

Hydrothermal Synthesis, Characterization, and Enhanced Antibacterial Activity of ZnO Nanoparticles against Waterborne Pathogens

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Abstract. Clean water access remains a global challenge due to the high costs and hazardous byproducts from conventional chemical disinfectants. This study explores zinc oxide (ZnO) nanoparticles as an innovative antibacterial agent for water disinfection, leveraging their high surface area and ability to generate reactive oxygen species (ROS) that disrupt bacterial cell walls. ZnO nanoparticles were synthesized via hydrothermal method and characterized using X-ray diffraction (XRD), UV-vis spectroscopy, and scanning electron microscopy (SEM). XRD confirmed high-purity hexagonal wurtzite ZnO with nine distinct peaks, UV-vis revealed an absorbance peak at 350-380 nm, and SEM showed spherical morphology. Antibacterial efficacy was tested against *Escherichia coli* (Gram-negative) and *Bacillus cereus* (Gram-positive) using disk diffusion assays. *B. cereus* exhibited greater sensitivity, suggesting ZnO nanoparticles primarily disrupt positively charged cell membranes via ROS-mediated oxidative stress. These findings highlight ZnO nanoparticles' potential as a sustainable water treatment solution, though further research is needed to address scalability and long-term stability.

1. INTRODUCTION

Water is essential for all living organisms, yet securing clean, safe water—free from microbes and pathogens—remains a formidable global challenge driven by rapid population growth, industrialization, climate change, and urbanization (Tiwarei et al., 2008). Water contamination arises from multiple sources including inadequate sewage treatment, industrial effluents, marine pollution, radioactive waste, agricultural runoff, and improper waste disposal, all exerting profound negative impacts on ecosystems and contributing indirectly to air pollution through volatile emissions (Yaqoob et al., 2020). Contaminated water contains elevated microbial loads, rendering it unsuitable for drinking, irrigation, or recreation, and heightens public health risks as pathogenic microorganisms enter the human body primarily through ingestion, sparking outbreaks of waterborne diseases such as diarrhea—which tragically claims 2,195 infant lives daily worldwide (Centers for Disease Control and Prevention, 2015).

Current disinfection strategies seek to neutralize harmful bacteria and pathogens via physical methods like filtration, exclusion, or adsorption, and chemical approaches involving agents dosed at specific concentrations for adequate contact time (Al-Issai et al., 2019). Chemical disinfection, particularly chlorination, has gained widespread adoption for its rapidity and cost-effectiveness in municipal water treatment. However, precise dosing is critical: excess chlorine reacts with natural organic matter to form carcinogenic disinfection by-products (DBPs) such as trihalomethanes and haloacetic acids, implicated in cancer, reproductive disorders, and developmental issues. Compounding these drawbacks, traditional methods prove increasingly ineffective against resilient pathogens like *Cryptosporidium*, a protozoan oocyst highly resistant to chlorine that survives standard treatment processes and contaminates treated supplies, underscoring the limitations of conventional technologies in ensuring pathogen-free water amid evolving microbial resistance (Leclerc et al., 2002). Consequently, there is an urgent need for advanced, DBP-free disinfection alternatives (Li et al., 2008).

Nanotechnology offers a transformative solution through engineered nanoparticles, which are biocompatible, economically viable, and uniquely sized (1–100 nm) to provide expansive surface areas for superior reactivity, enabling deep penetration into bacterial cells and highly efficient microbial inactivation via reactive oxygen species (ROS) generation (Khan et al., 2016; Wahab et al., 2013). Unlike conventional agents, nanoparticles circumvent growing microbial resistance while avoiding harmful by-products, with broad applicability in catalysis, medicine, sensing, biology, and environmental remediation—particularly water purification (Ayati et al., 2014). Among metal oxide nanoparticles, zinc oxide (ZnO) stands out as an exceptional antibacterial agent owing to its nanoscale dimensions, high surface area-to-volume ratio, broad-spectrum toxicity against diverse microorganisms (including bacteria, fungi, and viruses), and inherent environmental benignity coupled with distinctive optical, electrical, and photocatalytic properties (Dimapilis et al., 2018; Ghorbani et al., 2015; Sirelkhatim et al., 2015).

Numerous synthesis routes exist for ZnO nanoparticles, including sol-gel, microemulsion, thermal decomposition, spray pyrolysis, microwave irradiation, precipitation, and hydrothermal processes (Talam et al., 2012; Ong et al., 2018). This study employs the hydrothermal method, which utilizes sealed vessels under elevated temperatures (typically 100–200°C) and autogenous pressure to crystallize nanoparticles from aqueous precursors, offering key advantages: simplicity (basic autoclave equipment), low operational costs, precise control over particle size/morphology, scalability, and eco-friendliness without toxic solvents or high-energy inputs. The hydrothermal approach yields high-purity, crystalline ZnO with tunable properties ideal for disinfection applications (Madathil et al., 2007).

