Evaluation of Biofilm Eradication in Foodborne Pathogens by Green Chemistry and Traditional Silver Nanoparticles

Authors: Ana Alejandra Gómez Ramos

Submitted: 22. September 2025
Published: 22. September 2025
Volume: 12
Issue: 5

Affiliation: Universidad San Francisco de Quito, Quito, Ecuador

Languages: English
Keywords: Biofilm eradication; Silver nanoparticles (AgNPs); Foodborne pathogens; Green synthesis; Food safety.
Categories: Life Sciences, Medicine, Demetrios Project
DOI: 10.17160/josha.12.5.1089

Abstract:

Biofilms, formed by foodborne pathogens such as Escherichia coli, Salmonella enterica serovar Typhimurium, Bacillus cereus, and Staphylococcus aureus, present significant challenges due to their resistance to conventional antimicrobial treatments. In this study, we evaluated the biofilm eradication efficiency of silver nanoparticles (AgNPs) synthesized through green and traditional methods. Green AgNPs were produced using eco- friendly plant extracts, while traditional AgNPs utilized chemical reduction with sodium borohydride ($NaBH_4$). The results demonstrated that traditional AgNPs achieved higher biofilm eradication rates at lower concentrations. For example, S. Typhimurium biofilms exhibited an 82.54% eradication rate at 0.5 mM, while green AgNPs required a higher concentration of 5 mM to achieve a comparable 81.95% eradication rate. Similarly, for Escherichia coli, traditional AgNPs at 0.5 mM achieved 82.93% eradication, whereas green AgNPs at 5 mM showed a slightly lower rate of 71.58%. In contrast, Bacillus cereus and Staphylococcus aureus, both Gram-positive bacteria, demonstrated greater

JOSHA Journal of Science, Humanities and Arts

September 2025 Volume 12, Issue 5

Evaluation of Biofilm Eradication in Foodborne Pathogens by Green Chemistry and Traditional Silver Nanoparticles

Ana Alejandra Gómez Ramos
ana.gomez.ramos.20@gmail.com
Universidad San Francisco de Quito, Quito, Ecuador

Abstract

Biofilms, formed by foodborne pathogens such as *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Bacillus cereus*, and *Staphylococcus aureus*, present significant challenges due to their resistance to conventional antimicrobial treatments. In this study, we evaluated the biofilm eradication efficiency of silver nanoparticles (AgNPs) synthesized through green and traditional methods. Green AgNPs were produced using eco- friendly plant extracts, while traditional AgNPs utilized chemical reduction with sodium borohydride ($NaBH_4$). The results demonstrated that traditional AgNPs achieved higher biofilm eradication rates at lower concentrations. For example, *S. Typhimurium* biofilms exhibited an 82.54% eradication rate at 0.5 mM, while green AgNPs required a higher concentration of 5 mM to achieve a comparable 81.95% eradication rate. Similarly, for *Escherichia coli*, traditional AgNPs at 0.5 mM achieved 82.93% eradication, whereas green AgNPs at 5 mM showed a slightly lower rate of 71.58%. In contrast, *Bacillus cereus* and *Staphylococcus aureus*, both Gram-positive bacteria, demonstrated greater resistance, requiring higher concentrations of AgNPs for effective biofilm removal. These results highlight the influence of bacterial cell wall structure on treatment efficacy. This study underscores the potential of AgNPs, particularly green-synthesized variants, as eco- friendly alternatives to conventional antimicrobials. While green AgNPs require optimization to match the efficacy of

traditional methods, their reduced environmental impact and safety profile make them promising candidates for biofilm management in food processing and related industries.

Keywords: Biofilm eradication; Silver nanoparticles (AgNPs); Foodborne pathogens; Green synthesis; Food safety.

Significance Statement

Foodborne diseases remain a pressing global public health issue, largely driven by the persistence of biofilms that protect pathogenic bacteria from conventional treatments. This work evaluates green-synthesized and traditional silver nanoparticles as innovative alternatives for eradicating biofilms formed by *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Bacillus cereus*, and *Staphylococcus aureus*. The results demonstrate that while traditional AgNPs achieve higher efficacy at lower concentrations, green- synthesized AgNPs offer an eco-friendly solution with reduced environmental impact, highlighting their potential for sustainable food safety strategies. By bridging nanotechnology and green chemistry, this study contributes to advancing antimicrobial innovation and underscores the importance of environmentally responsible approaches to combat biofilm-related contamination.

This manuscript is derived from the author's Bachelor's thesis submitted on December 18th, 2024 at Universidad San Francisco de Quito (USFQ), as a requirement for the Biotechnology Engineering degree.

