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Biogenic Silver Nanocatalyst for Efficient Removal of Ampicillin, Ciprofloxacin, and Tetracycline from Water

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ABSTRACT: Water pollution from wastewater contaminated with bacterial pathogens and antibiotic residues results in severe public health threats. Nanomaterials have widely been employed as suitable alternatives for wastewater treatment due to their high surface area-to-volume ratio. In this study, we explored the antibacterial capabilities and antibiotic removal potential of green synthesized silver nanoparticles (AgNPs).The antimicrobial ability of the green synthesized AgNPs was assessed against the multi-drug resistant (MDR) isolates of enteroaggregative *E. coli* (EAEC), non-typhoidal *Salmonella* (NTS) species *S.* Typhimurium and *S.* Enteritidis and methicillin-resistant *Staphylococcus aureus* (MRSA) and their antibiotic removal was assessed against three classes of antibiotics namely ampicillin, ciprofloxacin, and tetracycline. The antibacterial results indicated minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) to be 7.80 µg/mL, and 15.625 µg/mL respectively. The photocatalytic antibiotic removal results indicated that AgNPs could effectively remove all three different classes of antibiotics and the removal of ampicillin and ciprofloxacin was better achieved as compared to that of tetracycline and the removal peaked at a particular point of time for the tested antibiotics, beyond which there were no appreciable elimination.

Keywords: Ampicillin, Antibiotic removal, Antimicrobial resistance, Ciprofloxacin, Nanoparticle, Silver, Tetracycline.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as an alarming public health threat(Prajescu *et al.*, 2023).Urbanization and intensive animal husbandry practices have resulted in the extensive use of antibiotics as therapeutic candidates in human and veterinary practice, which can not completely be degraded either by animals or humans. Consequently, the antibiotic resistome present in the environment has created considerable research attention, as higher concentrations of antibiotics promote the transfer of antibiotic-resistant genes and drug-resistant bacteria. Eventually, the judicious use of antibiotics has received considerable attention because of their potential hazards to public health, particularly to the environment (Asadzadeh *et al.*, 2021; Gupta *et al.*, 2024). The majority of the antibiotics are discharged to the treatment system which could affect the bioactivity of functional flora, resulting in the deterioration of effluent quality. Moreover, the inadequate treatment of antibiotics with the discharged effluents would result in secondary pollution (Gini *et al.*, 2023; Lu *et al.*, 2020). Several approaches such as photocatalytic degradation, oxidation, chlorination, floatation, biodegradation, electrochemical methods, and micro-extraction could effectively remove a substantial quantity of antibiotics from wastewater (Neha *et al.*, 2021). The disadvantages associated with these approaches include prolonged time, vigorous operating conditions, and cost involved are mitigated with the adsorption technique ranging from macro to nanoscale range (Lu *et al.,* 2020; Neha *et al.*, 2021). Owing to the smaller size, high surface area-to-volume ratio, presence of numerous active sites, and high pollutant removal efficiency, nano-adsorbents are widely employed (Sahu *et al.*, 2021). Over the years, the use of nanomaterials as efficient photocatalysts has received considerable attention. However, the heterogeneous photocatalytic methodsemploying nanomaterials have demonstrated an effective capacity to degrade antibiotics (Batra *et al.*, 2022). Traditionally, silver nanoparticles (AgNPs) act as efficient antibacterial agents through electrostatic interaction (Atram *et al*., 2023; Ezeuko *et al*., 2021). Moreover, AgNPs exhibit catalytic, binding, optical as well as electrical properties (Ali *et al*., 2021). Therefore, we hypothesized that AgNPs could effectively act as nano-adsorbents for removing antibiotics from water. It is in this regard that this study is envisaged to assess the efficacy of AgNPs synthesized from a potential probiotic strain for the photocatalytic removal of routinely used antibiotics (ampicillin, ciprofloxacin, and tetracycline) in humans as well as veterinary practice from wastewater. This study appears to be the first of its kind to report the removal of antibiotics from probiosynthesised AgNPs.

MATERIALS AND METHODS

A. Green synthesis of AgNPs

The green synthesis of AgNPs was performed using a cell-free supernatant of a potential probiotic strain- *Lactobacillus acidophilus* MTCC 10307 as described earlier in our study (Abishad *et al*., 2022). The synthesis of AgNPs obtained was verified using a UV-Vis spectrophotometer (Thermo Fisher Scientific, USA).