The primary objective of this research is to synthesize ZnO nanoparticles (ZnO NPs) via the hydrothermal method and rigorously assess their antibacterial potency against prevalent waterborne pathogens resistant to aqueous environments and conventional disinfectants—specifically Gram-positive *Bacillus cereus* (with its thick peptidoglycan layer and positive cell wall charge) and Gram-negative *Escherichia coli* (featuring a thin peptidoglycan layer and lipopolysaccharide outer membrane). We hypothesize that hydrothermally synthesized ZnO NPs will effectively inhibit microbial growth through ROS-mediated oxidative

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stress, membrane disruption, and intracellular damage, with potentially differential efficacy based on bacterial cell wall architecture. Nanoparticles will be characterized using X-ray diffraction (XRD) for phase purity and crystallinity, UV-vis spectroscopy for optical bandgap confirmation, and scanning electron microscopy (SEM) for morphological analysis. Antibacterial performance will be evaluated via standard disk diffusion assays. Ultimately, this work aims to validate ZnO NPs as a viable, sustainable component for next-generation water treatment systems, fostering safer, cleaner water accessible for global public consumption while addressing persistent challenges in disinfection efficacy and environmental safety.

2. METHODOLOGY

2.1. Materials and Equipment

All chemicals and reagents, including zinc chloride (ZnCl_2), sodium hydroxide (NaOH), ethanol, sterilized distilled water, nutrient broth, and Mueller-Hinton agar (MHA), were of analytical grade and used without further purification. Bacterial strains (*Escherichia coli* and *Bacillus cereus*) were obtained from Zayed University laboratories. Equipment included a magnetic stirrer, round-bottom flask, glass beakers, graduated cylinders, weighing balance, droppers, pipettes, stainless steel spatula, hydrothermal autoclave reactor with Teflon chamber, sonicator (ultrasonic water bath), incubator, hot air oven, TESCAN VEGA scanning electron microscope (SEM), Shimadzu X-ray diffractometer (XRD), and Cary 100 UV-Vis spectrometer—all sourced from Zayed University facilities.

2.2. Synthesis of ZnO Nanoparticles

ZnO nanoparticles were synthesized via the hydrothermal method. Separate solutions of 5 g ZnCl_2 and 2.932 g NaOH were prepared in 100 mL distilled water each and stirred magnetically until fully dissolved. NaOH solution was slowly added dropwise to the ZnCl_2 solution under constant stirring, adjusting the pH to 8–11. The initially transparent mixture turned milky white, indicating precipitation. The reaction mixture was transferred to a Teflon-lined hydrothermal autoclave reactor and heated in a hot air oven at 180°C for 12 h, then cooled naturally to room temperature. The resulting white precipitate was repeatedly washed with ethanol and distilled water, centrifuged at 4000 rpm for 5 min (repeated 3–4 times to remove impurities), and dried overnight in a hot air oven at 150°C .

2.3. Characterization of ZnO Nanoparticles

Synthesized ZnO nanoparticles were characterized for crystalline, morphology, and optical properties. X-ray diffraction (XRD; Shimadzu) confirmed phase purity and crystal structure using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$). Scanning electron microscopy (SEM; TESCAN VEGA) analyzed particle morphology and surface features. UV-Vis spectroscopy (Cary 100) determined absorbance spectra in the 200–800 nm range to verify nanoparticle formation via the characteristic excitonic peak.

2.4. Antibacterial Activity Assessment

Antibacterial efficacy was evaluated against Gram-negative (*E. coli*) and Gram-positive (*B. cereus*) strains using the disk diffusion method on Mueller-Hinton agar (MHA) plates. ZnO nanoparticle stock suspension (1000 $\mu\text{g/mL}$) was prepared by dispersing 30 mg dried powder in 30 mL sterilized water, serially diluted to test concentrations (100, 150, 200, 300, 400, 500 $\mu\text{g/mL}$; Table 1), and sterile filter paper disks (6 mm) were soaked in each dilution via sonication for 2 h to ensure adsorption.

MHA plates were prepared by dissolving 19 g MHA powder in 500 mL distilled water, stirring for homogeneity, autoclaving at 121°C for 30 min, cooling to -45°C , and pouring into sterile Petri dishes to solidify. Bacterial suspensions (adjusted to 0.5 McFarland standard) were spread evenly across plates using a sterile glass spreader and air-dried. ZnO-loaded disks were placed centrally on inoculated plates and gently pressed for agar penetration (20 min incubation at room temperature). Plates, including nanoparticle-free controls, were inverted and incubated at 37°C for 24 h (duplicates per concentration). Zones of inhibition (ZOI) were measured radially (mm) post-incubation.

Table 1. The different concentrations used of ZnO NPs.

Concentration ($\mu\text{g/mL}$)	ZnO stock solution (mL)	Sterilized water (mL/g)
100	1	9
150	1.5	8.5
200	2	8
300	3	7
400	4	6
500	5	5

Note: The total concentration of ZnO NPs stock solution = 1000 $\mu\text{g/mL}$.