Introduction

Biofilms are structured communities of microorganisms embedded within a self-produced extracellular polymeric substance (EPS) matrix, adhering to surfaces and resisting environmental stresses. (Carrascosa et al., 2021; Jara-Medina et al., 2024). This protective matrix enables microbes to thrive under adverse conditions, such as UV exposure, dehydration, lack of nutrients, and antimicrobial agents among others, making biofilms a significant concern in environmental, industrial, and medical contexts (Pang et al., 2023; Zhou et al., 2022). Biofilms can be formed on biotic and abiotic surfaces, including food processing equipment, medical devices, and living tissues, posing severe public health risks due to their enhanced antimicrobial resistance (Galié et al., 2018; Cangui-Panchi et al., 2023; Cangui-Panchi et al., 2022).

Biofilms can harbor various pathogenic bacteria, including *Escherichia coli, Salmonella enterica* serovar Typhimurium, *Bacillus cereus*, and *Staphylococcus aureus*, which present unique challenges due to their resistance profiles and survival strategies. *Escherichia coli* is a typical and commensal Gram-negative bacterium commonly associated with gut microbiota. However, certain pathogenic strains, known as *E. coli* pathotypes, can cause foodborne illnesses (Cangui-Panchi et al., 2023). Its ability to form biofilms on living and non-living surfaces enhances its resistance to environmental stresses and disinfectants, making cross- contamination in food processing environments a persistent issue. Pathotypes, such as enterohemorrhagic *Escherichia coli*, can cause severe gastrointestinal diseases, including hemolytic uremic syndrome (Zhou et al., 2022). Biofilm formation facilitates *E. coli's* survival, even under rigorous sterilization protocols (Galié et al., 2018).

Another genus containing pathogenic serovars is *Salmonella* causing global foodborne outbreaks. Most Gram-negative pathogens are able to form resilient biofilms in diverse environments, including food processing plants (Kundu et al., 2024). *Salmonella enterica* serovar Typhimurium is one of the most prevalent serovars implicated in global foodborne illnesses. This bacterium is highly adept at forming biofilms, particularly on food processing surfaces such as stainless steel, polyethylene, and other industrial materials (Pang et al., 2023). Its biofilm matrix is primarily composed of curli fimbriae and cellulose that not only facilitates adhesion to surfaces but also enhances resistance to environmental stressors, including disinfectants and desiccation (Carrascosa et al., 2021). The biofilm's protective

structure enables *S. Typhimurium* to persist under harsh conditions, leading to contamination of raw and processed foods, such as poultry, eggs, and dairy products. Moreover, the pathogen's ability to withstand sublethal concentrations of antimicrobials often results in increased resistance and cross-protection against other disinfectants such as oxidizing agents, denaturing agents, surfactants, and enzyme-based compounds (Pang et al., 2023). The persistence and adaptability of *S. Typhimurium* in biofilms pose significant challenges in ensuring food safety and public health, emphasizing the need for innovative control strategies.

Bacillus cereus, a Gram-positive bacterium, is also able to establish biofilms in submerged or surface environments. These biofilms can secrete toxins such as hemolysins HlyI and HlyII, and enzymes like proteases and phospholipases, which cause spoilage and foodborne illnesses (Majed et al., 2016). Moreover, its ability to form highly adhesive spores contributes to its biofilm resilience, making it resistant to thermal processes (Galié et al., 2018). Another Gram-positive opportunistic pathogen *is Staphylococcus aureus* forming robust biofilms on biotic and abiotic surfaces, like mucosal membranes, skin, and industrial materials like stainless steel. *S. aureus* biofilms are particularly resistant to antimicrobial agents due to their high EPS content, which includes polysaccharide intercellular adhesin and several proteins like fibrinogen-binding protein (Idrees et al., 2021; Galié et al., 2018). These biofilms also contribute to food contamination and complicated clinical treatments.

Therefore, several treatments are applied to eradicate these pathogenic biofilms during food processing. One of the suggested alternative treatments for biofilm eradication is the application of nanoparticles, particularly silver nanoparticles (AgNPs), which have emerged as promising agents for biofilm control (Liu et al., 2023). Traditional synthesis of AgNPs often employs chemical methods, such as the use of sodium borohydride as a reducing agent. However, these methods are energy-intensive, hazardous, and produce toxic by-products, limiting their environmental sustainability and safety for food industry applications (Ratan et al., 2021). In nanotechnology, green chemistry has partially replaced traditional synthesis methods, which are energy-intensive, hazardous, and generate toxic by-products. Green synthesis of nanomaterials, using clean and eco-friendly methods, has gained popularity due to its simplicity and reliance on biological systems. This approach has facilitated the application of nanoparticles in fields such as environmental remediation, photocatalysis, sensors, solar cells, and energy storage. Also, the biocompatibility and antimicrobial properties of these

nanoparticles have enabled their use in biomedical applications, including diagnostics, wound healing, immunotherapy, regenerative medicine, and targeted drug delivery. Nonetheless, challenges remain in selecting green raw materials, optimizing synthesis conditions, ensuring quality and stability, and assessing long-term properties for future use (Souza et al., 2021). Green AgNPs could enhance biofilm inhibition and eradication, particularly against Gram- negative bacteria. Their small size and high surface area enable efficient penetration and interaction with microbial cells, disrupting biofilm integrity (Shakir, 2016). So, the present study aims to compare the antibiofilm activity between AgNPs from traditional and green methodologies against well- known foodborne pathogens.

