrado na Figura 1A, a fase de atraso de E. coli é inferior a 4 h para o grupo controle. No entanto, a incubação com CA25AgB prolongou significativamente a fase de atraso para 24 h, na dose de 250 mg/L e mais de 30 h para doses de 500, 750 e 1000 mg/L. Para S. aureus, a fase de retardo foi estendida de 1 a 4 horas na dose de 250 mg/L e mais de 48 h para doses de 500, 750 e 1000 mg/L (Figura 1B). Parece que o CA25AgB tem um efeito maior nas bactérias Gram-positivas do que nas Gram-negativas. A fase de atraso de C. albicans foi inferior a 4 h para o grupo controle, mas foi estendida para 24 h quando exposta a 500 mg/L de composto CA6AgB. Nas doses de 1000, 1500 e 2000 mg/g, a fase de atraso foi estendida para 48 h, Figura 1C. Esses resultados estão de acordo com os apresentados na determinação da CIM, que mostrou atividade...
Figure 1 – Growth curves of E. coli exposure to AC25AgB composite (a); S. aureus exposure (b) and C. albicans (c) exposure to AC6AgB composites. Control represents bacterial culture without any Ag formulations (black). Bacterial growth is measured as the optical density at 600 nm. Incubation time = 0 h corresponds to the time at which the composite is added to the bacterial broth.

Figura 1 – Curva de crescimento do E. coli exposto ao compósito CA25AgB (a); S. aureus (b) e C. albicans (c) exposta ao compósito CA6AgB. Controle representa a cultura das bactérias sem prata (preto). Crescimento bacteriano é medido através da densidade ótica em 600 nm. Tempo de incubação = 0 h corresponde ao tempo em que os compósitos foram adicionados no caldo bacteriano.
Antimicrobial activity and toxicity of activated carbon with Ag

Phytotoxicity

Seedling Elongation Inhibition (SEI)

The effect of composites on Cucumis sativus growth was evaluated by seed germination in contact with suspensions containing increasing concentrations of the materials. Root length measurements were used to calculate the value (SEI %). Positive values represent growth inhibition, and negative values represent growth stimulation.

The effect of composite on seedling growth was calculated by SEI are shown in Figure S3 and Figure 2. The antimicrobiana nas mesmas concentrações. Os efeitos de inibição do crescimento dos outros compósitos são mostrados nas informações suplementares (SI4). Resultados semelhantes foram encontrados para carbono à base de casca de arroz com nanopartículas de prata no tempo de contato composto com 18 h para E. coli (55). Em outro estudo da actividade antibacteriana com nanoesferas de carbono carregadas com nanopartículas de prata, o crescimento de E. coli foi totalmente inibido em uma concentração superior a 50 mg/L (56).

Inhibition of the elongation of seedlings (IAP)

O efeito dos compósitos no crescimento de Cucumis sativus foi avaliado por germinação de sementes em contato com suspensões contendo concentrações crescentes dos materiais. As medidas do comprimento da raiz foram usadas para calcular o valor IAP (%). Valores positivos representam inibição do crescimento e valores negativos representam estimulação do crescimento.

O efeito do compósito no crescimento de plântulas cal-

![Figure 2 - Influence of composites on seedling elongation Inhibition (SEI)]. Values are means of three replicates ± SD. Different letters indicate significant differences (ANOVA followed by Tukey’s test, p < 0.05), at concentration of 10,000 mg/L.

![Figura 2 – Influência dos compositos sobre a inibição do alongamento da plantula (IAP)]. Os valores representam a média de três réplicas ± DP. Diferentes letras indicam diferenças significativas (teste ANOVA seguido de teste Tukey’s , p <0,05), na concentração de 10.000 mg/L.
AC had a growth stimulation effect, with no relationship between the concentration of H$_2$SO$_4$ used in the activation of termite feces and SEI, and higher stimulation at the concentration of 10,000 mg/L (29.6 ± 16.1%) for AC25. The composites containing silver nanoparticles (prepared using NaBH$_4$) showed inhibition at all concentrations (10-1000 mg/mL), without a dose-dependent effect and without significant differences (p<0.05) between them for composites (Figure S3).

Composites prepared with the lowest amount of H$_2$SO$_4$ (AC6Ag, AC2AgB and AC6AgS) at the dose of 10,000 mg/g showed the highest inhibitory effect on SEI, with inhibition of around 60%. This demonstrates that the silver-impregnated active carbons showed toxicity to C. sativus in the elongation stage. Similar results were observed for root growth of cucumber seeds when using Ag-NP (57). The same behavior has also been reported for the use of Ag-NP and AgNO$_3$, resulting in a severe reduction in growth parameters of C. sativus (58).

Germination index (GI)

The GI can assume values greater or lower than 100%, where a value equal to 100% indicates that the average seedling length and germination rate between a specific treatment and negative control are the same. With values of between 80% and 120%, the effects are similar to those produced by the negative controls, while values greater than 120% indicate biostimulation, and values lower than 80% indicate inhibition (7).