3. RESULTS AND DISCUSSION

3.1. UV-Vis Spectrophotometer of ZnO NPs

There are different techniques to confirm the preparation of zinc oxide nanoparticles. Two milliliters of the prepared solution were captured by using an ultraviolet-visible spectrophotometer to conform the absorbance spectra of zinc oxide nanoparticles. Two milliliters of distilled water were used as a control. As demonstrated in (Figure 1) the UV-vis absorption spectrum of the ZnO NPs sample shows an absorbance peak with a wavelength intensity in the ultraviolet within the range of 350–380 nm. Similar results were obtained by Barzinjy *et al.*, 2020. Overall, when the color of the prepared solution changed from colorless to white color, then this technique can easily demonstrate the synthesis of zinc oxide nanoparticles.

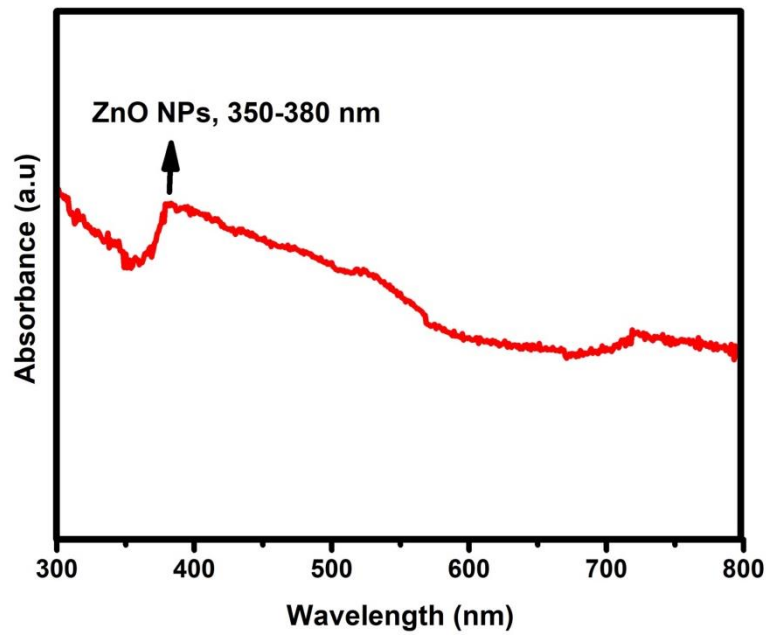


Figure 1. UV-vis absorption spectrum of the synthesized ZnO powder.

3.2. X-Ray Diffraction of ZnO NPs

X-ray diffraction (XRD) was used to analyze the crystalline nature and purity of the synthesized zinc oxide nanoparticles. Therefore, the X-ray diffraction pattern of the synthesized ZnO NPs powder is shown in (Figure). It was found that there are nine diffraction peaks with (2θ) values of 34.25° , 36.10° , 47.38° , 56.46° , 63.03° , 66.64° , 67.07° , and 68.91° corresponding to the crystal planes of (100), (002), (101), (102), (110), (103), (200), and (112), (201). In addition, all of diffraction peaks have been precisely identified as the hexagonal wurtzite structure of zinc oxide nanoparticles as shown in (Figure 3). Furthermore, the observed results shows that the absence of any XRD peaks other than ZnO peaks suggests that the produced nano powder was devoid of impurities. Similar results were obtained by Talam et al., 2012 and Oladiran et al., 2013.

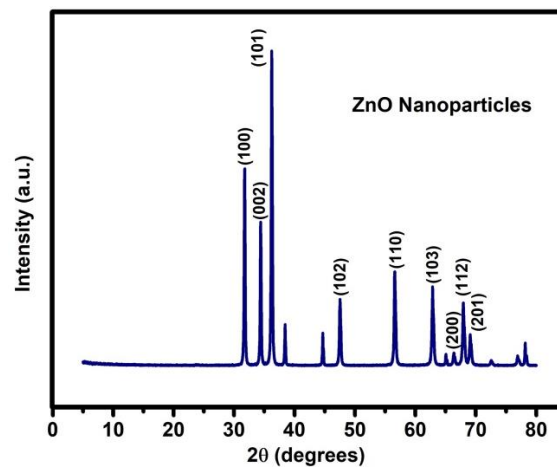


Figure 2. XRD pattern of synthesized ZnO nanoparticles.

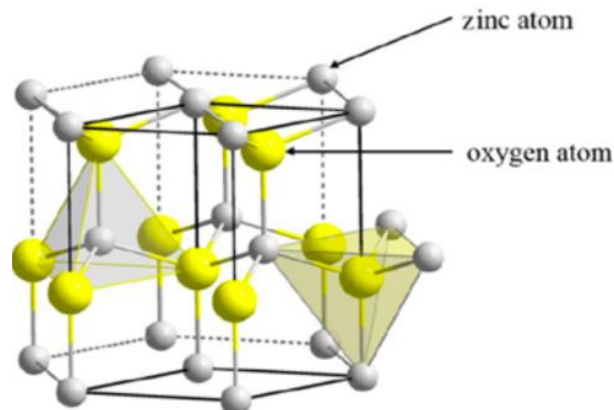


Figure 3. Hexagonal wurtzite structure of ZnO (Barzinjy et al.,2020).

