Antibacterial Activity of Biosynthesized Zinc Oxide Nanoparticles Against Bacterial Contamination in Wastewater

Wafaa Razzaq Hilal[1], and Atheer Saieb Naji[2]

[1,2] Department of environmental pollution, college of environmental sciences, Al-Qasim Green university, Hilla, Iraq.

Email: wafaarazzaq95@gmail.com

Abstract

In this study biosynthesis of zinc oxide via aqueous olive leaves (olea europea) was investigated. The prepared nanoparticles were characterized by different technique which include FTIR to provide information about functional group, XRD to give structure was hexagonal phase, average size was (13.7) (16.4) (17) nm of sample A, B, C of ZnoNPs respectively.FESEM to provide average particles size was 49. 96, 68.87, 76.69 nm of samle A, B, C of ZnoNPs respectively, the shape of nanoparticles was spherical. EDX which give elemental composition of nanoparticles which confirm the presence of Zn element.The antibacterial effect was investigated and confirmed by CFU(colony forming unit). ZnoNPS causing 100% mortality of E –coli and have activity on total count of bacteria.

Keywords: zinc oxide nanoparticles, biosynthesis, antibacterial activity

1. Introduction

Microbial contamination is one of the most critical aspects of water pollution, especially pathogenic bacteria, such as enteric pathogens, which are often responsible for water-borne disease. Contamination of water is also a critical environmental issue since it has a rapid and significant negative impact on human health and biodiversity in aquatic ecosystems. [1]. Because of the indiscriminate use of antimicrobial medications, microbes have acquired resistance to many antibiotics, posing a significant difficulty in the treatment of infectious diseases. With the rise in resistance of many microbes to currently employed antimicrobials, as well as the high expense of synthetic compound synthesis, also several adverse effects, there is a need to explore alternatives. [2] The synergistic impact of antibiotics in plant extracts against resistant bacteria opens up new options for the treatment of infectious disease, making the plant a viable drug development candidate.[3]. For centures, plants have been considered an important source of medicinal compounds, and many unique medication components have been extracted from natural plant sources. Traditionally, many of these plants and their extracts were utilized in medicine. Secondary metabolites with antimicrobial activities, such as tannins, terpenoids, alkaloids, and flavonoids, are abundant in plants [4].

Plant-mediated nanoparticle synthesis has recently attracted a lot of attention due to its inherent advantages such as speed, simplicity, environmental friendliness, and lower costs[5] Nanotechnology is a branch of nanoscience that deals with synthesis and control of matter at the nanoscale. [6]. Nanoparticles are particles with a diameter of less than 100 nanometres and a mass of 20–15000 atoms. Nanoparticles (NPs) come in a variety of shapes, including spheres, triangles, hexagons, rods, wires, and tubes, and can be made from a variety of materials.

One of the most interesting and promising metallic nanomaterials is zinc (Zn) and its oxide (ZnO). Zinc is a fairly active element and simultaneously a strong reducing agent; according to its reduction potential it can easily oxidize, forming zinc oxide [7].which is very helpful in preparation of zinc oxide nanoparticles. Zn plays an important part in biological activities such as DNA replication, DNA repair, and cell cycle progression[8]. Compared to traditional Zn sources, they have higher bioavailability and biocompatibility [9] Because of their improved semiconducting properties, ZnONPs are becoming increasingly important in optics and electronics. [10]photoelectrical [11] and catalytic properties. ZnO is an inorganic compound with a large band gap n-type semiconductor (3.37 eV) with a hexagonal wurtzite crystal structure (60 meV). [12]Zinc oxide (ZnO) is a "generally regarded as safe" (GRAS) chemical with low toxicity in humans, according to the US Food and Drug Administration. ZnONPs are more biocompatible than TiO2 and have a strong photocatalytic activity. [13]. Because of its antibacterial properties, zinc oxide is commonly utilized in the food sector to maintain colors and prevent rotting. Because of its high surface-to-volume ratio, ZnO NPs show considerable antibacterial action [14]. Precipitation, hydrothermal, solvothermal, and sol gel procedures are among the laboratory approaches for ZnO synthesis that have been documented.[15].