The results of GI are presented in Figure 3. The active carbon without silver presented a general stimulation tendency, at the concentration of 10000 mg/L, for an effect greater than 120% (130±2.5%) for AC25, without significant difference (p> 0.05). The activated carbons prepared using NaBH$_4$ showed a negative effect on germination and growth, with values ranging from 35-60%, depending on the amount of acid used for the preparation. On the other hand, there was no correlation with the amount of silver contained in the composites. This effect was only observed at the concentration of 10000 mg/L (Figure S2). The composites containing silver nanoparticles, prepared with reducing agent or sucrose, also had negative effects, with GI < 80% for all composites. The GI results showed the same tendency, as shown by the SEI, with low GI values being attributed to inhibition of root growth. Similar results were previously found using Ag-NP in C. sativus germination, where a significant decrease in GI was observed (57).

Índice de germinação (IG)

O IG pode assumir valores maiores ou menores que 100%, o valor igual a 100% significa que o comprimento médio das plântulas e a taxa de germinação entre um tratamento específico e o controle negativo são os mesmos. Com valores entre 80% e 120%, os efeitos são semelhantes aos produzidos pelos controles negativos, enquanto valores superiores a 120% indicam bioestimulação e valores inferiores a 80% indicam inibição (7).

Os resultados do IG são apresentados na Figura 3. O CA sem prata apresentou uma tendência geral de estimulação, na concentração de 10000 mg/L, para um efeito superior a 120% (130 ± 2,5%) para CA25, sem diferença significativa (p > 0.05). Os CAs preparados com NaBH$_4$ apresentaram efeito negativo na germinação e crescimento, com valores variando de 35 a 60%, dependendo da quantidade de ácido utilizado na preparação. Por outro lado, não houve correlação com a quantidade de prata contida nos compósitos. Este efeito foi observado apenas na concentração de 10000 mg/L (Figura S2). Os compósitos contendo nanopartículas de prata, preparados com redutor ou sacarose, também tiveram efeitos negativos, com IG < 80% para todos os compósitos. Os resultados do IG mostraram a mesma tendência, como demonstrado pelo SEI, com baixos valores de IG atribuídos à inibição do crescimento radicular. Resultados semelhantes foram encontrados anteriormente usando NPs-Ag na germinação de C. sativus, houve uma diminuição significativa no IG (57).
**Dry weight**

The impact of the composites on the dry weight of *C. sativus* was measured. The results are shown Table 4. Seedling dry weight after 7 days of incubation with composites was normalized according to the number of germinated seeds of *C. sativus*. According to Table 5, no significant difference (p> 0.05) was found between treatments and between treatment and negative control for all composites at a concentration of 10 g/L. The results differ from those reported for *C. sativus* treated with Ag-NP and AgNO$_3$, in which there was a significant reduction in dry weight (58).

**Chlorophyll content**

Chlorophyll A (Chl A) and chlorophyll B (Chl B) play a fundamental role in photosynthesis: they both absorb at the same wavelength range that is necessary to transform light energy into chemical energy. Because chlorophyll is extremely important for the metabolic process, a decrease in the chlorophyll content can be problematic for the plant. Table 4 shows the effects of composites addition to *C. sativus* on the ChlA and ChlB, respectively.
ChlB contents. The ChlA content of *C. sativus* exposed to suspensions of the composite at a concentration of 10 g/L showed a reduction with significant difference (*p* < 0.05) for AC6AgB (52 % reduction), AC12AgB (44 % reduction) and AC12AgS (31 % reduction) when compared to the control. Similar results were found for *Crambe abyssinica* (59), *Oryza sativa* L. (60) with inhibition of ChlB synthesis, and *Lycopersicon esculentum* (61) with inhibition of both ChlA and ChlB synthesis, and *Physalis peruviana* L. total chlorophyll (62) when exposed to silver nanoparticles. Chlorophyll content, which acts as a biomarker of the photosynthetic activity of a plant, may be reduced when exposed to metals. Decreased chlorophyll contents indicate disturbed chlorophyll synthesis which may have serious implications on the synthesis of organic food material (63). The decrease in chlorophyll contents in tissues of metal-treated plants is dependent on several factors, such as a disturbance in pigment synthesis, pigment degradation, a *C. sativus* no conteúdo de ClA e ChlB. O conteúdo de ClA de *C. sativus* exposto a suspensões do compósito na concentração de 10 g/L mostrou uma redução com diferença significativa (*p* < 0.05) para CA6AgB (redução de 52%), CA12AgB (redução de 44%) e CA12AgS (redução de 31%) quando comparado ao controle. Resultados similares foram encontrados para *Crambe abyssinica* (59), *Oryza sativa* L. (60) com inibição da síntese de ClB, e *Lycopersicon esculentum* (61) com inibição da síntese tanto da ClA quanto da ClB, e *Physalis peruviana* L. com redução da clorofila total (62) quando expostas a nanopartículas de prata. O teor de clorofila, que atua como um biomarcador da atividade fotossintética de uma planta, pode ser reduzido quando exposto a metais. O conteúdo reduzido de clorofila indica uma síntese de clorofila alterada, que pode ter sérias implicações na síntese de material alimentar orgânico (63). A diminuição do conteúdo de clorofila nos tecidos de plantas tratadas com metal depende de vários factores, como distúrbios na síntese de