B. In vitro photocatalytic antibiotic degradation ability of AgNP

The *in vitro* photocatalytic potential of the AgNPs to degrade the test antibiotics (ampicillin, ciprofloxacin, and tetracycline; Sisco Research Laboratories Pvt. Ltd., India) was investigated on exposure to sunlight (Baaloudj *et al*., 2021). Briefly, the aqueous solution of individual antibiotics (20 ppm) was mixed with AgNPs to attain a final concentration of 2 mg/mL. To obtain an adsorption-resorption isotherm, the antibiotic-NP solutions were then kept for 15 min in the dark using a magnetic stirrer at 300 rpm (Neaution Technologies Pvt. Ltd., India). The mixture was exposed separately to sunlight (52,100 lx) and the aliquots drawn at specific intervals (0, 15, 30, 60, 90, and 120 min) were centrifuged for 15 min at 5000 rpm to settle down the AgNPs. The absorbance of the supernatant was recorded at 269, 277, and 357 nm for ampicillin, ciprofloxacin, and tetracycline, respectively, using a UV-Vis spectrophotometer. Finally, the degradation of antibiotics (in %) was estimated (Lin and Cen, 2022) as

$$(A_{max} - A_0)/\ (A_{max}) \times 100$$

wherein A_{max} denotes the absorbance of the test sample at a specific time point, whereas A_0 denotes the absorbance of control.

RESULTS AND DISCUSSION

A. Green synthesis of AgNPs

The decline phase cell-free supernatant of potential probiotic bacterial strain *L. acidophilus* and aqueous solution of silver nitrate (0.10M) in the ratio of 1:4 was used for the green synthesis of AgNPs. The formation of the AgNPs was visualized by the change in the color of the solution to brownish white from an initial colorless solution. Moreover, UV-Vis spectroscopy performed to detect the surface plasmon resonance of the AgNPs (Singh *et al*., 2021) exhibited a characteristic peak at 430 nm (Fig. 1), which indicated the synthesis of AgNPs.

B. In vitro photocatalytic antibiotic degradation ability of AgNPs

The *in vitro* photocatalytic ability of the green synthesized AgNPs to degrade ampicillin, ciprofloxacin, and tetracycline on exposure to sunlight is represented in Fig. 2. There was a reduction in the absorption amplitude of the parent compound collected on the predefined period when treated with probiosynthesised AgNPs on exposure to different light sources. The elevation in the optical density readings obtained from the UV-Vis spectroscopy (Fig. 2), as observed in this study, could likely be due to the increased production of the break-down products from the antibiotics over time and was directly proportional to each other (Lin and Cen 2022). However, in this study, a highly significant ($P < 0.001$) degradation of tetracycline was observed when treated with AgNPs, while a significant ($P < 0.05$) degradation of ampicillin as well as ciprofloxacin (Fig. 3) was observed. In addition, it was also observed that the degradation of the tested antibiotics reached a maximum at a one-time point, beyond which there was no significant difference in the degradation (Fig. 3). The degradation of ampicillin (Fig. 3) and ciprofloxacin (Fig. 3) was found to reach its maximum (nearly 70%) at 90 min of exposure with AgNPs. Interestingly, with a degradation efficiency of 12%, tetracycline exhibited a mild degradation on treatment with AgNPs (Fig. 3). It has been reported that adsorption and photocatalysis constitute the primary mechanisms for the degradation of antibiotics upon treatment with nanomaterials, including AgNPs. Besides, it could be inferred that the degradation of antibiotics could largely be attributed to photocatalytic degradation rather than adsorption (Baaloudj *et al*., 2021; Dao *et al*., 2018). This phenomenon has been explained by the production of ROS such as $^{\bullet}OH$, $O2^{\bullet}$, and holes (h^+) from the surface of AgNPs when irradiated with light sources, producing simple non-toxic molecules like CO_2, water, and other smaller degradation products (Asadzadeh *et al*., 2021). The change in degradation percentage was noticed as a measure of the elimination of the parent compound to form metabolites in the due process and their further degradation to form simpler molecules like water, carbon dioxide, etc. The change in degradation percentage has a significant positive correlation with their parent compound concentrations (Ding *et al*., 2020). The elevation in the optical density readings obtained from UV-Vis spectroscopy, as observed in this study, could likely be due to the increased production of the break-down products from the antibiotics over time and was directly proportional to each other (Lin and Cen 2022). The present study evaluated the ability of AgNPs to degrade antibiotics of different structural and functional classes. The antibiotics selected were ampicillin (penicillin class), ciprofloxacin (quinolone class), and tetracycline (protein synthesis inhibitor) which all have different structural morphologies (Bryskier, 2005).

