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Zinc Oxide Nanoparticles—Synthesis, Characterization and Antibacterial Activity

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The ever increasing demand for seafood has lead aquaculture farmers to resort to intensive culture practices leading to stress of the animals and enhanced susceptibility to diseases. Vibriosis is one of the most prevalent bacterial diseases. Antibacterial activity of zinc oxide nanoparticles was tested in this study. Zinc oxide nanoparticles were synthesised by chemical precipitation method using Zinc acetate and varying concentrations (0.1%, 0.5%, 1%) of soluble starch and compared with the commercial ZnO nanoparticles for the inhibition of *V. anguillarum* pathogenic bacteria in aquaculture environment. The synthesized ZnO nanoparticles were confirmed using X-ray Diffraction analysis and the average size of the particles was 50 nm. Field Emission-Scanning Electron Microscopy images showed hexagonal morphology of the synthesised nano-ZnO. Flourescence Transform Infrared Spectroscopy analysis confirmed binding of starch to nano-ZnO. Energy dispersive X-ray analysis determined the elemental composition of nano-ZnO and Ultraviolet-Visible spectroscopy analysis revealed significant blue shift and ascertained the stability of the particles. Synthesized ZnO nanoparticles had higher bactericidal activity than commercial. Antibacterial assay revealed that nano-ZnO synthesised using 0.5% starch had the most efficient antibacterial activity against *V. anguillarum* with MIC of 30 μg/ml and MBC of 40 μg/ml and zone of inhibition of 15 mm. SEM image of *V. anguillarum* treated with and without ZnO nanoparticles confirmed the bacterial cell damage by ZnO nanoparticles. The results concluded that nano-ZnO is an effective bactericidal agent on *V. anguillarum* and can be used in aquaculture to effectively combat bacterial diseases.

Keywords: ZnO Nanoparticles, Antibacterial Activity, *V. anguillarum,* Minimum Inhibitory Concentration, Minimum Bactericidal Concentration.

1. INTRODUCTION

The significance of aquaculture in the context of global food security and the socioeconomic development of a country is now fully appreciated worldwide. Aquaculture is fast becoming a major industry with continuously growing demand as supply from natural resources indicate a declining trend and demand for fisheries products are increasing. The increase in global demand for aquaculture food has led farmers to adopt intensive culture practices that are detrimental to pond water quality which in turn leads to enhanced susceptibility of cultured animal to several diseases.

Disease outbreaks in aquafarming are an important limiting factor affecting production and trade. Vibriosis, one of the most prevalent bacterial diseases in aquaculture, caused by members of the genus *Vibrio*, infects a wide range of aquatic organisms such as penaeid shrimp,[1] several fish species[2] and molluscs.[3] Salmon, trout, turbot, plaice and eels are some of the fishes commonly affected by vibriosis.[4,5] Moreover, vibriosis can cause sea food borne gasteroentitis in human beings. Hence, an effective antibacterial agent to control vibriosis is necessary to prevent the disease in aquaculture.

The use of antimicrobial drugs in farms is a well-known control measure against microbial pathogens. However, they have side effects that affect fish, shrimp and the aquaculture environment.[6] Antibiotics can cause immunomodulation in fish[7] and its residues in food products can induce antibiotic resistance and is a cause of serious concern for public health. Hence, only a few antibiotics have been

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licensed for use in aquaculture.[8] Consequently, nanoparticles and their composites are emerging as a viable alternative.

Inorganic and organic metal oxide nanoparticles are widely used as antimicrobial agents.[9,10] When compared to organic metal oxide nanoparticles, inorganic metal oxide nanoparticles are more stable, robust and have longer shelf life. Nano ZnO have excellent mechanical strength,[11] antistatic,[12] antibacterial,[13] UV absorption[14,15] and photocatalytic[16] properties. Among the inorganic metal oxides, nano-ZnO are non-toxic, biosafe, biocompatible and heat resistant and are being used as drug carriers, cosmetics and fillings in medical materials.[17,18] Another advantage of using nano-ZnO as antibacterial agent is that, they effectively inhibit activity of pathogenic microbes at small concentration.

