ENSURING SAFE WATER ACCESS THROUGH MYCOGENIC SILVER NANOPARTICLES ENCAPSULATED IN SODIUM ALGINATE

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ABSTRACT

The development of effective and cost-efficient water disinfection methods is a key objective outlined by the United Nations in its 2030 Agenda for Sustainable Development. Currently, silver nanoparticles (AgNPs), particularly those derived from biological sources, are emerging as an attractive alternative due to their economic and environmental advantages. In this study, we evaluated the efficacy of AgNPs synthesized by marine fungi and encapsulated in alginate gel for disinfecting water contaminated with *Escherichia coli* and *Pseudomonas aeruginosa*. Our results demonstrated remarkable antimicrobial activity, with reductions exceeding 70 % in the concentration of these pathogenic bacteria within 60 minutes for *E. coli* and 20 minutes for *P. aeruginosa*. The alginate gel encapsulation effectively stabilized and controlled the release of AgNPs without significant toxicity to the bacteria, validating their efficacy. This research highlights the potential of biologically derived, more specifically mycogenic, encapsulated AgNPs as a promising approach for water disinfection, providing an effective solution for managing microbiological contamination in water resources.

Keywords: Mycogenic silver nanoparticles. Alginate immobilization. *Escherichia coli*. *Pseudomonas aeruginosa*. Water disinfection.

1 INTRODUCTION

Access to clean water and basic sanitation is a fundamental human right, essential for maintaining dignified health, housing, nutrition, and environmental preservation [1]. However, unequal access and inadequate provision of safe drinking water expose populations to extreme vulnerability, increasing the risk of waterborne diseases such as COVID-19 [2]. Data from the Brazilian Institute of Geography and Statistics (IBGE) and the Annual Evaluation Report of Plansab reveal that many Brazilian municipalities still fail to meet water potability standards.

The presence of fecal pathogens in treated water, including certain strains of *Escherichia coli*, is a common cause of diarrheal diseases, posing a serious public health challenge [3,4]. These pathogens serve as indicators of fecal contamination and the potential presence of other enteric pathogens [5,6]. Additionally, the opportunistic pathogen *Pseudomonas aeruginosa*, found in moist environments and water distribution networks, further complicates water quality assurance [7,8]. This Gram-negative bacterium forms biofilms resistant to disinfection, contributing to its persistence in adverse environments [9]. *P. aeruginosa* is associated with severe health conditions due to its antibiotic resistance [10,11]. In summary, both bacteria pose significant challenges to ensuring safe drinking water, necessitating effective treatment and control strategies to protect public health [12,13].

In this context, water disinfection plays a crucial role in reducing microbiological contamination-related diseases. However, conventional disinfection techniques may generate unwanted byproducts and financial challenges. Nanotechnology, specifically the use of silver nanoparticles (AgNPs), emerges as a promising alternative due to their antimicrobial properties [14]. Immobilizing AgNPs is essential to ensure stability and reduce environmental and human health risks associated with their release it the environment [15].

The synthesis of AgNPs by fungi, offers a sustainable and cost-effective route. Fungi can synthesize extracellular enzymes that have a crucial role on the production of AgNPs providing significant advantages in terms of cost and processing [14,15]. Recent research also explores the potential of AgNPs in wastewater disinfection, suggesting their ecologically sound use as an effective alternative to mitigate microbiological contamination [16].

In summary, developing efficient, sustainable, and economically viable water disinfection processes is crucial for universal access to safe drinking water and aligns with the Sustainable Development Goals (SGD) set by the 2030 Agenda. This study proposes the synthesis and evaluation of antimicrobial action by mycogenic AgNPs encapsulated in sodium alginate, aiming to combat bacterial pathogens contaminations and promote water safety (aligned with SDG Goal 6 – "Ensure availability and sustainable management of water and sanitation for all").

2 MATERIAL & METHODS

AgNPs mycosynthesis: the production process of AgNPs was carried out extracellularly using the *Aspergillus niger* IBCLP20 strain (supplied by the Culture Collection of the Institute of Biosciences - Campus of Litoral Paulista). This strain was originally isolated from marine sediment collected at the Araçá Bay, São Sebastião-SP. From (Malt extract Glucose Yeast extract Peptone Agar (MGYP; final composition: 3 g/L malt extract, 10 g/L glucose, 3 g/L yeast extract, 35 g/L bacteriological peptone) medium-inoculated Petri dishes, 6 mm plugs were taken from the peripheral area of grown fungal colonies and inserted into the central region of new plates containing Potato Dextrose Agar (PDA). After a 7-day incubation period, at 30 °C, the grown colonies were used as the inoculum source for the assays. The fungal supernatant was prepared by inserting 5 IBCLP20 strain plugs into a 250 mL Erlenmeyer flask containing 50 mL of liquid MGYP. After 72 hours under standard agitation conditions (150 rpm), the grown biomass was filtered and washed with 300 mL of sterile deionized water. Approximately 5 g of wet biomass were transferred to a new 250 mL Erlenmeyer flask containing 50 mL of sterile deionized water and maintained under 150 rpm agitation until filtration, which occurred after 72 hours using a Millipore filter paper (3 µm). Finally, the resulting supernatant was transferred to a new 250 mL Erlenmeyer flask for the addition of a 1.0 mM silver nitrate ($AgNO_3$) solution. This mixture was kept in a light-free environment under 150 rpm agitation for 72 hours, after which the AgNPs were formed.