Methods

Bacteria isolates and growth conditions

Silver nanoparticles on green chemistry were studied for the ability to eradicate biofilm formation against foodborne bacteria, specifically Gram-positive bacteria such as *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923, and Gram-negative bacteria as *Escherichia coli* ATCC 25922 and *Salmonella enterica* serovar Typhimurium ATCC 14028 from the bacterial collection of Institute of Microbiology of the Universidad San Francisco de Quito (IM-USFQ). All bacterial species were stored at -80 °C and grown on Mueller-Hinton agar (MHA) medium at 37 °C for 24 hours before each assay.

Biofilm eradication assays

A culture of the bacterial species was grown overnight, followed by the preparation of an inoculum with a concentration of $1x10^7$ colony-forming units (CFU)/mL using tubes with 7 mL of saline solution, based on an initial 0.5 McFarland turbidity standard (equivalent to $1x10^8$ CFU/mL), and then diluted by a factor of 10. The inoculum was then centrifuged at 400 rpm for 10 minutes, and the pellet was re-suspended in the same volume of sterile Mueller-Hinton broth (MHB). Afterward, 200 µL of inoculum plus media were added to each well, except the negative control (containing only the media). The plate was then incubated at 37°C for 24 hours under aerobic conditions to allow biofilm formation. Following incubation, the media was carefully removed and replaced with 200 µL of sterile media. The biofilms were then treated with fresh media containing 1× and 2× of the previously determined minimum inhibitory concentrations (MIC) from each antimicrobial treatment including: Green (AgNPs), Traditional (AgNPs), Silver ions (Ag+), and NaBH4

(Cabascango, 2023). The plate was incubated once more at 37°C for 24 hours under aerobic conditions, and the wells were gently washed with phosphate-buffered saline (PBS; at pH 7.4) through a 45-degree angle, avoiding contact with the bottom of the wells. Depending on the pathogen and protocol, one or two additional washing steps with PBS (pH 7.4) were performed.

Biomass evaluation

To evaluate biomass eradication by the different nanoparticle treatments, the wells in the 96-well plates were washed once with 200 µL of PBS. Optical density (OD) values were measured at 570 nm using a microplate spectrophotometer (ELISA Elx808, Biotek, Winooski, USA), as previously outlined by Atiencia et al. (2022). This method showed no significant difference between crystal violet (CV) staining and PBS suspension techniques for measuring biomass formation, based on a modified version of Gulati et al. (2018)'s method.

Statistical analysis

All experimental data was statistically analyzed on RStudio software through non-parametric tests such as the Wilcoxon test. It was performed to compare differences between positive control and both treatments/buffer/Ag ions during the biofilm eradication assays. The applied R software was the newly released version on September 23, 2024, from the official web page (https://posit.co/download/rstudio-desktop/) using the following libraries packages: "rstatix", "readxl", "dplyr", "data.table", and "knitr".

Results

Evaluation of biofilm eradication on foodborne pathogens

The analysis of biofilm eradication in foodborne bacteria using silver nanoparticles synthesized through green chemistry (green AgNPs) versus traditional methods revealed significant results across different bacterial strains.

1. Gram-positive bacteria

Concerning *Bacillus cereus* ATCC 11778, the green AgNPs at 5.0 mM (2× MIC) demonstrated 72.62% biofilm eradication (p-value = 3.38e-05), while the traditional AgNPs treatment at 1.0 mM (2× MIC) eradicated 70.02% of biofilm (p-value =

3.38e-05). Ag+ ions at 1.0 mM showed 69.51% eradication (p-value = 3.42e-05), while the lowest efficacy was again observed with $NaBH_4$ (buffer) at 1.0 mM, which achieved only 16.60% eradication (p-value = 2.56e-03). These findings are summarized in Table 1a.

When evaluating *Staphylococcus aureus* ATCC 25923, The green AgNPs at 2.5 mM (2× MIC) demonstrated 53.10% of biofilm eradication (p-value = 3.6e-05) but showed a lower percentage of 41.61% at 1.0 mM (1× MIC) with a (p-value = 3.59E-02). Traditional AgNPs at 1.0 mM (2× MIC) and 0.25 mM (0.5× MIC) yielded superior eradication results of 78.13% (p-value = 3.57e-05) and (p-value=3.57e-05). Ag+ ions at 1.0 mM showed an eradication rate of 78.13% (p-value = 3.59e-05), being similar to traditional AgNPs at 1.0 mM. As expected, $NaBH_4$ (buffer) exhibited the lowest eradication percentage, with only 18.16% eradicated (p-value = 2.43e-04), as shown in Table 1b.

Table 1. Evaluation of biofilm eradication in Gram-positive pathogens.