Zinc oxide has been established as antibacterial agent against number of bacterial pathogens including *Streptococcus agalactia*,[19] *Escherichia coli*,[20,21] *Staphylococcus aureus*,[22] *Salmonella typhimurium*,[23] and also against fungi such as *Botrytis cinerea* and *Penicillium expansum*.[24] Though studies of antibacterial activity of ZnO nanoparticles against *Vibrio* species is still in infancy, ZnO nanoparticles have been proved to be effective antibacterial agents against *Vibrio cholerae*,[25] *Vibrio fischeri*[26] and *Vibrio harveyi*.[27] The present work focuses on synthesising stable nanocrystalline ZnO and testing its antibacterial activity against *Vibrio anguillarum.*

2. MATERIALS AND METHODS

2.1. Synthesis and Characterization of ZnO Nanoparticles

ZnO nanoparticles were synthesised by chemical precipitation method[28] using Zinc acetate dihydrate as the starting material, different concentration of soluble starch (0.1%, 0.5%, 1%) as capping agent sodium hydroxide as reducing agent and at a temperature of 100 °C. In the aqueous synthesis of ZnO nanoparticles, the initial precipitate of the reaction is $Zn(OH)_2$, which by subsequent modifications produces ZnO nanoparticles. Synthesis of ZnO was carried out at high temperature because increasing the temperature leads to increasing the concentration of ZnO nanoparticles and decreasing of its particle size and prevents its aggregation.[29] The growth units were, $Zn(OH)_4^{2-}$, so alkaline pH was used which favoured ZnO nanoparticle formation.[30] Commercial nano-ZnO (50 nm) procured from Sigma Aldrich was used for comparative study with prepared nano-ZnO. Nano-ZnO synthesised from 1% starch, 0.5% starch, 0.1% starch and commercial nano-ZnO will be referred as nZnO_1S, nZnO_0.5S, nZnO_0.1S and nZnO_C respectively. Structural characteristics of synthesized ZnO nanoparticles were tested for the confirmation by X-ray diffraction (XRD) using Rigaku Miniflex IIC X-ray diffractometer, field emission-scanning electron microscopy (FE-SEM) using Hitachi SU6600 High Resolution Analytical FE-SEM, fluorescence transform infrared spectroscopy (FTIR) using Perkin-Elmer FTIR spectrophotometer and Energy dispersive X-ray (EDAX) using EMAX, Horiba.

To confirm the stability, prepared nano-ZnO was dispersed in de-ionised water using ultra-sonication and UV-Visible spectrum was recorded from 200–800 nm using UV-Visible spectroscopy (Shimadzu UV-1700).

2.2. Antibacterial Activity of Nano-ZnO

Broth dilution method was carried out to study the antimicrobial activity as per CLSI guidelines.[31] *Vibrio anguillarium* (gifted by Indo-Norway project) was used as test organism. It was grown in 50 ml of APW (Alkaline peptone water) containing 2% NaCl for 24 hours @ 37 °C. Concentration of bacteria was measured @ 600 nm using spectrophotometer and adjusted to 0.5 McFarland turbidly standard. MIC is defined as the lowest concentration of antibacterial agent that reduces the viability of the bacterial inoculum by $\geq$99.9%. To determine the MIC, 100 μl of bacterial suspension with the concentration of 10^8 CFU/ml was inoculated in 10 ml of APW medium containing 2% salt with nano ZnO suspension of various concentrations (20–80 μg/ml) and incubated at 37 °C for 24 hours. Negative control tube contained only sterile broth and positive control tube contained inoculated broth without nano-ZnO. After incubation period, turbidity was measured based on OD value at 600 nm using UV-Visible spectrophotometer. To determine the MBC, the dilutions representing the MIC were plated on APW agar medium and viable bacterial cells were enumerated after 24 hours of incubation at 37 °C.

The efficiency of MBC was further evaluated by agar well diffusion assay.[32] APW agar plates were prepared for testing antibacterial activity and the prepared inoculum of bacteria was spread on plates (100 μl). 5 mm wells were made using sterile microtips and filled with 50 μl of ZnO nanoparitcles and incubated at 37 °C for 24 hours. In each plate, a well without addition of nano-ZnO suspension served as positive control. After incubation, the zone of inhibition was measured and recorded.

