

A NANOCOMPOSITE BASED ON MICROBIAL SYNTHESIS OF HUMIC PRECURSORS AND OF METAL NANOPARTICLES USED AS AN ANTIMICROBIAL COMPOUND IN SOIL

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Abstract: Soil humic precursors could be considered the most active and mobile fraction and are highly significant to a series of biochemical processes in all types of soil. The microbial biosynthesized humic precursors attracted increasing attention on green synthesis of nanocomposite compounds realized between biopolymers and metal nanoparticles. Silver nanoparticles are the most used engineered nanocomposite serving as antimicrobial agents.

In the present study we used aqueous solution of humic precursors synthesized by four microbial consortia (C1-C4) and selected on the basis of high quantities of exometabolites with structural similarities to soil humic acid fraction. The humic precursors were used as capping agents of silver nanoparticles in the nanocomposite synthesis.

Biosynthesized humic precursors act as reductive and stabilizative agents of nanoparticles which are found between 5-300nm in size and with spherical preponderant shape.

The presence of humus precursors and the biosynthesized silver nanoparticles was confirmed by FTIR and UV-Vis.

At a given precursor concentration, the efficiency of nanocomposite synthesis increased with particle concentration and time of reaction, property which can be attributed to the high reduction capacity of humic precursors.

The induced antimicrobial effect of exposure to nanocomposites differs due to the size, time of preparation and stability.

Stabilization of nanocomposite by specific metal-ligand bonds was obtained in the solution for three months without any precipitate.

The antimicrobial effect of nanocomposites was estimated under laboratory agar well diffusion tests against mycotoxigenic soil fungal isolate *Aspergillus niger*

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(A27). The green synthesis of nanocomposite material with the best antimicrobial effect against test fungus was realized by microbial consortium C3 and C4.

1. Introduction

Silver (Ag) has a strong toxicity to a broad spectrum of microorganisms. Due to their antimicrobial properties, silver nanoparticles (AgNPs) were obtained by nanotechnologies involving reducing agents of chemical or biological nature (Sarsar et al., 2014; Elbeshehy et al., 2015; Khodashenas and Ghorbani, 2015) and used in biomedical applications, food preservation and other ecological disinfection processes (Prabhu and Poulouse, 2012; Matei et al., 2015).

Humic precursors have a variety of components including quinone, phenol, catechol and sugar moieties. The functional groups which contribute most to surface charge and reactivity of prehumic substances are phenolic and carboxylic groups. The molecules form supramolecular structures held together by non-covalent forces, such as Van der Waals force, π - π , and CH- π bonds.

Nanocomposites made of humic precursors and silver nanoparticles could be an alternative in production of bactericidal products (Akaighe et al., 2011). By the ratio of large surface, volume, and high active surface, metal nanoparticles have properties to influence the growth of catalytic activity.

Nanocomposites made from humic precursors biosynthesized by microbial consortia and silver nanoparticles attempt to capitalize matrix characteristics for obtaining antimicrobial nanocomposites and selecting efficient consortia. The AgNPs toxicity depends on their size and shape because the control of these parameters becomes a crucial factor during their synthesis and stabilization (Ramirez et al., 2009; Cunha et al., 2017).

Non-toxic nanocomposites with antimicrobial activity can be synthesized by reducing silver nitrate in the presence of biopolymers (Lam et al., 2018).

The nanocomposites with polyvalent and synergistic antimicrobial effects can effectively kill soil pathogenic microflora without the appearance of adaptive resistance (Xiaomei et al., 2017; 2018; Matharu et al., 2018).

The antimicrobial properties of such nanocomposites can be also exploited in products such as antimicrobial sprays, cosmetics, detergents, food supplements (Tran et al., 2013).

Research has been carried out to assess the influence of biopolymers as humic precursors from four microbial consortia (C1-C4) as a silver reducing and capping agents for synthesis of nanocomposite materials as new efficient antimicrobial agents.

2. Material and methods

Microorganisms -A mycotoxigenic soil fungal isolate of *Aspergillus niger* (A27) obtained from laboratory collection of INCDPAPM-ICPA Buc. was used for assessing antimicrobial activity of prepared nanocomposites.

The humic precursors were biosynthesized by four soil microbial consortia C1-C4 (including 22 isolates of bacteria and fungi belonging to genera *Pseudomonas*, *Lactobacillus*, *Streptomyces*, *Fusarium* and *Botrytis*) selected on the basis of quantities of exometabolites and the structural similarities of compositions with standard humic acid fractions, as presented in previous published results (Matei et al., 2016).