(a) *Bacillus cereus* ATCC 11778

Component	Concentration	Eradication (%)	Biomass (%)	p-value	Significance
Green AgNPs	5.0 mM	72.62	27.38	3.38e-05	***
	2.5 mM	51.83	48.17	3.41e-05	***
Traditional AgNPs	1.0 mM	70.02	29.99	3.38e-05	***
	0.5 mM	56.30	43.70	3.37e-05	***
Ag+ ions	1.0 mM	69.51	30.49	3.42e-05	***
	0.5 mM	40.43	59.57	3.41e-05	***
$NaBH_4$ buffer	1.0 mM	16.60	83.40	2.56e-03	**
	0.5 mM	18.52	81.48	1.477e-05	***
Control (+)	-	0.00	100.00	-	
Control (-)	-	100.00	0.00	-	

(b) *Staphylococcus aureus* ATCC 25923

Component	Concentration	Eradication (%)	Biomass (%)	p-value	Significance

Green AgNPs	2.5 mM	53.10	46.90	3.6e-05	***
	1.0 mM	41.61	58.39	3.59e-02	*
Traditional AgNPs	1.0 mM	78.13	21.87	3.57e-05	***
	0.5 mM	62.16	37.84	3.57e-02	*
Ag+ ions	1.0 mM	78.13	21.87	3.59e-05	***
	0.5 mM	65.54	34.47	3.59e-05	***
$NaBH_4$ buffer	1.0 mM	18.16	89.77	2.43e-04	***
	0.5 mM	10.23	81.84	1.01e-02	*
Control (+)	-	0.00	100.00	-	
Control (-)	-	100.00	0.00	-	

Eradication percentages on Gram-positive on each treatment: Green AgNPs, Ag+ ions, Traditional AgNPs, and buffer. Also, the statistical analysis was realized illustrating p-values as *** when $\alpha \leq 0.001$, ** when $\alpha \leq 0.01$, and * when $\alpha \leq 0.05$.

2. Gram-negative bacteria

For *Escherichia coli* ATCC 25922, the green AgNPs at 5.0 mM (2× MIC) showed a remarkable eradication rate of 71.58%, while the green AgNPs at 2.5 mM concentration (1× MIC) eradicated 55.26%. Both concentrations evidenced highly significant eradication values when compared to controls (Table 2a), more precisely, p-values of 3.55e-05 and 3.59e-05, respectively. On the other hand, the traditional AgNPs at 0.5 mM exhibited an eradication efficacy of 82.93% with a p-value of 3.57e-05, showing comparable effectiveness to the green AgNPs. In comparison, Ag+ ions at 0.5 mM eradicated 59.49% (p-value = 3.60e- 05), demonstrating moderate efficacy. $NaBH_4$ buffer showed the lowest eradication percentage at 40.42% (p-value = 3.59e-05). As expected, the negative controls showed no biofilm eradication, thus validating the experimental evaluation of the present study.

Regarding *Salmonella enterica* serovar Typhimurium ATCC 14028, the green AgNPs treatment at 5.0 mM resulted in an eradication rate of 81.95% (p-value = 3.40e-05), while the traditional AgNPs at 0.5 mM (2× MIC) achieved a slightly higher efficacy with 82.54% biofilm eradication (p- value = 3.35e-05). Ag+ ions at

0.5 mM showed an eradication rate of 71.73% (p-value = 3.26e- 05), slightly lower than the green and traditional AgNPs. In contrast, $NaBH_4$ buffer exhibited again the lowest eradication efficacy at 21.92% (p-value = 3.41e-05). Lower concentrations of the traditional AgNPs showed decreased efficacy, as expected. These findings are illustrated in Table 2b.

Table 2. Evaluation of biofilm eradication in Gram-negative pathogens.

(a) *Escherichia coli* ATCC 25922

Component	Concentration	Eradication (%)	Biomass (%)	p-value	Significance
Green AgNPs	5.0 mM	71.58	28.42	3.55e-05	***
	2.5 mM	55.26	44.74	3.59e-05	***
Traditional AgNPs	0.5 mM	82.93	17.08	3.57e-05	***
	0.25 mM	68.48	31.53	3.57e-05	***
Ag+ ions	0.5 mM	59.49	40.51	3.6e-05	***
	0.25 mM	54.33	45.67	3.59e-05	***
$NaBH_4$ buffer	1.0 mM	40.42	59.58	3.59e-03	***
	0.5 mM	23.11	76.89	1.477e-05	***
Control (+)	-	0.00	100.00	-	
Control (-)	-	100.00	0.00	-	

(b) *Salmonella enterica serovar* Typhimurium ATCC 14028

Component	Concentration	Eradication (%)	Biomass (%)	p-value	Significance
Green AgNPs	5.0 mM	81.95	18.05	3.4e-05	***
	2.5 mM	70.60	29.40	3.42e-02	***
Traditional AgNPs	1.0 mM	82.54	17.46	3.35e-05	***
	0.5 mM	52.48	47.52	3.41e-02	***
Ag+ ions	0.5 mM	71.73	28.27	3.26e-05	***

	0.25 mM	66.90	33.10	3.42e-05	***
$NaBH_4$ buffer	1.0 mM	21.92	78.08	3.41e-05	***
	0.5 mM	19.20	80.80	2.54e-03	**
Control (+)	-	0.00	100.00	-	
Control (-)	-	100.00	0.00	-	

Eradication percentages on Gram-negative on each treatment: Green AgNPs, Ag+ ions, Traditional AgNPs, and buffer. Also, the statistical analysis was realized illustrating p-values as *** when $\alpha \leq 0.001$, ** when $\alpha \leq 0.01$, and * when $\alpha \leq 0.05$.