2.3. SEM Analysis of Bacteria

Scanning Electron Microscopy (SEM) analysis was performed to investigate the morphology of bacteria with and without treatment of nano-ZnO. For this, the bacterial cultures were centrifuged at 2500 rpm for 10 minutes and 10% formalin (1 ml formalin +9 ml Phosphate buffered saline, pH 7.2) was added and incubated overnight at room temperature for fixation. This preparation was centrifuged at 2500 rpm for 10 minutes, supernatant was discarded and the pellets were dehydrated with increasing concentrations of ethanol (30%, 50%, 70%, 100%). The samples were incubated in each ethanol concentration for 10 minutes and in 100% ethanol for 1 hour and were put on stub and air

dried. 3 nm gold was sputtered onto the bacterial samples and the SEM analyses of the samples were done.[20]

3. RESULTS AND DISCUSSION

3.1. Confirmation of Synthesized Nano-ZnO

The peak positions and the relative peak intensities in the XRD of the synthesized nano-ZnO matched with the JCPDS No. 79-2205 (Joint Committee on Powder Diffraction Standard). The strong peaks appeared at 31.78, 34.44 and 36.27 deg in the (100), (002) and (101) planes respectively and the other lower intense peaks at 47.55, 56.59, 62.84, 66.35, 67.92, 69.05, 72.52 and 76.91 were associated with (102), (110), (103), (200), (112), (201), (004) and (202) planes respectively (Fig. 1). In accordance with the JCPDS card number all the peaks belonged to hexagonal or wurtzite structure. It is noteworthy that only a single phase of ZnO was formed and no characteristic peaks of any impurities were detected, suggesting that high-quality ZnO was obtained without calcination. There was no crystalline peak associated with starch, which indicated that the component existed in amorphous phase, which could improve the hydrophilicity of nano-ZnO. Table I shows the average crystallite size of nano-ZnO with different concentrations of starch obtained using Scherrer's formula as

$$D = k\lambda/\beta\cos\theta$$

where: k—constant taken to be 0.91, λ—Wavelength of X-ray source (CuKα = 1.5406 Å), β—Full width half maximum (FWHM) of the diffraction peak, θ—Diffraction angle (Deg).

The particle size of ZnO nanoparticles decreased with increase in starch concentration from 0.1 to 0.5%. However, with further increase in concentration of starch (1%), the particle size also increased. This could be due to forced aggregation caused by starch at high concentration and hence formation of large particles (Table I).[33]

FE-SEM images of nano-ZnO (Fig. 2) revealed hexagonal faceted rod shaped nano-ZnO formation with homogeneity in particle size and morphology and the particles were mono-dispersed. Polysaccharides like starch form

Table I. Average crystallite size of nano-ZnO using Scherrer's formula.

Type	nZnO_1S	nZnO_0.5S	nZnO_0.1S
Average crystallite size (nm)	55.67	44.09	51.16

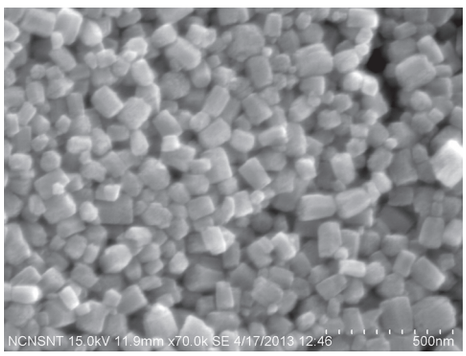

Figure 2. FE-SEM image of nano-ZnO with starch (nZnO_0.5S).

complexes with divalent metal ions due to their high number of coordinating functional groups (hydroxyl and glucoside groups). The soluble starch in its helical form is quick to protect the formed ZnO nanoparticles and prevent aggregation and growth by the action of steric hindrance.[28] Probably, majority of the zinc ions were closely associated with the starch molecules. Hence, nucleation and initial crystal growth could have occurred within regions of both high starch and Zn concentration, leading to the formation of nanoparticles.[33] Moreover, soluble starch improved the stability of ZnO in water and prevented further aggregation of ZnO, resulting in the formation of ZnO nanoparticles encapsulated by soluble starch.[34] In addition, polysaccharides present dynamic supra-molecular associations facilitated by inter and intra-molecular hydrogen bonding, which acted as templates for the growth of nanoparticles.[35] As template, soluble starch formed more interactions with zinc ions, which controlled the growth of nano-ZnO.