| Activated carbon / Carvão ativado | Chlorophyll A / Clorofila A (mg/g) | Chlorophyll B / Clorofila B (mg/g) | Evans blue / Azul de Evans (O.D. / D.O. 595 nm) | Dry weight / Peso seco (mg) |
|----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------------------|-----------------------------|
| Control / controle               | 14.9 ± 4.4                        | 6.7 ± 2.7                         | 0.44 ± 0.12                                   | 117 ± 30                    |
| AC6                              | 13.8 ± 5.3                        | 6.5 ± 2.4                         | 0.42 ± 0.13                                   | 147 ± 10                    |
| AC12                             | 12.1 ± 4.4                        | 6.4 ± 2.2                         | 0.43 ± 0.23                                   | 151 ± 2                     |
| AC25                             | 11.4 ± 5.1                        | 7.3 ± 2.9                         | 0.43 ± 0.22                                   | 152 ± 9                     |
| AC50                             | 14.8 ± 1.6                        | 6.4 ± 1.3                         | 0.42 ± 0.20                                   | 161 ± 2                     |
| Control / controle               | 15.9 ± 3.0                        | 7.2 ± 1.4                         | 0.56 ± 0.05                                   | 142 ± 19                    |
| AC6Ag                            | 12.5 ± 1.9                        | 6.4 ± 0.8                         | 0.44 ± 0.01                                   | 123 ± 16                    |
| AC12Ag                           | 15.6 ± 0.2                        | 7.2 ± 0.4                         | 0.38 ± 0.11                                   | 146 ± 2                     |
| AC25Ag                           | 13.4 ± 0.2                        | 6.0 ± 0.8                         | 0.35 ± 0.10                                   | 148 ± 3                     |
| AC50Ag                           | 14.6 ± 1.6                        | 7.0 ± 1.4                         | 0.32 ± 0.01                                   | 140 ± 5                     |
| Control / controle               | 16.5 ± 2.1                        | 8.0 ± 0.8                         | 0.40 ± 0.15                                   | 131 ± 8                     |
| AC6AgB                           | 8.0 ± 3.2*                        | 4.7 ± 1.6                         | 0.47 ± 0.29                                   | 151 ± 5                     |
| AC12AgB                          | 9.2 ± 1.2*                        | 4.7 ± 1.1                         | 0.41 ± 0.16                                   | 135 ± 18                    |
| AC25AgB                          | 11.8 ± 2.4                        | 5.3 ± 1.1                         | 0.45 ± 0.22                                   | 135 ± 27                    |
| AC50AgB                          | 9.9 ± 4.0                         | 5.5 ± 0.7                         | 0.35 ± 0.12                                   | 152 ± 13                    |
| Control / controle               | 15.9 ± 3.0                        | 7.4 ± 1.7                         | 0.56 ± 0.05                                   | 138 ± 17                    |
| AC6AgS                           | 11.8 ± 1.6                        | 5.8 ± 0.9                         | 0.47 ± 0.01                                   | 147 ± 2                     |
| AC12AgS                          | 11.0 ± 2.0*                       | 5.4 ± 0.6                         | 0.50 ± 0.18                                   | 136 ± 14                    |
| AC25AgS                          | 12.4 ± 1.6                        | 5.7 ± 0.1                         | 0.40 ± 0.12                                   | 131 ± 15                    |
| AC50AgS                          | 13.6 ± 2.3                        | 6.7 ± 0.2                         | 0.45 ± 0.18                                   | 133 ± 17                    |
and direct inhibition of the enzymatic steps coupled with chlorophyll biosynthesis (64, 65).

**Cytotoxicity**

The cytotoxicity of treated and untreated *C. sativus* was studied by root cell viability assay using Evans blue dye. This dye is a marker of membrane cell integrity. Table 4 shows the absorbance of the solutions obtained after staining the root with Evans blue and discoloring with methanol. *C. sativus* treated with composite, at a dose of 10000 mg/g and the values of the control plants, which had the same ability to fix the Evans blue dye. These results therefore indicate that there is no loss of plasma membrane integrity with exposure to composites, as the values for the treated plants were similar to those of the control plants. These results differed from those of potato seedlings treated with Ag-NP and AgNO₃, which showed a significant increase in Evans blue absorption in both treatments (66).

**Toxicity of composites to Artemia Salina**

The results for the mortality rate of *A. salina* exposed to different composites at different time intervals (24 and 48 h) are shown in Table 5. In the positive control, exposure to K₂Cr₂O₇ showed 100% mortality within 24 h. In the groups treated with different composites, the mortality rates after 24 h ranged from 0-3%. These mortality rates were below 11% after 48 h, except for that of AC12AgB, which was 15%. The LD50 value for all composites must therefore be greater than the concentration tested (1000 mg/L).