Overall, the present findings underscore the effectiveness of both green and traditional AgNPs, when compared to Ag+ ions, in biofilm eradication across various bacterial pathogens with statistically significant results. In contrast, $NaBH_4$ buffer consistently showed the lowest eradication efficacy across all strains, reinforcing its limited antimicrobial activity. These results highlight the potential of green and traditional AgNPs as viable antimicrobial agents in foodborne pathogen management.

Discussion

General overview

Silver nanoparticles (AgNPs) have shown substantial potential in reducing mature biofilms formed by both Gram-positive and Gram-negative foodborne bacteria. The results illustrate strain-specific differences in biofilm eradication efficiency, largely attributed to structural variations between Gram-positive and Gram-negative bacteria. The thick peptidoglycan layer in Gram-positive species may hinder nanoparticle penetration, reducing eradication efficacy when AgNPs are used alone (Vaiwala et al., 2022). However, studies have reported that combining AgNPs with hydrogels, plant extracts, probiotic-derived compounds, or antibiotics can significantly enhance their antimicrobial performance (Miño et al., 2024; Machado et al., 2023), achieving up to 85% biofilm reduction in *Staphylococcus aureus* at 2× MIC (Bouryabaf et al., 2017). The effectiveness of AgNPs lies in their ability to penetrate extracellular polymeric substances (EPS),

which shield bacterial cells and limit the action of traditional antimicrobials (Idrees et al., 2021).

Gram-positive bacteria

Based on Araújo et al. (2012), *Bacillus cereus* biofilms were effectively disrupted by AgNPs, consistent with evidence showing up to a 5-log reduction in planktonic populations of diverse pathogens including *S. aureus, L. innocua, Salmonella enterica* Choleraesuis, *P. aeruginosa* and *E. coli*. This is notable given the resilience of *B. cereus*, which forms spores and exhibits strong resistance to environmental stressors. Chemically synthesized AgNPs using reducing agents such as $NaBH_4$ or surfactants like Dotab have been particularly effective in overcoming these defenses (Araújo et al., 2012). Likewise, for *S. aureus*, traditional AgNPs showed higher eradication at lower concentrations compared to green AgNPs, supporting their potential for industrial and clinical sanitation. These results for *B. cereus* and *S. aureus* are summarized in Fig 1.

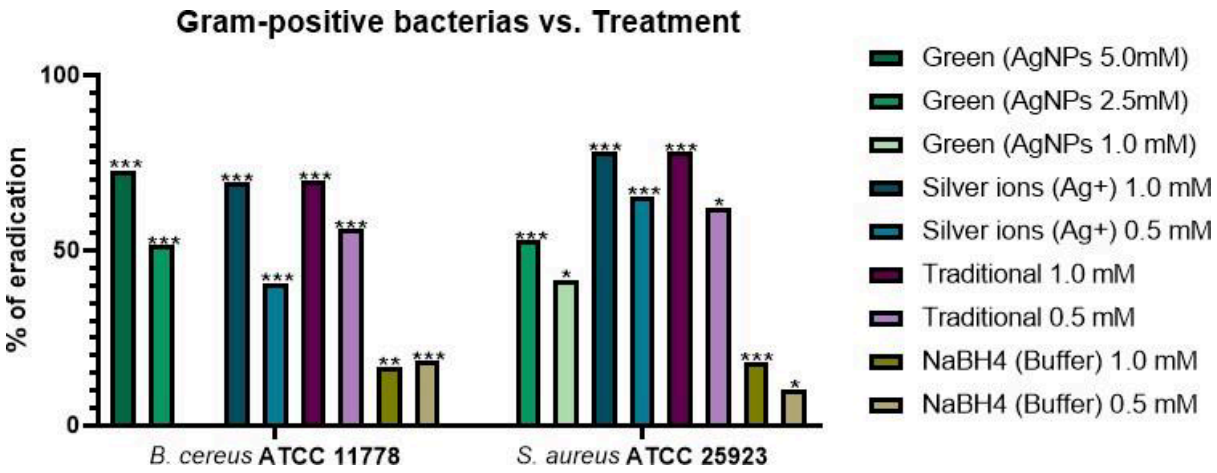


Fig 1. Biofilm eradication efficacy of silver nanoparticle treatments against Gram- positive bacteria. Percentage of biofilm eradication in *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923 under different treatments. Error bars represent mean ± SD (n=3). Statistical significance relative to untreated control: ***p < 0.001, **p < 0.01, *p < 0.05.