3.3. SEM and EDX Analysis of ZnO NPs

The Scanning Electron Microscope (SEM) was used to find the structural morphology and particle spreading of zinc oxide nanoparticles at two different magnification levels (5 and 10 mm) and the results are shown in (Figures 4) the SEM images confirm the formation of ZnO nanoparticles. The particles were uniform, and a spherical shape was observed. Moreover, among the many forms of zinc oxide nanostructures, spherical zinc oxide nanostructures have been found to have the greatest pollutants degradation rate due to their large oxygen vacancies (Ong et al., 2018). In addition, (Figures 5) shows the Energy Dispersive X-ray (EDX) which demonstrates the chemical composition of the prepared particle. Zinc and oxygen elements have been correctly identified. In addition, (Table 2) indicated the atomic percentage of the presented elements. The zinc atomic percentage is 52.57%, while the oxygen atomic percentage is 47.43%, which gives a total of 100%, confirming that no other impurities were observed.

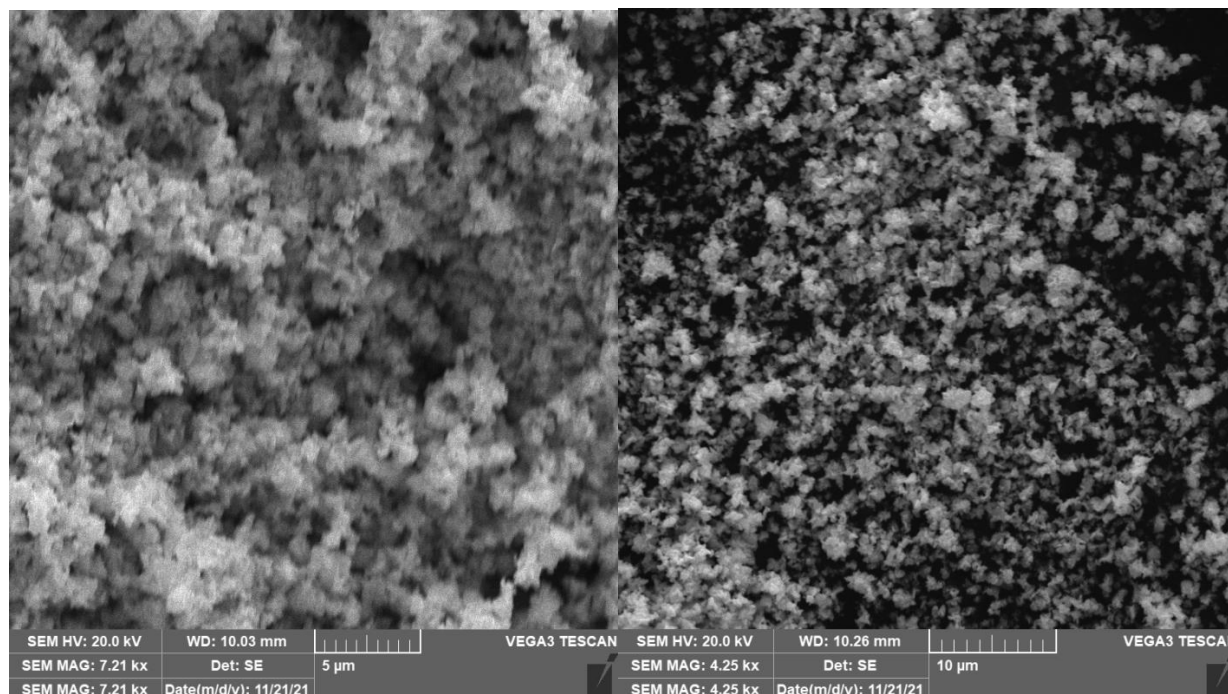


Figure 4. SEM images of zinc oxide nanoparticles with different magnifications (left 5 μm) and (right 10 μm).

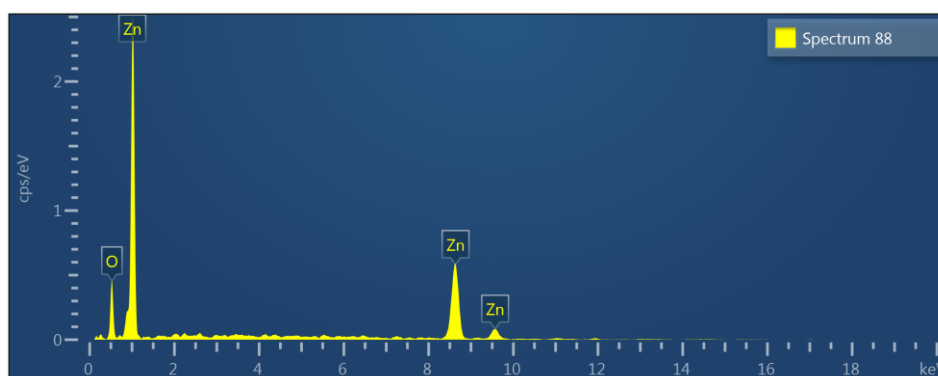


Figure 5. EDX analysis of zinc oxide nanoparticles.