The results showed that the composites were less toxic when compared to PVP/PEI-coated AgNPs, with mortality rates of about 70% after 48 exposure at 100 mg/L dose (67). Toxicity was lower than that reported for chitosan-silver nanoparticle-fluoride composites, with mortality rates above 60% at a concentration of 1000 mg/L (68). Toxicity was lower than that observed for PVP-stabilized AgNP and AgNw with mortality rates of 20% and 100%, respectively, at a concentration of 100 mg/L after 48 h of exposure (69).
Conclusion

The AC containing silver nanoparticles were prepared with termite feces using different concentrations of H$_2$SO$_4$, silver, and different chemical reducers. The characteristics of the composites depend on the conditions used in their preparation. The results show that these composites have good antimicrobial activity, with MICs of 500 µg/mL for AC25Ag, AC25AgB and AC6AgS against *S. aureus*, *E. coli* and *C. albicans*, respectively. AC25AgB delayed the growth of *S. aureus* and *E. coli* by 48 and 32 h, respectively. The phytotoxicity results showed deleterious effects on *C. sativus* germination parameters with composites containing high silver concentrations. The ecotoxicology tests in *A. salina* demonstrated non-toxicity for all composites in the tested dose. Based on the results above, the AC25AgB composite has the potential to be used in the disinfection of water contaminated with pathogenic microorganisms. More detailed studies on the stability of composites in relation to the release of Ag$^+$ or Ag$^0$ are being conducted for a possible application of these composites in the disinfection of water.

Conclusões

Os CA contendo nanopartículas de prata foram preparados com fezes de cupim usando diferentes concentrações de H$_2$SO$_4$, prata e diferentes redutores químicos. As características dos compósitos dependem das condições empregadas na sua preparação. Os resultados mostram que esses compósitos apresentam boa atividade antimicrobiana. Com CIM de 500 µg/mL para CA25Ag, CA25AgB e CA6AgS contra *S. aureus*, *E. coli* e *C. albicans*, respectivamente. O CA25AgB retardou o crescimento de *S. aureus* e *E. coli* em 48 e 32 h, respectivamente. Os testes de ecotoxicologia em *A. salina* não mostraram toxicidade dos compósitos na dose testada. Com base nos resultados acima o compósito CA25AgB apresenta potencial para ser utilizado na desinfeção de água contaminada com microrganismos patogênicos. Estudos mais detalhados sobre a estabilidade dos compósitos com relação a liberação do Ag$^+$ ou Ag$^0$ estão sendo conduzidos para uma possível aplicação destes compósitos na desinfeção de água.
**Authors Contributions Statement**

The authors Giovana Manesco and Carla Albertina Demarchi, were responsible for the preparation and characterization of the composites, they were also responsible for the antimicrobial and toxicity tests. The authors Jean Felipe Fossá Calisto and Rafael Martello were responsible for determining silver, supervised by Prof. Dr. Jacir Dal Marco. The studies of antimicrobial activity were supervised by Prof. Dr. Alexandre Bella Cruz. Prof Dr. Clovis Antonio Rodrigues supervised the synthesis, characterization, and cytotoxicity evaluation of the composites.

**Funding**

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Conselho Nacional de desenvolvimento Científico e Tecnológico- Brasil (CNPq). The authors thank the Governo do Estado of Santa Catarina for the scholarship of Art. 170 (Giovanna Manesco).

**Conflict of Interests**

The authors declare there are no financial and/or personal relationships that could present a potential conflict of interests.

**Abbreviations**

- AC6 - termite feces, activated with 6 g de $H_2SO_4$
- AC12 - termite feces, activated with 12 g de $H_2SO_4$
- AC25 - termite feces, activated with 25 g de $H_2SO_4$
- AC50 - termite feces, activated with 50 g de $H_2SO_4$
- AC6Ag - termite feces, activated with 6 g de $H_2SO_4$, impregnated with Ag nanoparticle without reducing agent
- AC12Ag - termite feces, activated with 12 g de $H_2SO_4$, impregnated with Ag nanoparticle without reducing agent
- AC25Ag - termite feces, activated with 25 g de $H_2SO_4$, impregnated with Ag nanoparticle without reducing agent
- AC50Ag - termite feces, activated with 50 g de $H_2SO_4$, impregnated with Ag nanoparticle without reducing agent
- AC6AgB - termite feces, activated with 6 g de $H_2SO_4$

**Declaração sobre as contribuições do autor**

As autoras Giovana Manesco e Carla Albertina Demarchi, foram as responsáveis pela preparação e caracterização dos compósitos, também foram responsáveis pelos testes antimicrobianos e de toxicidade. Os autores Jean Felipe Fossá Calisto e Rafael Martello foram responsáveis pela determinação de prata, orientados pelo Prof. Dr. Jacir Dal Marco. Os estudos de actividade antimicrobiana foram supervisionados pelo Prof. Dr. Alexandre Bella Cruz. O Prof Dr. Clovis Antonio Rodrigues orientou os trabalhos de preparação, caracterização e citotoxicidade dos compósitos.

**Financiamento**

Este estudo foi financiado em parte pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código Financeiro 001 e pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil (CNPq). Os autores agradecem ao Governo do Estado de Santa Catarina pela bolsa de estudos do art. 170 (Giovanna Manesco).

**Conflito de Interesses**

Os autores declaram que não há relações financeiras e/ou pessoais que possam representar um potencial conflito de interesses.