These results confirm that AgNPs are effective against resilient Gram-positive biofilms such as *B. cereus* and *S. aureus*, supporting their potential role in industrial and clinical sanitation strategies.

Gram-negative bacteria

For *Escherichia coli*, biofilm eradication rates exceeded 80% under silver nanoparticle treatment. Green-synthesized AgNPs derived from *Azadirachta indica* extracts achieved eradication levels of 84–96% at ~3.32 mM (2× MIC) (Zena & Alaa, 2023), comparable to the present results where traditional AgNPs reached 82% eradication at lower concentrations of 0.25–0.50 mM (More et al., 2023). By contrast, *S. Typhimurium* biofilms displayed higher resilience, requiring stronger treatments due to cellulose and curli fimbriae enhancing matrix robustness (Ramachandran et al., 2016). Similar findings showed only ~60% reduction at 125 µg/mL (~1.16 mM) AgNPs (Bouryabaf et al., 2017), in line with our observations, as shown in Fig 2.

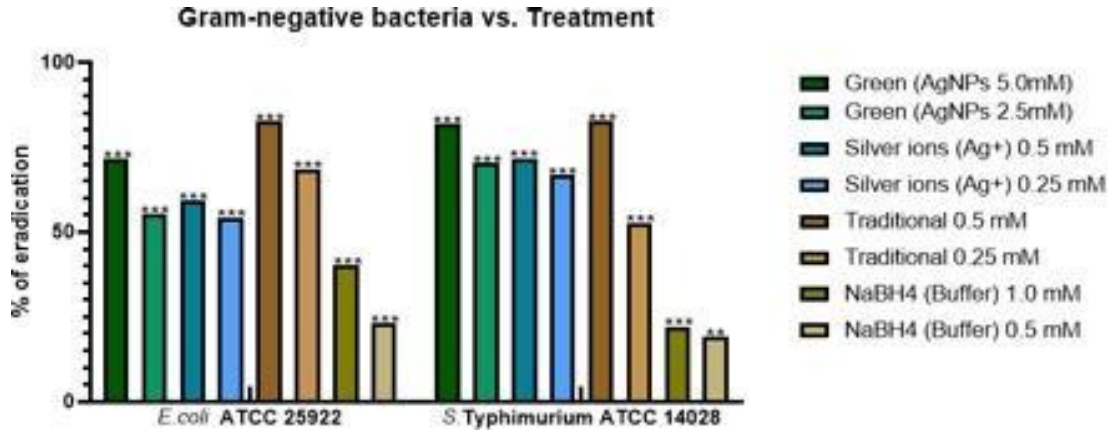


Fig 2. Biofilm eradication efficacy of various treatments against Gram-negative bacteria. Percentage of biofilm eradication in *Escherichia coli* ATCC 25922 and *Salmonella enterica* serovar Typhimurium ATCC 14028. Error bars represent mean ± SD (n=3). Significance as in Fig 1.

Overall, the results indicate that although AgNPs are effective against Gram-negative biofilms, the structural complexity of species like *S. Typhimurium* demands higher concentrations or longer exposure times. This highlights the importance of tailoring nanoparticle dosage and synthesis strategies to specific pathogens in order to maximize eradication efficacy.

Advantages of silver nanoparticles over conventional approaches

Beyond their antimicrobial efficacy, AgNPs offer distinct advantages over conventional disinfectants. For instance, sodium hypochlorite (NaOCl) requires

concentrations as high as 29.6 mM to achieve antimicrobial activity, whereas AgNPs achieve comparable effects at only 5 mM, providing a safer and more sustainable alternative (Ismail et al., 2019). Moreover, nanoparticle size critically influences activity: smaller AgNPs penetrate biofilms more effectively due to higher surface-area-to-volume ratios (Kundu et al., 2024). Green synthesis further enhances these benefits by replacing toxic reducing agents such as $NaBH_4$ with plant-derived compounds, thereby reducing environmental and health risks (Younus et al., 2024). This eco-friendly approach improves both cost-effectiveness and sustainability, making AgNPs particularly suitable for applications in food and healthcare systems (More et al., 2023; Cangui-Panchi et al., 2022; Galie et al., 2018).

Overall significance

Taken together, the present study underscores the broad potential of silver nanoparticles, both green and traditional, as promising antimicrobial agents for biofilm eradication. The advantage combined brought high efficacy, reduced toxicity, and sustainability, which make them innovative candidates to address biofilm-related challenges in diverse industrial and clinical contexts.

Conclusions

Both traditional and green-synthesized silver nanoparticles (AgNPs) demonstrated significant efficacy in eradicating biofilms, confirming their potential as antimicrobial agents. Traditional AgNPs achieved higher eradication at lower concentrations, while green AgNPs provide a more sustainable and environmentally friendly alternative, though requiring further optimization. Their ecological advantages make them attractive for future applications in food safety compared to conventional treatments. Future research should explore prolonged biofilm formation (48–72 h), combined treatment strategies, and broader testing against other microorganisms such as fungi. Moreover, assessing the cytotoxicity of both green and traditional AgNPs in food industry contexts is essential to ensure safety alongside efficacy. This study provides baseline evidence supporting the integration of eco- friendly nanoparticles into biofilm management strategies and serves as a foundation for applied and translational research in sustainable antimicrobial solutions.