Exometabolites were assessed by spectrophotometric method for absorbance at two wavelengths 465 and 665nm, with a standard working solution containing humic acid (HA) prepared in pyrophosphate alkali solvent.

Preparation of nanocomposites – The nanocomposite materials were synthesized by *in situ* chemical oxidation-polymerization of silver nitrate (1mM) with biopolymers from consortia C1-C4. The mixture was allowed to react for 48h under constant stirring at 25°C.

UV–Vis spectroscopy -The absorption spectrum of the reaction mixtures was recorded at room temperature using UV–Vis spectrophotometer (Rayleigh, UV-2100) at a resolution of 1 nm.

FT-IR spectroscopy -The products were analysed by Fourier transform infrared (FT-IR) spectroscopy using KBr disks with spectrophotometer over the 4000 to 400 cm⁻¹ range, at a rate of 16 nm/s (Gerasimowicz and Byler, 1985).

TEM- Transmission electron microscopy analysis of silver nanoparticles size and shape was performed using (TEM) JEM – 1400 (Jeol) microscope (80kV accelerating voltage), according to the method described by Dwivedi and Gopal (2010).

SEM-Field emission scanning electron microscopy was applied to observe the surface morphology of nanocomposite materials using a (SEM) JSPM – 5200 instrument (10kV accelerating voltage), accordingly to Paulo et al., (2012).

Antimicrobial activity assay -The antimicrobial activity of nanocomposite materials was tested against mycotoxigenic soil fungi *Aspergillus niger* (A27) using agar well diffusion method (Magaldi et al., 2004).

3. Results and discussion

Silver nanoparticle colloidal solutions were synthesized by using biopolymers as reducing agents. Since biopolymers were biosynthesized by microbial consortia C1-C4, the composition and reactivity were different from one to another.

Extracellular extracts with humic precursors biosynthesized by consortia C1-C4 are presented in Figure 1.

The biopolymers acted as reducing agents of the silver (Ag^+) ions.

For assessing the concentration of exometabolites, similar in structure with humic acids, in this study were used aqueous solutions of standard humic acids. Results evidenced the presence and the level of biopolymers in culture medium at all four microbial consortia (C1-C4). Also, every consortium was analysed for the quantities of exometabolites produced and for similarities with composition of humic acid subfractions.

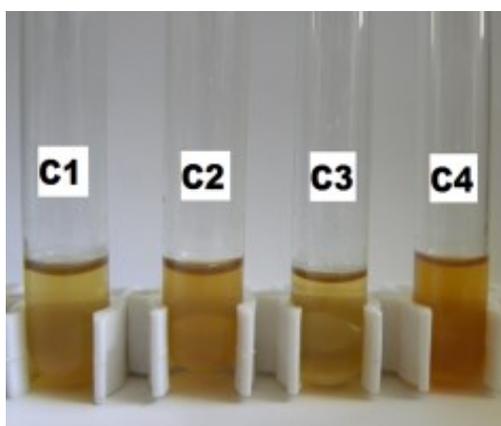


Fig. 1 Humic precursors biosynthesized by consortia C1-C4

The FTIR spectra of the biosynthesized humic precursors exhibited different absorbances due to the different qualitative compositions and the different spectra aspect was due to the relative intensity of some bands. Thus, the main bands attributions (according to Tatzber et al., 2007) comprise a broad band at $3600\text{-}3200\text{ cm}^{-1}$, which corresponds to the OH groups, organic phenols and acids H-linked; a band at $2930\text{-}2920\text{ cm}^{-1}$ from the tensioning of the C-H alkyl structures; a broad band at $1650\text{-}1630\text{ cm}^{-1}$ with the first pick at 1600 cm^{-1} and the second less picked at $1540\text{-}1550\text{ cm}^{-1}$ produced mainly by the aromatic groups C = C, C = O of amides (I), ketones and quinones; a broad band between 1120 and 980 cm^{-1} , centered at 1034 cm^{-1} , which generally characterize aromatic ethers as well as carbohydrates. All spectra of humic precursors showed a high intensity at the bands: 3400 , 1650 and 1034 cm^{-1} .

Biosynthesized humic precursors proved to be efficient reductive and stabilizative agents and formed silver nanoparticles with $5\text{-}300\text{ nm}$ in size and with spheric preponderant shape.