Alginate Gel Encapsulation: The biopolymer solution was prepared by dissolving 3.1 % (w/v) sodium alginate in 10 mL of mycogenic AgNPs suspension. Subsequently, the biopolymer solution (10 mL) was dripped into 100 mL of an aqueous calcium chloride solution (0.14 M) using a 20 mL intravenous infusion syringe and a 27 g intravenous infusion device [17,18]. The system was constantly agitated at 500 rpm for 1 minute. After the gelation process, the microcapsules were vacuum-filtered and stored at 4 °C.

Antimicrobial Activity in Batch Mode: a volume of 50 mL of distilled water contaminated inoculated with the bacterial pathogens *E. coli* IPT-245 or *P. aeruginosa* IPT-236 at a concentration of 1 x 10^4 CFU/mL was poured into a 250 mL Erlenmeyer flasks. Subsequently, 8.8 g of encapsulated AgNPs were added to each flask. The samples were kept in an orbital shaker, at 37 °C and 150 rpm, and 10 µL withdrawals were made at 20 and 30-minute intervals. These samples were then incubated for 0, 30, 60, and 90 minutes for *E. coli*, and 0, 20, 30, 40, and 60 minutes for *P. aeruginosa* at 37 °C and 150 rpm in an orbital shaker. Subsequently, the cell counts were quantified using Tryptic Soy Agar (TSA) plates. These assays were performed in triplicate.

3 RESULTS & DISCUSSION

Given that water disinfection is essential for reducing diseases associated with pathogenic microorganisms transmitted through this route, and that strains of *E. coli* and *P. aeruginosa* are frequently found in post-treated waters worldwide [19], antimicrobial assays were conducted to assess the potential of encapsulated AgNPs produced via a mycological route. These AgNPs were derived from marine fungi isolated by our research group, and their initial interaction times were evaluated. Additionally, cytotoxicity assays were performed using the AgNPs, their precursor ($AgNO_3$), and the immobilizing agent, sodium alginate (see Figure 1).

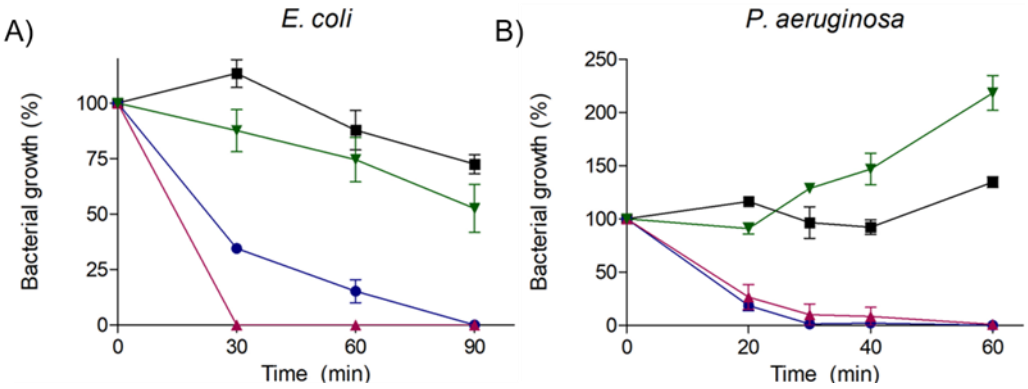

Figure 1. Antimicrobial action of AgNPs (silver nanoparticles) during the initial exposure times on *Escherichia coli* and *Pseudomonas aeruginosa*. Cells of *E. coli* (A) and *P. aeruginosa* (B) at a concentration of 104 CFU/mL were inoculated in sterile distilled water without any compound (■) or containing 8.8 g of encapsulated compound: alginate (▼), $AgNO_3$ (▲), or AgNPs (●).

The results indicate a potent antimicrobial effect of AgNPs during the initial contact with both bacterial strains. Specifically, *P. aeruginosa* exhibited 75 % cell death after 20 minutes of exposure, while *E. coli* showed similar effects after 60 minutes. These findings are significant as they demonstrate the high bactericidal potential of encapsulated AgNPs in treatment. Studies have also shown that AgNPs, at bactericidal concentrations, do not inhibit immune system cells in mammals and humans [19,20]. Additionally, AgNPs have been reported to prevent biofilm formation, a factor contributing to resistance against disinfectants, surface adhesion, and infection pathogenesis [20,21].

Furthermore, it is noteworthy that bacteria exposed solely to sodium alginate did not exhibit significant cytotoxic effects on *E. coli*. In the case of *P. aeruginosa*, there was even a stimulation of growth, which could serve as an indicator of biofilm formation aiding its dissemination under adverse environmental conditions [8,9].

4 CONCLUSION

Based on our results, the mycogenic AgNPs produced using marine fungi and immobilized with sodium alginate demonstrate high potential for water decontamination against *E. coli* and *P. aeruginosa* bacteria. The system's effectiveness was evident through a reduction of over 75 % CFUs in *P. aeruginosa* within just 20 minutes, and a similar reduction in *E. coli* within 60 minutes. Additionally, the sodium alginate gel efficiently immobilized the AgNPs without compromising bacterial viability. Interestingly, in the case of *P. aeruginosa*, it even promoted cellular growth. These findings suggest that combining these materials could be a promising approach for cost-effective and efficient water decontamination. However, further large-scale studies are essential to assess the method's viability and potential long-term effects on both the environment and human health.

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