**Abreviações**

- CA6 - fezes de cupin CA ativado com 6 g de $H_2SO_4$
- CA12 - fezes de cupin CA ativado com 12 g de $H_2SO_4$
- CA25 - fezes de cupin CA ativado com 25 g de $H_2SO_4$
- CA50 - fezes de cupin CA ativado com 50 g de $H_2SO_4$
- CA6Ag - fezes de cupin CA ativado com 6 g de $H_2SO_4$, impregnado com nanopartículas de Ag sem agente redutor
- CA12Ag - fezes de cupin CA ativado com 12 g de $H_2SO_4$, impregnado com nanopartículas de Ag sem agente redutor
- CA25Ag - fezes de cupin CA ativado com 25 g de $H_2SO_4$, impregnado com nanopartículas de Ag sem agente redutor
- CA50Ag - fezes de cupin CA ativado com 50 g de $H_2SO_4$, impregnado com nanopartículas de Ag sem agente redutor
- CA6AgB - fezes de cupin CA ativado com 6 g de $H_2SO_4$, impregnado com nanopartículas de Ag sem agente redutor
$H_2SO_4$ impregnated with Ag nanoparticle reduced with NaBH$_4$

AC12AgB - termite feces, activated with 12 g de $H_2SO_4$ impregnated with Ag nanoparticle reduced with NaBH$_4$

AC25AgB - termite feces, activated with 25 g de $H_2SO_4$ impregnated with Ag nanoparticle reduced with NaBH$_4$

AC50AgB - termite feces, activated with 50 g de $H_2SO_4$ impregnated with Ag nanoparticle reduced with NaBH$_4$

AC6AgS - termite feces, activated with 6 g de H2SO4 impregnated with Ag nanoparticle reduce with sucrose

AC12AgS - termite feces, activated with 12g de H2SO4 impregnated with Ag nanoparticle reduce with sucrose

AC25AgS - termite feces, activated with 25 g de H2SO4 impregnated with Ag nanoparticle reduce with sucrose

AC50AgS - termite feces, activated with 50 g de H2SO4 impregnated with Ag nanoparticle reduce with sucrose

impregnado com nanopartículas de Ag e reduzido com NaBH$_4$

CA12AgB - fezes de cupin CA ativado com 12 g de $H_2SO_4$ impregnado com nanopartículas de Ag e reduzido com NaBH$_4$

CA25AgB - fezes de cupin CA ativado com 25 g de $H_2SO_4$ impregnado com nanopartículas de Ag e reduzido com NaBH$_4$

CA50AgB - fezes de cupin CA ativado com 50 g de $H_2SO_4$ impregnado com nanopartículas de Ag e reduzido com NaBH$_4$

CA6AgS - fezes de cupin CA ativado com 6 g de $H_2SO_4$ impregnado com nanopartículas de Ag e reduzido com sacarose

CA12AgS - fezes de cupin CA ativado com 12g de $H_2SO_4$ impregnado com nanopartículas de Ag e reduzido com sacarose

CA25AgS - fezes de cupin CA ativado com 25 g de $H_2SO_4$ impregnado com nanopartículas de Ag e reduzido com sacarose

CA50AgS - fezes de cupin CA ativado com 50 g de $H_2SO_4$ impregnado com nanopartículas de Ag e reduzido com sacarose
References / Referências

1. Syafiuiddin A, Salmiati S, Hadibara T, Kueh ABH, Salim MR, Zaini MAA. Silver Nanoparticles in the Water Environment in Malaysia: Inspection, characterization, removal, modeling, and future perspective. Sci Reports. 2018;8(1):1–15.

2. Panáček A, Smekalová M, Věčerová R, Bogdanová K, Röderová M, Kolář M, Kilianová T, Froning JP, Havrdová M, Prucek R, Zbořil R. Kvitek L. Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Enterobacteriaceae. Colloid Surface B. 2016;142:392–399.

3. Booshehri AY, Wang R, Xu R. Simple method of deposition of CuO nanoparticles on a cellulose paper and its antibacterial activity. Chem Eng J. 2015;262:999–1008.

4. Akhibe L, Ouki S, Sarjo D. Disinfection and removal performance for Escherichia coli and heavy metals by silver-modified zeolite in a fixed bed column. Chem Eng J. 2016;295:92–98.

5. Panáček A, Smekalová M, Věčerová R, Kolář M, Röderová M, Dyčka F, Šebela M, Prucek R, Tomance O, Zbořil R. Bacterial resistance to silver nanoparticles and how to overcome it. Nat Nanotechnol., 2018;13(1):65–7.

6. Prashantha Kumar TKM, Mandlimath TR, Sangeetha P, Sakhthivel P, Revathiv SK, Ashok Kumar SK, Sahoo SK Highly efficient performance of activated carbon impregnated with Ag, Zno and Ag/ZnO nanoparticles as antimicrobial materials. RSC Advances. 2015;5(130):108034–108043.

7. Syafiuiddin A, Salmiati S, Kueh ABH, Salim MR, Hadibara T, Nur H. A review of silver nanoparticles: research trends, global consumption, synthesis, properties, and future challenges. J Chin Chem Soc. 2017;64(7):732–756.