Olive (Olea europaea L.), a member of the Oleaceae family, is native to tropical and warm temperate climates around the world. Olive is also regarded as a versatile crop with high output potential. The olive tree, which is known for its

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fruit and is also known as the olive, is an economically important source of olive oil in the Mediterranean region. The olive is mostly found along the coasts of the eastern Mediterranean Basin., southeastern Europe's, western Asia's, and northern Africa's coastal parts, as well as northern Iran near the Caspian Sea's southern end The olive tree is beneficial to both health and nutrition. Extracts derived from olive leaves have been used to boost health and preserve food for millennia. Olive oil is also a well-known folk remedy for fever and tropical diseases such as malaria.[16].

2. Experimental

2.1. preparartion of olive leaves extract

It was collected in the Al-Hussainiya District of Kerbalaa City and cleaned multiple times with distilled water to eliminate any dust particles before being sun dried for 18 days to remove moisture residue. In a 500 mL glass beaker, 10 g of dried fine cut olea europea leaf extract was combined with 400 mL of sterile distilled water. The mixture was then brought to a boil for 5 minutes, or until the aqueous solution's color changed from watery to yellow. The mixture was then allowed to cool to ambient temperature before being filtered with Whatman No. 1 filter paper and centrifuged for 2 minutes at 1200 rpm to remove biomaterials. To be used in future research, the extract is kept at room temperature.

2.2. biosynthesis of zinc oxide nanoparticles

It was prepared according to method describing by [17] .

1-In a typical reaction combination, 5-10 ml of aqueous yellow leaf extract of Olea europea is added to 300 ml of (2,4,6) mM aqueous zinc sulfate heptahydrate solution was named as sample A, B, C respectively. and stirred for 5 minutes at room temperature to generate a pale-yellow solution.

2-Then, with constant stirring at room temperature, a drop by drop of 1M sodium hydroxide solution was added to the mixture, and the yellow color of the mixture began to change to a yellowish-white suspension at pH 12.

3-The suspended particles are purified by dispersing them in sterile distilled water and centrifuging them three times. Before being employed in the final product, the white particles are washed with ethanol to remove any impurities. A white powder is obtained after 6 hours of drying at 60°C in a vacuum oven.

2.3. collection of wastewaters

The study included taking wastewater in glass container from al-maamerah wastewater treatment plant at treated sewage outlet from Hilla city in March 2022 and transport as soon as possible to laboratory for culturing.

2.4. Antibacterial activity tests

Plate count method was used in this study. Typical media include nutrient agar for general count and macconkey agar to count gram negative bacteria. Wastewater collected from Sewage and Cultured before and after treatment by different concentration of (0.5,1,2,5) mg/l as a powder for each sample of ZnoNPs. According to [18].The removal efficiency was determined by counting the number of colony forming unit and was defined as N = N1- N2/ N1 x 100 % where N1 and N2 , the number of colonies before and after treatment.

3. Result and Discussion

3.1. FTIR

is a simple analytical technique used to identify functional groups in solid nanoparticles.ZnO samples prepared using olea europea leaf extract was examined using FTIR analysis in a range of wavelengths (400-4000 cm-1). Figure (1) showed a wide absorption peak at 3337.68 cm-1 due to the stretching vibration of (O-H) bond because of the absorption of water molecules. The peaks at 2919.89, 2357.68, 1650.29, 1075.84, and 667.62 cm-1 were assigned to vibration of C-H, C=O (amide), C=C (alkenes), C-N (aliphatic and aromatic amines), and N-H (primary amine and amide) bonds, respectively. The appearance of absorption peaks confirming the presence of functional groups is due to the biomolecules present in the olea europea leaf extract that act as a reducing agent such as proteins. The characteristic peak at 552.10 cm-1 is due to the stretching vibration of the (Zn-O) bond[19]. The identical of the peaks of the spectrum for the prepared concentration indicates the success of biosynthesis ZnO nanoparticles. The results are similar to previous research prepared by [20]and[21].