The colour of reaction mixture between humic precursors and AgNO_3 changed from yellow to brown after 48 hours from the addition of AgNO_3 (1 mM) due to

excitation of Surface plasmon vibration in metal nanoparticles (Singh and Raja, 2011).

AgNPs synthesized using humic precursors from C1-C4 acting as reducers of silver ions were grouped in function of the sizes, in five categories: 5-10nm; 10-30nm; 30-100nm; 100-150nm; 150-200nm (Fig. 2).

For all C1-C4 humic precursors preponderant was the group of AgNPs with the sizes 5-10nm. In the case of humic precursors from consortium C4, the presence of AgNPs in the 5-10nm group represented 71% from all the mixture (Fig. 2) and with a prevalent spheric shape (Fig. 3).

Surface plasmon resonance occurs at 420-450nm as indicative of silver nanoparticles formation.

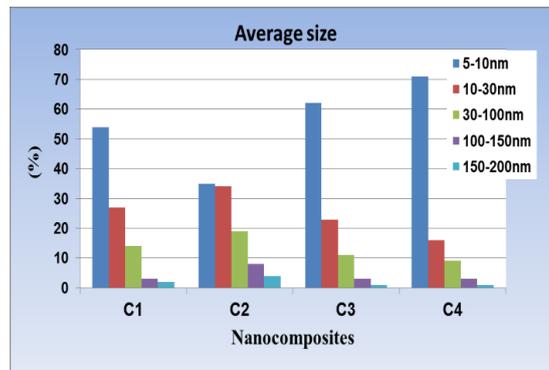


Fig. 2 Effect of reducers of consortia C1-C4 on the size of AgNPs

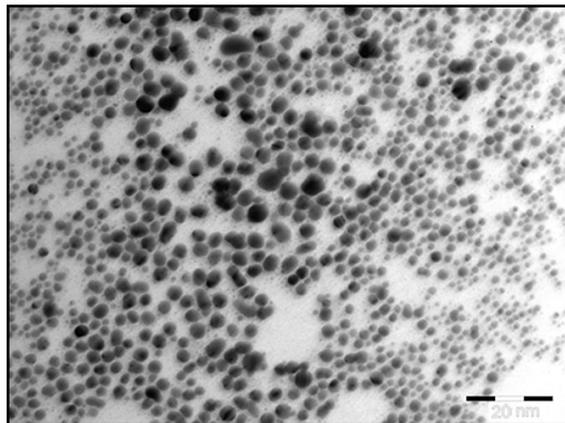


Fig. 3 TEM images of spherical AgNPs synthesized

with humic precursors from consortium C4

The biosynthesis of silver nanoparticles mediated by humic precursors from consortia C1-C4 was confirmed by UV-Vis spectroscopy at 430nm (Fig. 4). The reduction process of Ag^+ to Ag^0 and AgNPs formation was monitored by absorption and fluorescence spectral changes in addition to colour change of reaction mixture from yellow to brown. The results showed the maximum absorption peak at the wavelength of 430nm which refers to silver nanoparticles formation, according to statements from literature, that “AgNPS exhibit maximum UV-visible absorption in the range of 400-500nm because of surface plasmon resonance” (Sestry et al., 1997; Al-Zahrani and Al-Garni, 2019).

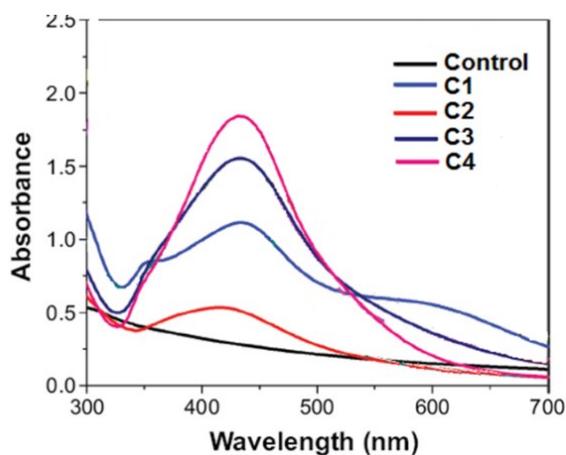


Fig. 4 UV-visible spectrum of AgNPs with humic precursors from consortia C1-C4

The humic precursors from consortia C1-C4 also acted as capping agents of silver nanoparticles in synthesis of nanocomposite materials (Fig. 5).



Fig.5 Nanocomposites between humic precursors from consortia C1-C4

and silver nanoparticles

As in a typical synthesis process, humic precursors existing in the resulted solution of colloidal Ag nanoparticles realized with these a complex mixture in aqueous solution. The mixture was allowed to react under constant stirring.