8. Fan M, Gong L, Huang Y, Wang D, Gong Z. Facile preparation of silver nanoparticle decorated chitosan cryogels for point-of-use water disinfection. Sci Total Environ. 2018;613:1317–1323.

9. Durán N, Durán M, de Jesus, MB, Seabra AB, Fávaro WJ, Nakazato G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. Nanomed-Nanotechnol. 2016;12(3):789–799.

10. Biswas P, Bandypadhyay R. Water disinfection using silver nanoparticle impregnated activated carbon: Escherichia coli cell-killing in batch and continuous packed column operation over a long duration. Water Res. 2016;100:105–115.

11. Panáček A, Kvitek L, Smekalová M, Věčerová R, Kolář M, Röderová M, Dyčka F, Šebela M, Prucek R, Tomance O, Zbořil R. Bacterial resistance to silver nanoparticles and how to overcome it. Nat Nanotechnol., 2018;13(1):65–7.

12. Prashantha Kumar TKM, Mandlimath TR, Sangeetha P, Sakhthivel P, Revathiv SK, Ashok Kumar SK, Sahoo SK Highly efficient performance of activated carbon impregnated with Ag, Zno and Ag/ZnO nanoparticles as antimicrobial materials. RSC Advances. 2014;5:108034–108043.

13. Kim MH, Cho D, Kwon OH, Park WH. Thermal fabrication and characterization of Ag nanoparticle-activated carbon composites for functional wound-dressing additives. J Alloys Compd. 2018;735:2670–2674.

14. Altintig E, Arabac G, Altundag H. Preparation and characterization of the antibacterial efficiency of silver loaded activated carbon from corncobs. Surf Coat Technol. 2016;304:63–67.

15. Ortiz-Ibarra H, Torres-Vitela R, Gómez-Salazar S, Casillas N, Ponce de León C, Walsh FC. Enhancement of antibacterial efficiency at silver electrodeposition on coconut shell activated carbon by modulating pulse frequency. J Solid St Electroch., 2018;22(3):749–759.

16. Sudhakar P, Soni NH. Catalytic reduction of Nitrophenols using silver nanoparticles-supported activated carbon derived from agro-waste. J Environ Chem Eng. 2018;6(1):28–36.

17. Marková Z, Šišková K, Filip J, Šafářová K, Prucek R, Panáček A, Kolář M, Zbořil R. Chitosan-based synthesis of magnetically-driven nanocomposites for antibacterial and hyperthermic applications. Curr App Phys. 2015;15(11):1482–1487.

18. Filippo E, Serra A, Buccolieri A, Manno D. Green synthesis of silver nanoparticles with sucrose and maltose: Morphological and structural characterization. J Non-Cryst Solids. 2010;356(6–8):344–350.

19. Syafiuiddin A, Hadibara T, Kueh ABH, Salim MR, Hadibara T, Nur H. A review of silver nanoparticles: research trends, global consumption, synthesis, properties, and future challenges. J Chin Chem Soc. 2017;64(7):732–756.

20. Panáček A, Smékalová M, Večeřová R, Bogdanová K, Röderová M, Kolář M, Kilianová T, Froning JP, Havrdová M, Prucek R, Zbořil R. Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Enterobacteriaceae. Colloid Surface B. 2016;142:392–399.

21. Biswas P, Bandypadhyay R. Water disinfection using silver nanoparticle impregnated activated carbon: Escherichia coli cell-killing in batch and continuous packed column operation over a long duration. Water Res. 2016;100:105–115.

22. Panáček A, Smekalová M, Věčerová R, Kolář M, Röderová M, Dyčka F, Šebela M, Prucek R, Tomance O, Zbořil R. Bacterial resistance to silver nanoparticles and how to overcome it. Nat Nanotechnol., 2018;13(1):65–7.

23. Prashantha Kumar TKM, Mandlimath TR, Sangeetha P, Sakhthivel P, Revathiv SK, Ashok Kumar SK, Sahoo SK Highly efficient performance of activated carbon impregnated with Ag, Zno and Ag/ZnO nanoparticles as antimicrobial materials. RSC Advances. 2014;5:108034–108043.

24. Kim MH, Cho D, Kwon OH, Park WH. Thermal fabrication and characterization of Ag nanoparticle-activated carbon composites for functional wound-dressing additives. J Alloys Compd. 2018;735:2670–2674.

25. Altintig E, Arabac G, Altundag H. Preparation and characterization of the antibacterial efficiency of silver loaded activated carbon from corncobs. Surf Coat Technol. 2016;304:63–67.

26. Ortiz-Ibarra H, Torres-Vitela R, Gómez-Salazar S, Casillas N, Ponce de León C, Walsh FC. Enhancement of antibacterial efficiency at silver electrodeposition on coconut shell activated carbon by modulating pulse frequency. J Solid St Electroch., 2018;22(3):749–759.

27. Sudhakar P, Soni NH. Catalytic reduction of Nitrophenols using silver nanoparticles-supported activated carbon derived from agro-waste. J Environ Chem Eng. 2018;6(1):28–36.