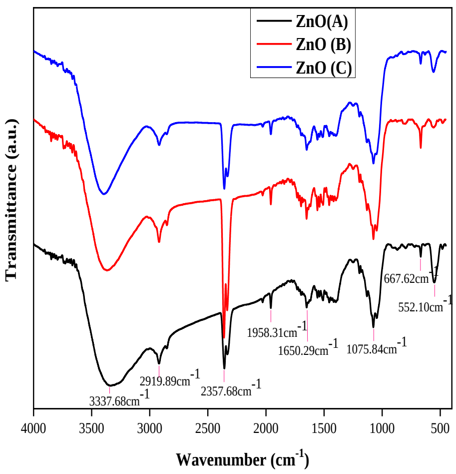

Figure .1. FTIR of different sample of ZnoNPs

3.2.XRD

ZnO nanoparticles were analyzed using X-ray diffraction as shown in Figures (2). The XRD spectra of ZnO NPs at sample A showed low crystallinity with the presence of impurities due to the appearance of several peaks in the spectrum.The diffraction peaks at 2θ = 31.37, 34.06, 35.93, 47.20, 56.26, 62.59, and 67.65 assigned to the hkl planes (100), (002), (101), (102), (110), (103), and (112), respectively. As it was found that there is a great be identical between the apparent peaks with the standard data (JCPDS card 36-1451), and this confirms that ZnO NPs have a hexagonal wurtzite structure[22]. The crystalline size values calculated from the Debye-Scherrer equation for sample A, B, C of ZnoNPs was 13.7, 16.4, and 17nm, respectively.

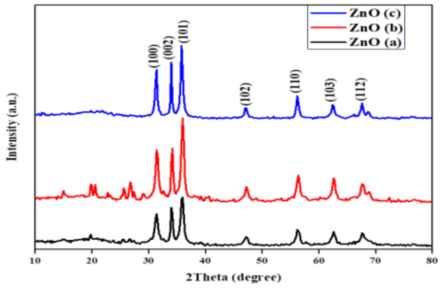

Figure .2. XRD of different sample of ZnoNPs

Table (1): Crystalline data of ZnoNPs						
Sample	2θ	d (nm)	I %	FWHM	Crystal size (nm)	Average size
Sample A	19.82	0.447	11.9	0.522	15	13.7
	31.37	0.284	66.2	0.637	13	
	34.06	0.262	44.4	0.355	24	
	35.93	0.249	100	0.597	14	
	47.20	0.192	25.5	0.811	10	
	56.26	0.163	40.2	0.718	12	
	62.59	0.148	35	0.736	12	
	67.65	0.138	34.8	0.916	10	
Sample B	19.89	0.446	17.3	0.433	19	16.4
	26.79	0.332	22.2	0.43	19	
	31.44	0.284	60.1	0.56	15	
	34.14	0.262	59.9	0.331	26	
	35.99	0.249	100	0.515	16	
	47.23	0.192	17.9	0.691	12	
	56.37	0.163	30.3	0.642	14	
	62.62	0.148	27.7	0.645	14	
	67.68	0.138	18.1	0.727	13	
Sample C	31.34	0.285	67.8	0.448	18	17
	33.96	0.263	22.7	0.575	14	
	35.82	0.250	100	0.442	19	
	47.17	0.192	22.2	0.674	13	
	56.22	0.163	37.7	0.514	17	
	62.44	0.148	29.3	0.706	13	
	67.59	0.138	23	0.518	18	

3.3. FESEM

the surface morphology of ZnO nanoparticles was determined using FESEM analysis. Figure (3) shows the morphology of ZnO nanoparticles, as it shows oval structures of irregular shapes with a high physical contact between the particles, and this leads to the distribution of the particles in the form of lumps. It was observed that the spherical shapes were unclear. This result is consistent with the previous studies[23][24].The average nanoparticles size of the samples A,B,C was 49. 96, 68.87, 76.69 nm respectively.

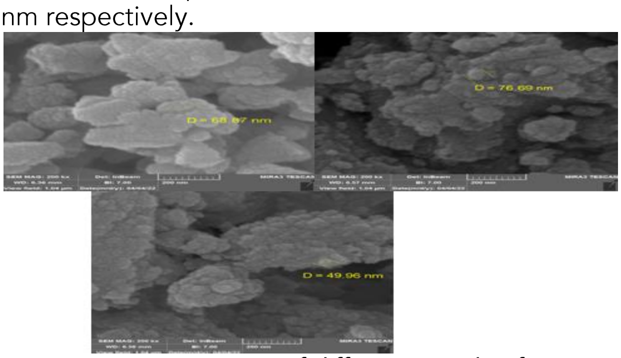

Figure.3. FESEM images of different sample of ZnONPs

3.4.EDX: The EDX analysis of sample A shows a decrease in the weight ratio of zinc element up to 10.34%, oxgen ratio was 21.16% and the appearance of several additional peaks due to present of the boron, carbon, sulfur, and sodium elements. The appearance of pattern peaks of sample B where the zinc and oxygen elements were observed in weight ratios that are 23.78% and 15.18, respectively. This indicates the completion of ZnO synthesis.The presence of carbon, nitrogen, phosphorous and sulfur elements in weight ratios 14.48%, 1.60%, 1.35%, 10.90% and is due to the materials that constitution of olea europea leaf extract as a reducing agent. The high weight ratios of zinc and oxygen elements indicate the success of the biosynthesis of zinc oxide nanoparticles. The presence of sodium element in the pattern is due to the use of sodium hydroxide during the synthesis process. In EDX analysis of sample C an increase in the weight ratio of zinc (33.66%) was observed due to the increase in the concentration of zinc sulfate heptahydrate solution and ratio of oxygen was 12.09 % [25].