References

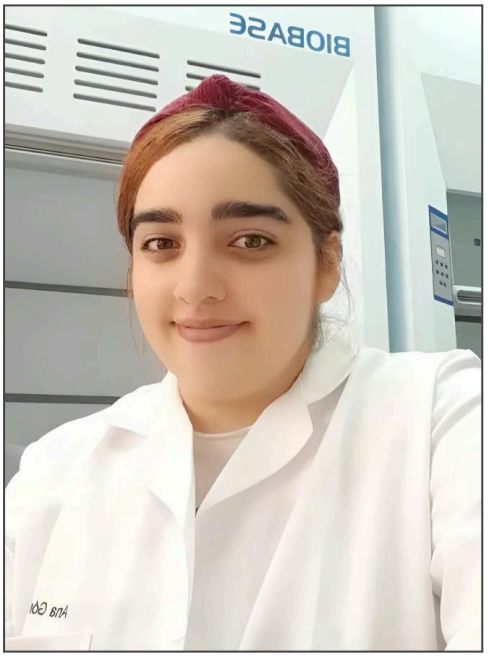
1. Araújo, E. A., Andrade, N. J., Da Silva, L. H. M., Bernardes, P. C., Teixeira, A. V. N. C., De Sá, J. P. N., Fialho, J. F. Q., Jr., & Fernandes, P. E. (2012). Antimicrobial effects of silver nanoparticles against bacterial cells adhered to stainless steel surfaces. Journal of Food Protection, 75(4), 701–705. https://doi.org/10.4315/0362- 028X.JFP-11-276
2. Bouryabaf, L. S., Moradi, M., Tajik, H., & Badali, A. (2017). Biofilm removal and antimicrobial activities of agar hydrogel containing colloid nano-silver against Staphylococcus aureus and Salmonella typhimurium. Journal of Medical Bacteriology, 6(3, 4), 51–58. https://www.researchgate.net/publication/321289343_Biofilm_Removal_and_Antimicrobial_Activities_of_Agar_Hydrogel_Containing_Colloid_Nano-Silver_against_Staphylococcus_aureus_and_Salmonella_typhimurium
3. Cabascango, D. (2023). Evaluating antimicrobial activity of silver nanoparticles and their interaction with Bursera graveolens extract by green chemistry on foodborne bacteria. Universidad San Francisco de Quito. https://repositorio.usfq.edu.ec/handle/23000/13606
4. Cangui-Panchi, S. P., Ñacato-Toapanta, A. L., Enríquez-Martínez, L. J., Salinas-Delgado, G. A., Reyes, J., Garzon-Chavez, D., & Machado, A. (2023). Battle royale: Immune response on biofilms - host-pathogen interactions. Current research in immunology, 4, 100057. https://doi.org/10.1016/j.crimmu.2023.100057
5. Cangui-Panchi, S. P., Ñacato-Toapanta, A. L., Enríquez-Martínez, L. J., Reyes, J., Garzon- Chavez, D., & Machado, A. (2022). Biofilm-forming microorganisms causing hospital- acquired infections from intravenous catheter: A systematic review. Current research in Microbial Sciences, 3, 100157. https://doi.org/ 10.1016/j.crmicr.2022.100175
6. Carrascosa, C., Raheem, D., Ramos, F., Saraiva, A., & Raposo, A. (2021). Microbial Biofilms in the Food Industry—A Comprehensive Review. International Journal of Environmental Research and Public Health, 18(4), 2014. https://doi.org/10.3390/ijerph18042014
7. Galié, S., García-Gutiérrez, C., Miguélez, E. M., Villar, C. J., & Lombó, F. (2018). Biofilms in the food industry: Health aspects and control methods. Frontiers in Microbiology, 9, 898. https://doi.org/10.3389/fmicb.2018.00898
8. Idrees, M., Sawant, S., Karodia, N., & Rahman, A. (2021). Staphylococcus aureus biofilm: Morphology, genetics, pathogenesis and treatment strategies.