28. Marková Z, Šišková K, Filip J, Šafářová K, Prucek R, Panáček A, Kolář M, Zbořil R. Chitosan-based synthesis of magnetically-driven nanocomposites for antibacterial and hyperthermic applications. Curr App Phys. 2015;15(11):1482–1487.

29. Filippo E, Serra A, Buccolieri A, Manno D. Green synthesis of silver nanoparticles with sucrose and maltose: Morphological and structural characterization. J Non-Cryst Solids. 2010;356(6–8):344–350.

30. Biswas P, Bandypadhyay R. Water disinfection using silver nanoparticle impregnated activated carbon: Escherichia coli cell-killing in batch and continuous packed column operation over a long duration. Water Res. 2016;100:105–115.

31. Park SJ, Ko YS, Jung H, Lee C, Woo K, Ko GP. Disinfection of waterborne viruses using silver nanoparticle-decorated silica hybrid composites in water environments. SciTotal Environ. 2018;625:477–485.

32. Dixit D, Gangadharan D, Popat KM, Reddy CRK, Trivedi M, Gadhavi DK. Synthesis, characterization and application of green seaweed mediated silver nanoparticles (AgNPs) as antibacterial agents for water disinfection. Water Sci Technol. 2018;78(1):235–246.

33. World Health Organization, Drinking-water. https://www.who.int/news-room/fact-sheets/detail/drinking-water, 2018 (accessed 14 April 2019).

34. Iannone MF, Groppa MD, de Sousa ME, Fernández van Raap MB, Benavides MP. Impact of magnetite iron oxide nanoparticles on wheat (Triticum aestivum L.) development: Evaluation of oxidative damage. Environ Exp Bot. 2016;131:77–88.

35. Libralato, G. The case of Artemia spp. in nanocotoxicology. Mar Environ Res. 2014;101(1):38–43.
36. Gercel O, Ozcan A, Ozcan AS, Gercel HF. Preparation of activated carbon from a renewable bio-plant of Euphorbia rigida by H2SO4 activation and its adsorption behavior in aqueous solutions. Appl. Surf. Sci. 2007;253(11): 4843–4852.

37. Cox M, El-Shafey EI, Pechugin AA, Appleton Q. Preparation and characterisation of a carbon adsorbent from flax shive by dehydration with sulfuric acid. J Chem Technol Biotech 1999;74(11): 1019–1029.

38. Chahm T, Souza LF, Santos NR, Silva BA, Rodrigues CA. Use of chemically activated termite feces a low-cost adsorbent for the adsorption of norfloxacin from aqueous solution. Water Sci Technol. 2019;79(2): 291–301.

39. Hamed MM, Ali MMS, Holiel M. Preparation of activated carbon from doun stone and its application on adsorption of 60Co and 152+154Eu: Equilibrium, kinetic and thermodynamic studies. J Environ Radiat. 2016;164:113–124.

40. Jiang Z, Liu Y, Sun X, Tian F, Sun F, Liang C, You W, Han C, Li C. CActivated carbons chemically modified by concentrated H2SO4 for the adsorption of the pollutants from wastewater and the dibenzothiophene from fuel oils. Langmuir, 2003;19(3): 731–736.

41. Karagöz S, Tay T, Ucar S, Erdem M. Activated carbons from waste biomass by sulfuric acid activation and their use on methylene blue adsorption. Bioresources Technol. 2009;99(14): 6214–6222.

42. Brudey T, Largitte L, Jean-Marius C, Tant T, Dumensil PC, Lodewyckx P. Adsorption of lead by chemically activated carbons from three lignocellulosic precursors. J Anal Appl Pyr. 2016;120:450–463.

43. Bohli T, Ouederni A. Improvement of oxygen-containing functional groups on olive stones activated carbon by ozone and nitric acid for heavy metals removal from aqueous phase. Environ Sci Pol Res. 2016;23(16): 15852–15861.

44. Arevalo-Fester J. Efficiency study of silver nanoparticles (AgNPs) supported on granular activated carbon against Escherichia coli. J Nanomed Res. 2014;1(2):1-5.

45. Jawad AH, Rashid RA, Ishak MAM, Wilson LD. Adsorption of methylene blue onto activated carbon developed from biomass waste by H2SO4 activation: kinetic, equilibrium and thermodynamic studies. Desalin Water Treat. 2016;57(52):25194–25206.

46. Kuster SO, Barana A, Chauanbha B, BoAli AAK, Essa M, Alsadai A. Silver nanoparticle-loaded activated carbon as an adsorbent for the removal of mercury from arabian gas-condensate. Arab J Sci Eng. 2019;44(7): 6285–6293.

47. Ewais HA, Obaid AY. Adsorption characteristics of toxic chromium(VI) from aqueous media onto nanosized silver nanoparticles-treated activated carbon. Sep Sci Technol. 2019;54(4):494–506.

48. Veisi H, Kavian M, Hekmati M, Hemmati S. Biosynthesis of the silver nanoparticles on the graphite oxide’s surface using Pistacia atlantica leaves extract and its anti-oxidant activity against some human pathogens. Polyhedron, 2019;161: 338–345.

49. Xiu Z, Ma J, Alvarez PJJ. Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. Environ Sci Technol. 2011;45: 9003–9008.