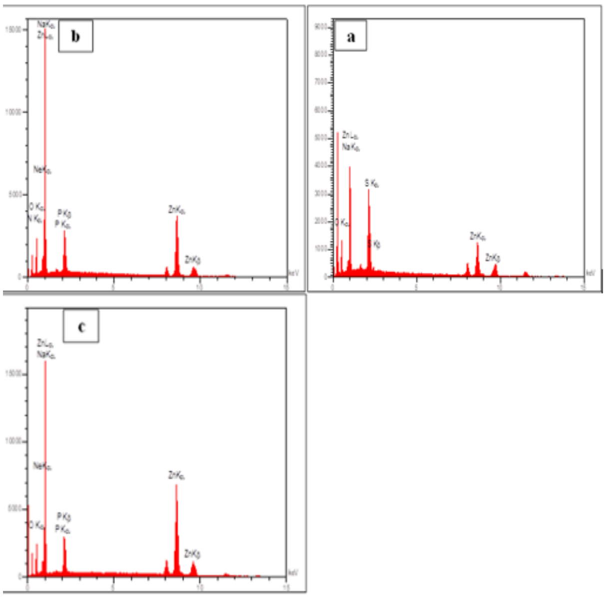

Figure.4. The EDX pattern of ZnO nanoparticles

Antibacterial activity of ZnoNPs

The total count of bacteria was 1600 CFU/ ml and total count of E-coli was 1120 CFU/ ml before treatment.

Table .2. the removal efficiency of ZnoNPs

Concentration of nanoparticles	Removal efficiency of total count bacteria for sample A	Removal efficiency of total count of sample B	Removal efficiency of total count of sample C
0.5 mg/l	54 %	48 %	42 %
1 mg/l	62 %	54 %	56 %
2 mg/l	68 %	61 %	52 %
5 mg/l	73 %	70 %	63%

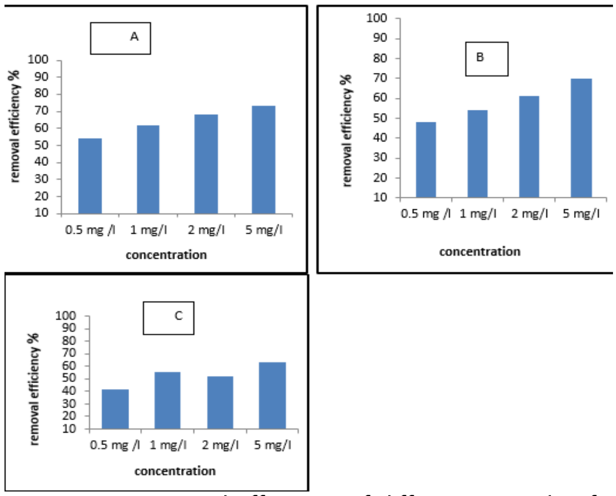

Figure .5. removal efficiency of differernt sample of ZnoNPs

Upon treatment removal efficiency was 100% against E-coli in all studied concentration. ,ZnoNPs was exhibited antibacterial activity against total count of bacteria as in table 2 by inactivating large number of bacteria , the removal efficiency increase when the concentration of nanoparticles increase .The bactericidal efficacies of ZnO nanoparticles with different particle sizes against E-coli. ,this result agreement with [26] were explained ZnoNPs causing 100% mortality of E-coli. Numerous research predominatingly support the idea that ZnO nanoparticles also exhibit antibacterial activity that is concentration dependent in addition to particle size dependence. According to [27], an increase in nanoparticle concentration led to a greater antibacterial effect because more H2O2 was being created on the surface of ZnO. The antibacterial activity of ZnO nanoparticles has been explained by the interaction of ZnO nanoparticles with bacteria, which leads to the destruction of the bacterial surface[28]. This study demonstrated that the presence of ZnO nanoparticles caused damage to the E. coli cell membrane. After ZnO destroys or captures the cell membrane, the nanoparticles are likely to stay firmly adhered on the surface of the dead bacteria, inhibiting further antibacterial action. [29]. the creation of extremely reactive species including OH^-, H2O2, and $O2^-$ Since both UV and visible light can activate ZnO with disorder, electron-hole pairs (eh+) can be produced. The holes divided the molecules of H2O into OH and H+. In order to produce hydrogen peroxide anions (HO2-) radicals, dissolved oxygen molecules are converted to, which then reacts with hydrogen to produce (HO2) with electrons. They then form H2O2 molecules through a reaction with hydrogen ions. The produced H2O2 can enter the bacterial cell membrane and destroy it [30].