Table 2. EDX analysis of ZnO nanoparticles.

Spectrum 88/ element	Line type	Weight %	Weight %	Atomic%
O	K series	18.09	1.86	47.43
ZN	K series	81.91	1.86	52.57
Total		100		100

3.4. Antimicrobial Activities of ZnO NPs

Generally, bacteria are made up of a cell membrane, a cell wall, and cytoplasm. The cell wall surrounds the cell membrane, and it is largely made up of a peptidoglycan layer that consists of amino acids and sugars. Moreover, the cell wall structure has a significant impact on bacteria's resistance or susceptibility to antibacterial. Moreover, antimicrobial activity is any agent that restricts microbial activity, inhibits the formation of bacterial colonies, and may kill microorganisms. Both gram-negative and gram-positive bacteria may differ in their sensitivity to antibacterial agents due to the different bacterial cell structures. The gram-positive bacteria have a thick (20-80 nm) peptidoglycan layer with a negative charge, which makes it more attractive to Zn^{+2} ions due to their positively charged ions. Whereas gram-negative bacteria have a thin peptidoglycan layer (7-8 nm) and an outer membrane that contains lipopolysaccharides, which are important for the structural integrity of the bacteria, allowing them to be more resistant to antibacterial agents (Dimapilis et al., 2018). On the other hand, nanoparticles can easily pass through the peptidoglycan, and can cause huge destruction to the bacterial cell due to their smaller size. Therefore, in this experiment, the antibacterial activity of

zinc oxide nanoparticles was evaluated against gram-positive bacteria *Bacillus cereus* and gram-negative bacteria *Escherichia coli* by using the disk diffusion method as shown in (Figure 6), (Figure 7), and (Figure 8) respectively. In addition, a zone of inhibition test is a qualitative approach used to measure the sensitivity of microbes to antimicrobial agents. For instance, if zinc oxide nanoparticles stop or limit both bacteria from growing, there will be an area surrounding the disk which indicates that the bacteria have not grown sufficiently to be visible. Furthermore, the inhibition zone results were illustrated in (Table 3). Synthesized zinc oxide nanoparticles are more effective against positively charged bacterial cells (*Bacillus cereus*) compared to negatively charged bacterial cells (*Escherichia coli*). Moreover, according to (Table 3), by increasing the concentration of zinc oxide nanoparticles, the inhibition zone also increased. As shown in (Figure 3.4.1) at lower concentrations (100, 150, and 200 $\mu\text{g/mL}$), small or no inhibitory effects were observed. It might be due to zinc oxide nanoparticles that were placed on the disks are not well dispersed in the sonicator, resulting in a lower antibacterial effect and therefore, no inhibition zones were clearly observed. However, when a higher concentration (300, 400, and 500 $\mu\text{g/mL}$) of zinc oxide nanoparticles were placed on the disk and well dispersed for around 2 hours in the sonicator. Therefore, zinc oxide nanoparticles had better penetration through the (MHA) agar and consequently into bacterial cell due to their small size. According to the results, the highest inhibition zone was achieved for *Bacillus cereus* (4 mm to 8 mm) at the higher concentrations (300, 400, and 500 $\mu\text{g/mL}$).