50. Sun Q, Cai X, Li J, Zheng M, Chen Z, Yu CP. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity. Colloids and Surfaces A. 2014;444:226–231.

51. Dat MN, Lhin VN, Huy LA, Huong NT, Tu TH, Phuong NTL, Nam HM, Thanh MP, Hieu NH. Fabrication and antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus of silver nanoparticle decorated reduced graphene oxide nanocomposites. Mater Technol. 2019;34(7):369–375.

52. Wang W, Xiao K, He T, Zhu L Synthesis and characterization of Ag nanoparticles decorated mesoporous sintered activated carbon with antibacterial and adsorptive properties J Alloys Comp. 2015; 647:1007-1012.

53. Tuan TQ, Son NV, Dung HTK, Luong NH, Thuy BT, Anh NTV, Hoa ND, Hai NH. Preparation and properties of silver nanoparticles loaded in activated carbon for biological and environmental applications. J Hazard Mat. 2019;1(2):1-5.

54. Bohli T, Ouederni A. Improvement of oxygen-containing functional groups on olive stones activated carbon by ozone and nitric acid for heavy metals removal from aqueous phase. Environ Sci Pol Res. 2016;23(16): 15852–15861.

55. Yang F.C, Wu KH, Liu MJ, Lin WP, Hu MK. Evaluation of the antibacterial efficacy of bamboo charcoal/silver biological protective material. Mat Chem Phys. 2009;113(1):474–479.

56. Cui J, Yin Y, Lu Y, Li F Rice husk based porous carbon loaded with silver nanoparticles by a simple and cost-effective approach and their antibacterial activity. J Colloid Interf Sci. 2015;455:117–124.

57. Cui J, Hu C, Yang Y, Wu Y, Yang L, Wang Y, Liu Y, Jiang Z. Facile fabrication of carbonaceous nanospheres loaded with silver nanoparticles as antibacterial materials. J Mat Chem. 2012;22(22):811–826.

58. Barrera R, Casals E, Colón J, Font X, Sánchez A, Puntes V. Evaluation of the ecotoxicity of model nanoparticles. Chemosphere, 2009;75(7):850–857.

59. Tripathi A, Liu S, Singh PK, Kumar N, Pandey AC, Tripathi DK, Chauhan DK, Sahi S. Differential phytotoxic responses of silver nitrate (AgNO3) and silver nanoparticle (AgNPs) in Cucumis sativus L. Plant Gen. 2017;11: 255–264.

60. Ma C, Chiklara S, Mineo R, Long S, Susante C, White JC, Xing B, Dhanekar OP. Reduced silver nanoparticle photosynthesis in Crambe abyssinica with enhanced glutathione production by overexpressing bacterial γ-glutamylcysteine synthase. Environ Sci Technol. 2019; 45:10117–10126.

61. Mirzajani F, Askari H, Hamzelou S, Farzaneh M, Ghasempour A. Effect of silver nanoparticles on Oryza sativa L. And its rhizosphere bacteria. Ecotox Environ Saf. 2013; 88:48–54.

62. Songu, JH, Waldman B, Roh J, Kim Y, Yi J, Lee EJ. Functionalities of nanoparticle toxicity: A comparative study of the effects of TiO2, and Ag on tomatoes (Lycopersicon esculentum). Ecotox Environ Saf. 2013;93:60–67.

63. Timoteo CO, Paiva R, Reis MV, Claro PIC, Ferraz LM, Marconcini JM, Oliveira JE. In vitro growth of Physalis peruviana L. affected by silver nanoparticles. 3 Biotech, 2019; 9:145-150.

64. Rani PU, Yasar J, Loke KS, Dutta, D. Effect of synthetic and biosynthesized silver nanoparticles on growth, physiology and oxidative stress of water hyacinth: Eichhornia crassipes (Mart) Solms. Acta Phys Plant. 2016;38(2):1-8.

65. Nair PMG, Chung IM Physiological and molecular level effects of silver nanoparticles exposure in rice (Oryza sativa L.) seedlings. Chemosphere. 2014; 112: 105–113.

66. Karimi J, Mohsenzadeh S. Physiological effects of silver nanoparticles and silver nitrate toxicity in Triticum aestivum. Iran J Sci Technol Trans. A 2017;41(1):111–120.

67. Homae MB, Ehsanpour AA. Silver nanoparticles and silver ions: Oxidative stress responses and toxicity in potato (Solanum tuberosum L) grown in vitro. Hort. Environ. Biotechnol. 2016;57(6):544–553.

68. Lacave JM, Fanjul A, Bilbao E, Gutierrez N, Barrio I, Arostegui I, Cajaraville PC, Slamfod TCM, Rosenblatt A. Antimicrobial and cytotoxicity evaluation of colloidal chitosan – silver nanoparticles – fluoride nanocomposites. Int J Biol Macromol. 2016;93:896–903.

69. An HJ, Sarkheil M, Park HS, Yu J, Johari SA. Comparative toxicity of silver nanoparticles (AgNPs) and silver nanowires (AgNWs) on saltwater microcrustacean, Artemia salina. Comp Biochem Phys C. 2019;218:62-69.