Superoxides and hydroxyl radicals cannot enter the cell membrane because they are negatively charged particles, therefore they must stay in contact with the bacteria's outer surface. H2O2, on the other hand, can enter the cell[31].

4. Conclusion

Green synthesis of metal oxide nanoparticles by plant extract is novel approach in field of nanoscience and nanotechnology When used as a capping and reducing agent, plant extract reduces the metal ions, causing the formation of nanoparticles. ZnoNPs was successfully via green approach which is cost effectiveness and less time requirement. It can be concluded that the ZnoNPs constitute effective antibacterial agent against pathogenic microorganism. ZnoNPs have bacteriacidal effect against E-coli.

References

[1] I. Karaboze, F. Ucar, R. Eltem, G. Ozdmir, and M. Ates, "Determination of existence and count of pathogenic microorganisms in Izmir Bay," *JES*, vol. 26, pp. 1–18, 2003.

[2] J. Davies, "Inactivation of antibiotics and the dissemination of resistance genes," *Science (80-.).*, vol. 264, no. 5157, pp. 375–382, 1994.

[3] D. S. Fabricant and N. R. Farnsworth, "The value of plants used in traditional medicine for drug discovery.," *Environ. Health Perspect.*, vol. 109, no. suppl 1, pp. 69–75, 2001.

[4] M. Obeidat *et al.*, "Antimicrobial activity of crude extracts of some plant leaves," *Res. J.*