International Journal of Environmental Research and Public Health, 18(7602), 1–20. https://doi.org/10.3390/ijerph18147602

9. Ismail, A., Ahmed, S., & Hussain, T. (2019). Evaluation of silver nanoparticles for their antimicrobial activity against Gram-positive and Gram-negative bacteria including Salmonella Typhimurium. Journal of Nanobiotechnology, 17(1), 1–12.
10. Jara-Medina, N. R., Cueva, D. F., Cedeño-Pinargote, A. C., Gualle, A., Aguilera-Pesantes, D., Méndez, M. Á., Orejuela-Escobar, L., Cisneros-Heredia, D. F., Cortez- Zambrano, R., Miranda-Moyano, N., Tejera, E., & Machado, A.(2024). Eco- alternative treatments for Vibrio parahaemolyticus and V. Cholerae biofilms from shrimp industry through Eucalyptus (Eucalyptus globulus) and Guava (Psidium guajava) extracts: A road for an Ecuadorian sustainable economy. PLOS ONE, 19(8), e0304126. https://doi.org/10.1371/journal.pone.0304126
11. Liu, X., Yao, H., Zhao, X., & Ge, C. (2023). Biofilm formation and control of foodborne pathogenic bacteria. Molecules, 28(2432). https://doi.org/10.3390/molecules28062432
12. Machado, A., Zamora-Mendoza, L., Alexis, F., & Álvarez-Suarez, J. M. (2023). Use of Plant Extracts, Bee-Derived Products, and Probiotic-Related Applications to Fight Multidrug- Resistant Pathogens in the Post-Antibiotic Era. Future Pharmacology, 3(3), 535-567. https://doi.org/10.3390/futurepharmacol3030034
13. Majed, R., Faille, C., Kallassy, M., and Gohar, M. (2016) Bacillus cereus Biofilms—Same, Only Different. Front. Microbiol. 7:1054. https://doi.org/10.3389/fmicb.2016.01054
14. Miño, K., Pérez, A., Xaymara, T., Cabascango, D. G., Gordillo, C., Gordillo, A., Vispo, N., Machado, A., & Alexis, F. (2024). Antimicrobial cellulose hydrogels against gram- positive and gram-negative bacteria. Frontiers in Soft Matter, 4, 1362677. https://doi.org/10.3389/frsfm.2024.1362677
15. More, P. R., Pandit, S., Filippis, A. D., Franci, G., Mijakovic, I., & Galdiero, M. (2023). Silver Nanoparticles: Bactericidal and Mechanistic Approach against Drug Resistant Pathogens. Microorganisms, 11(2), 369. https://doi.org/10.3390/microorganisms11020369
16. Pang, X., Hu, X., Du, X., Lv, C., & Yuk, H. G. (2023). Biofilm formation in food processing plants and novel control strategies to combat resistant biofilms: The case of Salmonella spp. Food Science and Biotechnology, 32(1703–1718). https://doi.org/10.1007/s10068- 023-01349-3

17. Ramachandran, G., Aheto, K., Shirtliff, M. E., & Tennant, S. M. (2016). Poor biofilm- forming ability and long-term survival of invasive Salmonella Typhimurium ST313. Pathogens and disease, 74(5), ftw049. https://doi.org/10.1093/femspd/ftw049
18. Ratan, Z. A., Mashrur, F. R., Chhoan, A. P., et al. (2021). Silver nanoparticles as potential antiviral agents. Pharmaceutics, 13(2034). https://doi.org/10.3390/pharmaceutics13122034
19. Shakir, A. A. (2016). Silver nanoparticles as antimicrobial against Salmonella Typhi, Proteus mirabilis and Bacillus cereus as a model of Gram-negative and Gram- positive bacteria. World Journal of Pharmaceutical Research, 5(10), 1448– 1456. https://doi.org/10.20959/wjpr201610-7063
20. Souza, G. M., Vieira, K. C. de O., Naldi, L. V., Pereira, V. C., & Winkelstroter, L. K. (2021). Green synthesized nanoparticles as a promising strategy for controlling microbial biofilm. In Nanotechnology for advances in medical microbiology, environmental and microbial biotechnology (pp. 1–28). Springer. https://doi.org/10.1007/978-981-15- 9916-3_1
21. Vaiwala, R., Sharma, P., & Ganapathy Ayappa, K. (2022). Differentiating interactions of antimicrobials with Gram-negative and Gram-positive bacterial cell walls using molecular dynamics simulations. Biointerphases, 17(6), 061008. https://doi.org/10.1116/6.0002087
22. Younus, M. M., Sayed, M. A., El Saied, M., & Abo El Naga, A. O. (2024). Catalytic reduction of toxic dyes over nickel oxide nanoparticles supported on CMK-3 catalyst. Scientific Reports, 14(16583). https://doi.org/10.1038/s41598-024-66243-2
23. Zena, A., & Aala, H. (2023). Efficiency of silver nanoparticle in removing Escherichia coli ATCC 25922 from drinking water distribution pipes. Result in Engineering, 17, 100988. https://doi.org/10.1016/j.ringeng.2023.100988
24. Zhou, F., Wang, D., Hu, J., Zhang, Y., Tan, B. K., & Lin, S. (2022). Control measurements of Escherichia coli biofilm: A review. Foods, 11(2469), 1–16. https://doi.org/10.3390/foods11162469

About the Authors



Ana Alejandra Gómez Ramos is a Biotechnology Engineer (USFQ, 2025) with research experience in antimicrobial strategies, biofilms, and green-synthesized nanoparticles for food safety applications. She completed her thesis on biofilm eradication using silver nanoparticles and continues to collaborate on microbiology and nanotechnology projects in Ecuador.