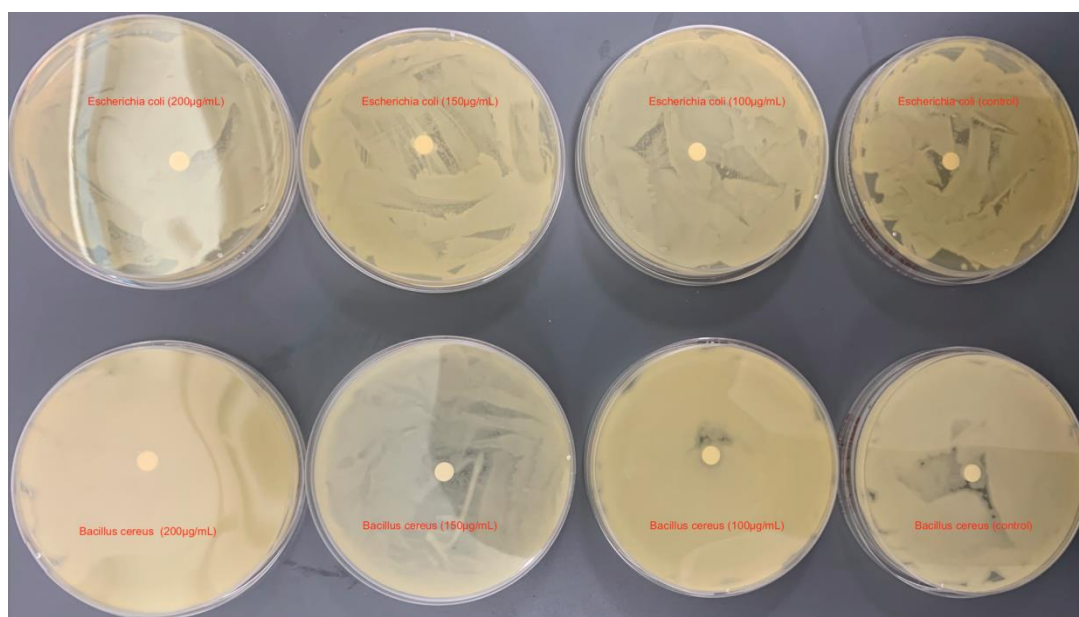


Figure 6. Antibacterial activity of zinc oxide nanoparticles against *Escherichia coli* and *Bacillus cereus*.

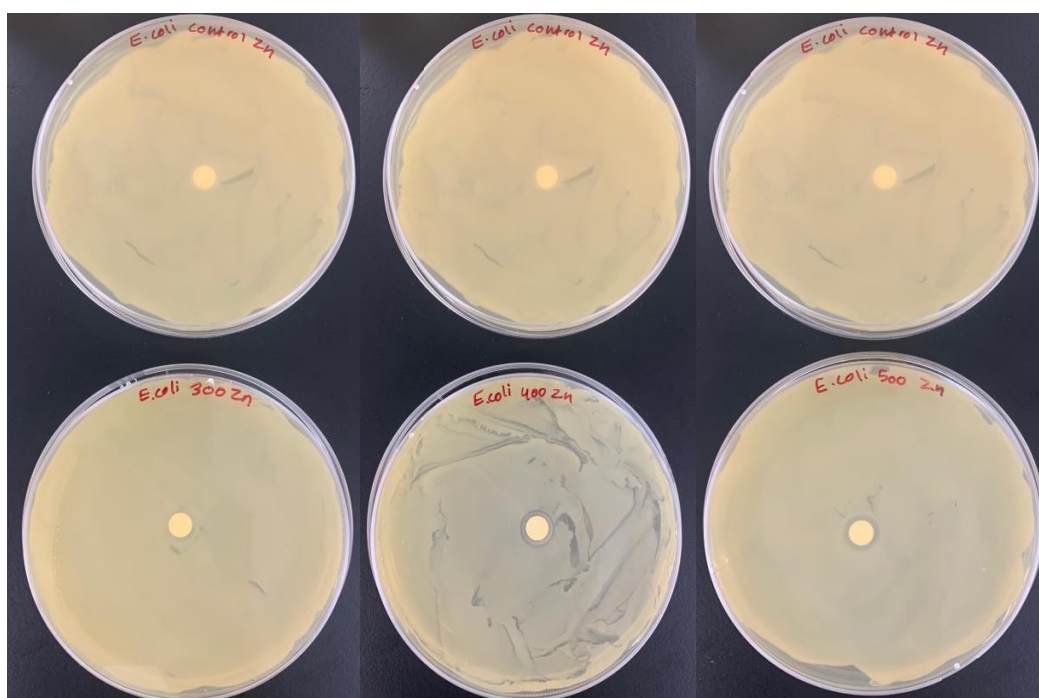


Figure 7. Antibacterial activity of zinc oxide nanoparticles against *Escherichia coli*.