Table 1: Comparative photocatalytic potential of AgNPs for degradation of ampicillin, ciprofloxacin and tetracycline on exposure to sunlight.

Antibiotic	Time of maximum degradation (min)	Degradation (%)
Ampicillin	90	71.22
Ciprofloxacin	90	69.08
Tetracycline	90	12.85

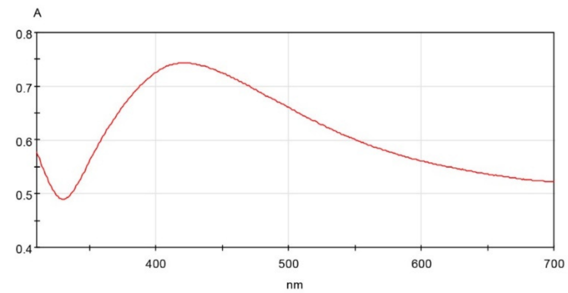

Fig. 1. Characterisation of AgNPs using UV-Vis spectroscopy.

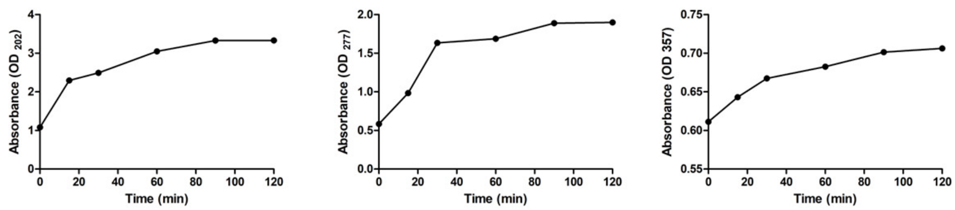

Fig. 2. Photocatalytic degradation of antibiotics treated with NPs under study on exposure to sunlight. Image (a) denotes ampicillin, (b) denotes ciprofloxacin and (c) denotes tetracycline, respectively.

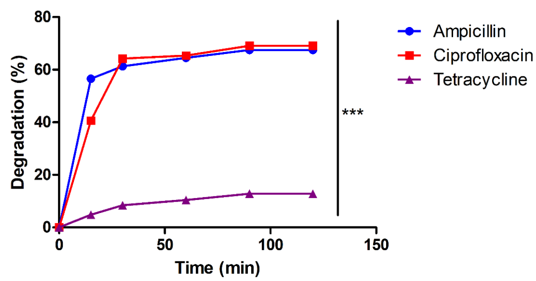

Fig. 3. Antibiotic degradation activity of green synthesized AgNPs against tested antibiotics on exposure to sunlight.

CONCLUSIONS

In short, this study evaluated the photocatalytic capability of green synthesized AgNPs for the removal of ampicillin, ciprofloxacin, and tetracycline under sunlight. This study successfully indicated the removal of ampicillin as well as ciprofloxacin in comparison to tetracycline by UV-Vis spectroscopy. Nonetheless, the removal of antibiotics peaked at a particular period beyond which the removal was negligible with the amount of time. The antibiotic removal experiments using AgNPs demonstrated that photocatalytic degradation outperforms adsorption in the removal of antibiotics. Further, in-depth chromatography-based kinetic studies are required to investigate the rate of antibiotic removal, which could be applied to wastewater treatment strategies.

FUTURE SCOPE

A significant gap in knowledge and practice among farmers in this study area regarding animal biosecurity and management.

There exists a significant gap in the knowledge and practice for the removal of antibiotics and their residues. These residues and their parent compounds could be a contributing factor to environmental pollution and have a direct effect on antimicrobial resistance. So their elimination is an area of importance. The usage of nanotechnology and nanoparticles in particular could be used as an efficient tool in the elimination of antibiotics and their residues. Also, the photocatalytic activity of the nanoparticles improves their efficiency. The biogenic preparation methods of AgNPs using the probiotic strains of *Lactobacillus acidophilus* ensure environmentally friendly preparation.

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