Microbiol., vol. 7, no. 1, pp. 59–67, 2012.
[5] H. Q. Raheem, A. Al-Thahab, and F. G. Abd, "Antibacterial activity of silver nanoparticles extracted from Proteus mirabilis and healing the wound in rabbit," *Biochem. Cell. Arch*, vol. 18, no. 1, pp. 97–104, 2018.
[6] N. Mude, A. Ingle, A. Gade, and M. Rai, "Synthesis of silver nanoparticles using callus extract of Carica papaya—a first report," *J. Plant Biochem. Biotechnol.*, vol. 18, no. 1, pp. 83–86, 2009.
[7] W. Maret, "Metals on the move: zinc ions in cellular regulation and in the coordination dynamics of zinc proteins," *Biometals*, vol. 24, no. 3, pp. 411–418, 2011.
[8] G. Bisht and S. Rayamajhi, "ZnO nanoparticles: a promising anticancer agent," *Nanobiomedicine*, vol. 3, no. Godište 2016, pp. 3–9, 2016.
[9] M. R. Hosseini and M. N. Sarvi, "Recent achievements in the microbial synthesis of semiconductor metal sulfide nanoparticles," *Mater. Sci. Semicond. Process.*, vol. 40, pp. 293–301, 2015.
[10] D. Sharma, S. Sharma, B. S. Kaith, J. Rajput, and M. Kaur, "Synthesis of ZnO nanoparticles using surfactant free in-air and microwave method," *Appl. Surf. Sci.*, vol. 257, no. 22, pp. 9661–9672, 2011.
[11] G. Nagaraju *et al.*, "Green, nonchemical route for the synthesis of ZnO superstructures, evaluation of its applications toward photocatalysis, photoluminescence, and biosensing," *Cryst. Growth Des.*, vol. 16, no. 12, pp. 6828–6840, 2016.
[12] N. Tripathy and D.-H. Kim, "Metal oxide modified ZnO nanomaterials for biosensor applications," *Nano Converg.*, vol. 5, no. 1, pp. 1–10, 2018.
[13] A. Sirelkhatim *et al.*, "Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism," *Nano-micro Lett.*, vol. 7, no. 3, pp. 219–242, 2015.
[14] J. T. Seil and T. J. Webster, "Antimicrobial applications of nanotechnology: methods and literature," *Int. J. Nanomedicine*, vol. 7, p. 2767, 2012.
[15] A. Kołodziejczak-Radzimska and T. Jesionowski, "Zinc oxide—from synthesis to application: a review," *Materials (Basel).*, vol. 7, no. 4, pp. 2833–2881, 2014.
[16] C. Soler-Rivas, J. C. Espín, and H. J. Wichers, "Oleuropein and related compounds," *J. Sci. Food Agric.*, vol. 80, no. 7, pp. 1013–1023, 2000.
[17] A. M Awwad, B. Albiss, and A. L Ahmad, "Green synthesis, characterization and optical properties of zinc oxide nanosheets using Olea europea leaf extract," *Adv. Mater. Lett.*, vol. 5, no. 9, pp. 520–524, 2014.
[18] A. M. El Saeed, M. Abd El-Fattah, and A. M. Azzam, "Synthesis of ZnO nanoparticles and studying its influence on the antimicrobial, anticorrosion and mechanical behavior of polyurethane composite for surface coating," *Dye. Pigment.*, vol. 121, pp. 282–289, 2015.
[19] M. Gupta, R. S. Tomar, S. Kaushik, R. K. Mishra, and D. Sharma, "Effective antimicrobial activity of green ZnO nano particles of Catharanthus roseus," *Front. Microbiol.*, p. 2030, 2018.
[20] T. Bhuyan, K. Mishra, M. Khanuja, R. Prasad, and A. Varma, "Biosynthesis of zinc oxide nanoparticles from Azadirachta indica for antibacterial and photocatalytic applications," *Mater. Sci. Semicond. Process.*, vol. 32, pp. 55–61, 2015.
[21] G. Sangeetha, S. Rajeshwari, and R. Venckatesh, "Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties," *Mater. Res. Bull.*, vol. 46, no. 12, pp. 2560–2566, 2011.
[22] V. Srivastava, D. Gusain, and Y. C. Sharma, "Synthesis, characterization and application of zinc oxide nanoparticles (n-ZnO)," *Ceram. Int.*, vol. 39, no. 8, pp. 9803–9808, 2013.
[23] H. Agarwal, S. V. Kumar, and S. Rajeshkumar, "A review on green synthesis of zinc oxide nanoparticles–An eco-friendly approach," *Resour. Technol.*, vol. 3, no. 4, pp. 406–413, 2017.
[24] D. Gnanasangeetha and D. SaralaThambavani, "One pot synthesis of zinc oxide nanoparticles via chemical and green method," *Res J Mater Sci*, vol. 2320, p. 6055, 2013.
[25] M. M. Chikkanna, S. E. Neelagund, and K. K. Rajashekarappa, "Green synthesis of zinc oxide nanoparticles (ZnO NPs) and their biological activity," *SN Appl. Sci.*, vol. 1, no. 1, pp. 1–10, 2019.
[26] W. Jiang, H. Mashayekhi, and B. Xing, "Bacterial toxicity comparison between nano-and micro-scaled oxide particles," *Environ. Pollut.*, vol. 157, no. 5, pp. 1619–1625, 2009.
[27] R. Jalal, E. K. Goharshadi, M. Abareshi, M. Moosavi, A. Yousefi, and P. Nancarrow, "ZnO nanofluids: green synthesis, characterization, and antibacterial activity," *Mater. Chem. Phys.*, vol. 121, no. 1–2, pp. 198–201, 2010.
[28] L. Zhang, Y. Jiang, Y. Ding, M. Povey, and D. York, "Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids)," *J. Nanoparticle Res.*, vol. 9, no. 3, pp. 479–489, 2007.
[29] O. Yamamoto, M. Komatsu, J. Sawai, and Z. Nakagawa, "Effect of lattice constant of zinc oxide on antibacterial characteristics," *J. Mater. Sci. Mater. Med.*, vol. 15, no. 8, pp. 847–851, 2004.
[30] M. Fang, J.-H. Chen, X.-L. Xu, P.-H. Yang, and H. F. Hildebrand, "Antibacterial activities of inorganic agents on six bacteria associated with oral infections by two susceptibility tests," *Int. J. Antimicrob. Agents*, vol. 27, no. 6, pp. 513–517, 2006.
[31] P.-C. Maness, S. Smolinski, D. M. Blake, Z. Huang, E. J. Wolfrum, and W. A. Jacoby, "Bactericidal activity of photocatalytic TiO2 reaction: toward an understanding of its killing mechanism," *Appl. Environ. Microbiol.*, vol. 65, no. 9, pp. 4094–4098, 1999.