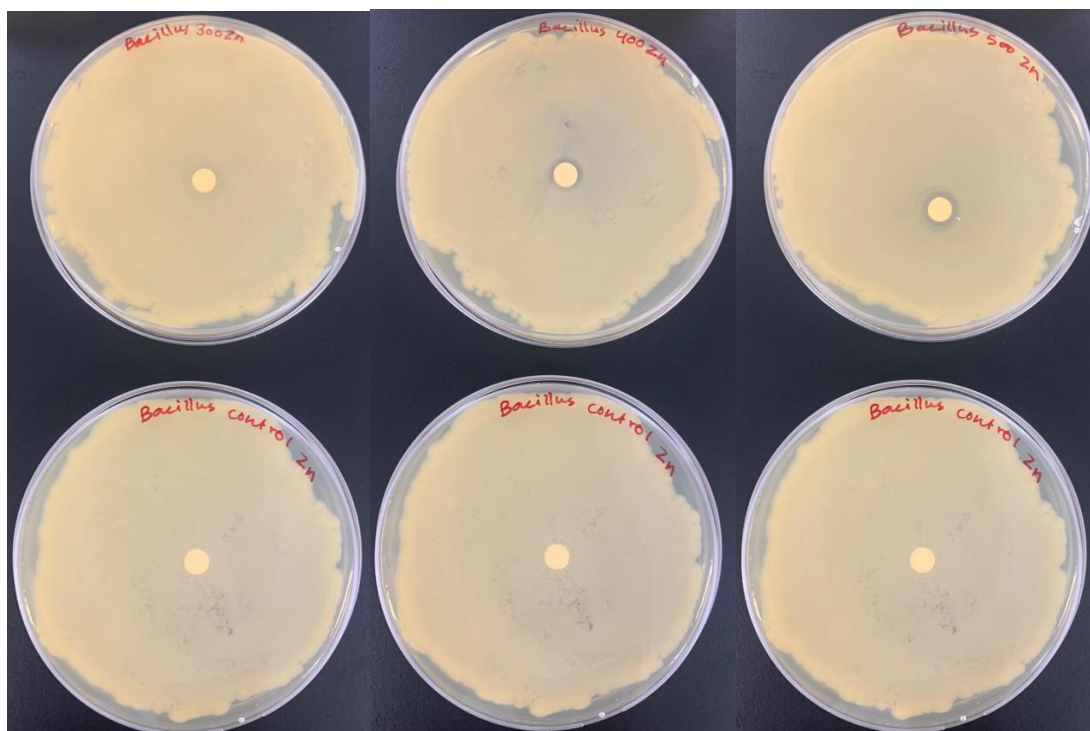
Figure 8. Antibacterial activity of zinc oxide nanoparticles against *Bacillus cereus*.

Table 3. The Inhibition zone of zinc oxide nanoparticles in six concentrations.

Concentrations($\mu\text{g/mL}$)	100	150	200	300	400	500
<i>Escherichia coli</i>	0mm	1mm	2mm	3mm	5mm	6mm
<i>Bacillus cereus</i>	1mm	1mm	2mm	4mm	5mm	8mm

3.5. Antimicrobial Mechanism

The antibacterial mechanism of nanoparticles remains under active investigation. However, several mechanisms underlying the antibacterial activity of zinc oxide nanoparticles have been well-explained and reported in the literature, as illustrated in Figure 9.

A primary mechanism involves the generation of reactive oxygen species (ROS)—chemical species acting as free radicals that inherently destroy cellular structures. ROS formation represents a key factor in the antibacterial efficacy of various metal oxide nanoparticles. Specifically, ZnO nanoparticles accumulate across bacterial cell surfaces, markedly increasing membrane permeability. This facilitates the release of highly toxic oxygen species, including hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radicals ($\text{OH}\cdot$). The resulting toxicity inflicts severe stress and destruction upon intracellular components such as carbohydrates, lipids, amino acids, nucleic acids, proteins, and DNA (Arora et al., 2020), ultimately enabling penetration into bacterial cells, inhibiting growth, and causing cell death through DNA degradation (Dimapilis et al., 2018).

Another critical mechanism entails the release of zinc ions, contributing to ZnO nanoparticle toxicity against *Escherichia coli* and *Bacillus cereus*. This effect stems from zinc ion solubility within bacteria-containing media: ZnO partially dissolves in water, liberating Zn^{2+} ions that damage cell membranes and penetrate intracellular contents. Nevertheless, ZnO's relative insolubility can hinder ion diffusion into the medium, thereby constraining its antibacterial potency (Sirelkhatim et al., 2015).

A third mechanism involves direct cell wall interaction, wherein ZnO nanoparticles bind to bacterial surfaces, elevating membrane permeability. This interaction triggers extensive damage and structural disorganization of the bacterial cell, culminating in its destruction (Dimapilis et al., 2018).