The morphology of the nanocomposite materials realized as a result of the complex mixture particularities is shown in Figure 6. These SEM images evidenced the level of porosity and structural organization of nanocomposite material which contains precursors coated silver nanoparticles.

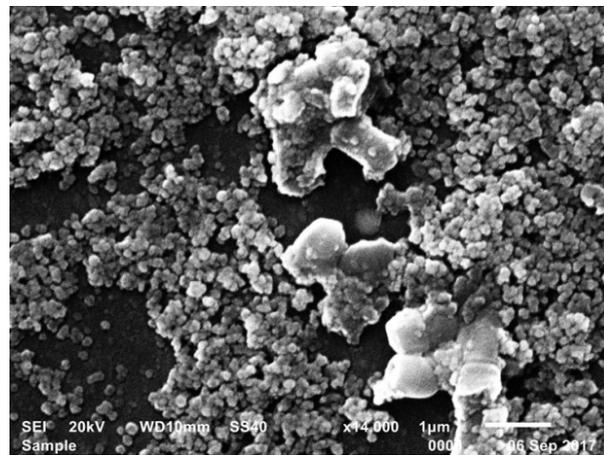


Fig. 6 SEM images of morphology of nanocomposite of consortium C3

The induced antimicrobial effect by exposure to nanocomposites differs as a function of their size, the time of preparation and stability.

The effect of nanocomposite materials on mycotoxigenic soil fungi was estimated in laboratory under the agar well diffusion tests against fungal isolate of *Aspergillus niger* (A27).



Fig. 7 Antifungal activity of nanocomposites against *Aspergillus niger*
a) Control; b) Consortium C1; c) Consortium C2; d) Consortium C3; e) Consortium C4;

The green synthesized nanocomposites revealed the best antimicrobial effect against fungal isolate in the case of microbial consortia C3 and C4. The average formed inhibition zone was of 12mm for C3 and 15mm for C4 (Fig. 7 and 8).

The results are similar to those reported for uniform spherical AgNPs of 1-10nm diameter (obtained using fruit waste material as reducing agent) that presented antimicrobial activity against *Salmonella* sp., *Proteus* sp., *Klebsiella* sp., *Candida* sp. and *Aspergillus* sp. (Kirunthika and Somanathan, 2014).

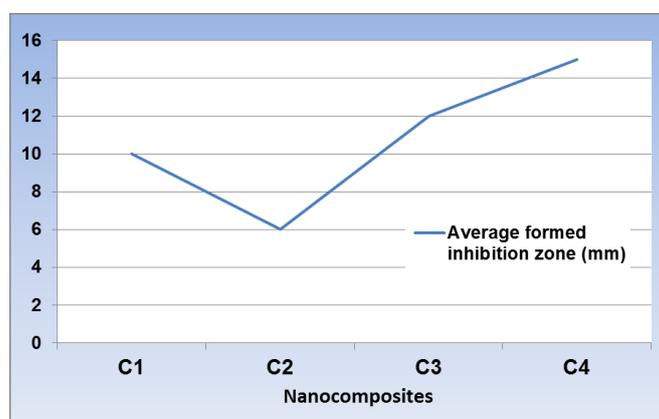


Fig. 7 Average of inhibition zones obtained from nanocomposites C1-C4 against mycotoxigenic fungus *Aspergillus niger* (A27)

The stability of nanocomposites was maintained in the solution for three months without any precipitate by the specific metal-precursors bonds.

Conclusion

The synthesis of silver nanoparticles under the consortia C1-C4 biopolymers was confirmed at 430nm.

AgNPs obtained had between 5-300nm in sizes and a spherical preponderant shape under the reductive and stabilizative humic precursors from consortia C1-C4.

Preponderant AgNPs synthesized using humic precursors from all consortia belonged to the size group of 5-10nm.

The percentage of the AgNPs presence in 5-10nm group was of 71% under the influence of humic precursors from consortium C4.

The SEM morphology of nanocomposites showed the porous structure of coated nanoparticles.

FTIR spectra of humic precursors of consortia C1-C4 showed a high intensity for bands 3400, 1650 and 1034 cm^{-1} .

The green synthesized nanocomposites with the best antimicrobial effect against mycotoxigenic fungal isolate *Aspergillus niger* (A27) were those realized with compounds produced by microbial consortia C4 and C3 with high average diameter of inhibition zones.

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