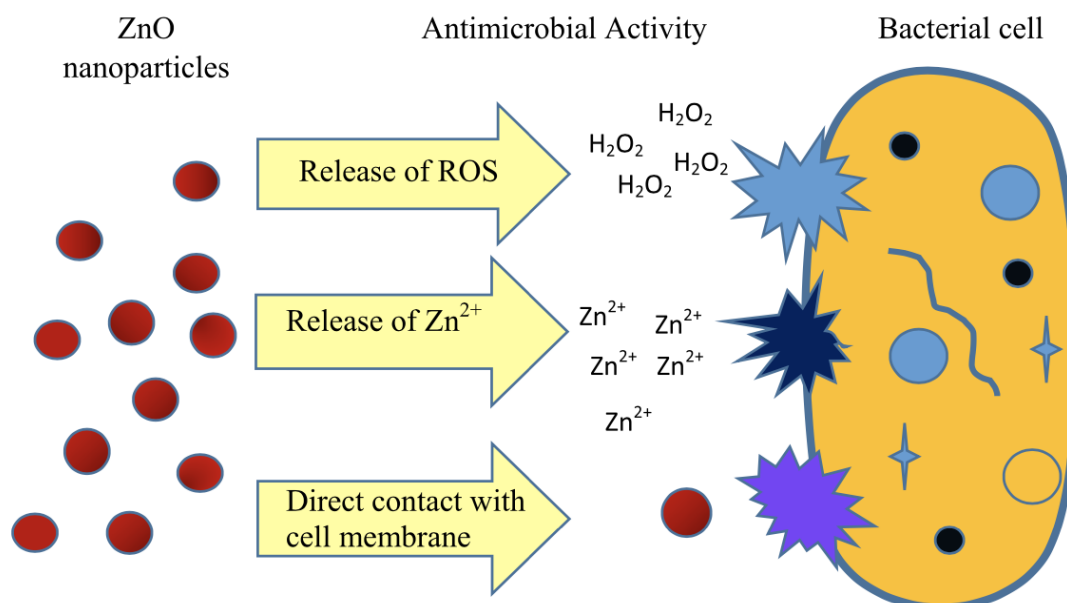


Figure 9. Antibacterial mechanisms of zinc oxide nanoparticles (Dimapilis et al., 2018).

4. STUDY LIMITATIONS

Several methodological challenges emerged during antibacterial testing, particularly in initial turbidity-based assays using UV-Vis spectrophotometry. Nutrient broth (2 mL) served as a blank (100% transmittance), while 2 mL aliquots containing *E. coli* or *B. cereus* with ZnO nanoparticles (100, 150, 200 µg/mL) were measured for bacterial killing efficiency. Initial readings showed rapid killing (e.g., *E. coli* at 100 µg/mL dropped from 54.3% to 100.3% transmittance after 1 h), likely due to nanoparticle interference or over-filtration.

Attempts to separate nanoparticles via syringe filtration before re-measurement yielded transmittance >100% across concentrations, indicating methodological flaws: manual filtration likely removed both nanoparticles and remaining bacteria, producing artifactually clear solutions that failed to quantify killing. Consequently, UV-Vis turbidity proved unreliable for this application.

The study pivoted to the standardized agar disk diffusion method, which provided reproducible zones of inhibition (ZOI). However, lower concentrations (100–200 µg/mL) yielded minimal or absent ZOI, attributed to inadequate nanoparticle dispersion and poor disk adsorption. This was resolved by testing higher concentrations (300–500 µg/mL) with prolonged sonication (2 h in ultrasonic water bath), enhancing dispersion and loading, which produced clear, concentration-dependent ZOI—demonstrating effective bacterial killing at elevated doses. Future work should employ advanced dispersion techniques (e.g., probe sonication), quantitative methods like colony-forming unit (CFU) counts or broth microdilution for minimum inhibitory concentration (MIC), and nanoparticle stability assessments to validate efficacy and overcome dispersion limitations.

Table 4. Stock solution of ZnO NPs in nutrient broth.

Concentration (µg/mL)	Nutrient Broth (mL)	Bacterial Culture(mL)	ZnO Stock Solution(mL)
Control	25	5	-
100	22	5	3
150	20.5	5	4.5
200	19	5	6

Note: The total concentration of ZnO NPs stock solution = 1000 µg/mL.

5. CONCLUSION AND RECOMMENDATIONS

Water pollution encompasses diverse contaminants, yet pathogenic bacteria pose the greatest waterborne health risk. Traditional disinfection methods like chlorination and ozonation increasingly reveal limitations, including formation of carcinogenic disinfection by-products (DBPs) such as trihalomethanes, bromates, and haloamides, alongside rising microbial resistance that diminishes their efficacy.

This study addressed these challenges by synthesizing zinc oxide (ZnO) nanoparticles via the hydrothermal method from zinc chloride and sodium hydroxide precursors. Nanoparticle formation was initially confirmed by the solution's color shift from colorless to milky white. UV-Vis spectroscopy revealed a characteristic absorbance peak at 350–380 nm, while X-ray diffraction (XRD) verified a pure hexagonal wurtzite structure with no extraneous peaks, indicating high purity. Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) analysis further elucidated spherical morphology and elemental composition.

Antibacterial assays demonstrated that synthesized ZnO nanoparticles exhibited concentration-dependent activity against both test strains, with markedly greater efficacy against Gram-positive *Bacillus cereus* compared to Gram-negative *Escherichia coli*. This superior performance arises from nanoparticles' nanoscale advantages over bulk materials, including elevated UV absorption, expansive surface area, and generation of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), which inflict oxidative stress, membrane damage, and cell death (Barzinjy et al., 2020).

While laboratory results are promising, nanotechnology's primary barrier to widespread water treatment adoption remains its predominant lab- and pilot-scale validation. Critical unknowns include nanoparticle stability in aqueous matrices, complete removal from large-scale effluents, cost-effectiveness, integration with existing infrastructure, and potential environmental/ecotoxicological risks. Future investigations must prioritize these challenges—enhancing ZnO stability, scalability, and safety profiling—to unlock sustainable, DBP-free disinfection against resistant pathogens at industrial scales.

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