Compositional analysis of soluble starch (Fig. 3) and nano-ZnO (Fig. 4) was carried out by FTIR measurement in the acquired range of 400–4000 cm^{-1}. It showed three

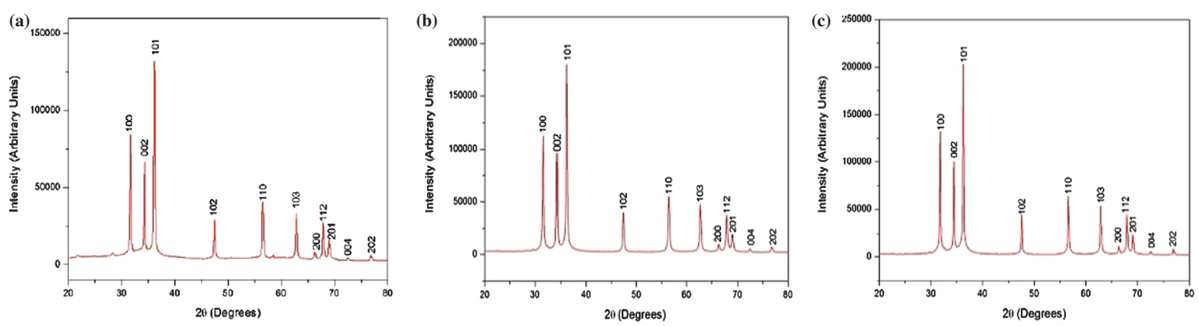

Figure 1. XRD of nano-ZnO synthesized using different concentration of starch: (a) nZnO_0.1S, (b) nZnO_0.5S, (c) nZnO_1S.

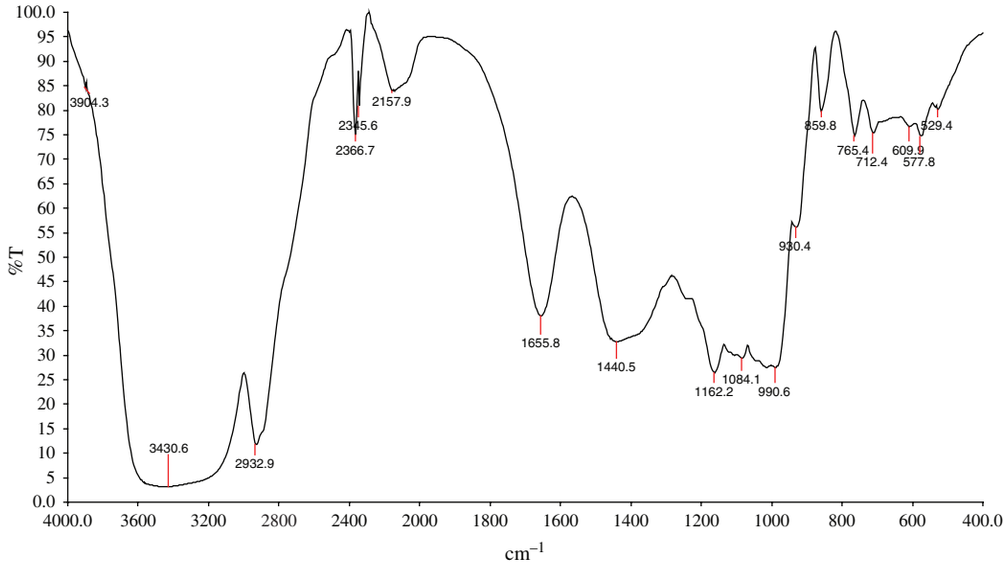

Figure 3. FTIR spectra of soluble starch.

peaks characteristic of –C–O– stretching in the fingerprint region of soluble starch. The peak at 1162 cm^{-1} belonged to the C–O bond stretching of the C–O–H group, and the two peaks at 1084 cm^{-1} and 990 cm^{-1} were attributed to C–O bond stretching of the C–O–C group in the an hydro glucose ring of starch.[36] The peak at 2932 cm^{-1} was characteristic of the C–H stretching associated with ring methane hydrogen atoms.[37] FTIR spectra of nano-ZnO (Fig. 4) showed high intensity broad band at 504 cm^{-1} resulting from the stretching of the zinc and oxygen bond. It also recorded the above peaks for soluble starch. Hence, in nano-ZnO particles there was strong binding of starch, but no obvious covalent bond formation between ZnO and starch was observed which coincides with the findings of Ma and Co-workers.[36]

The elemental composition of the material was confirmed through EDAX analysis. The EDAX spectrum (Fig. 5) showed Zn, O and C as elements in the sample. Table II shows the elemental composition of various synthesised nano-ZnO.

The absorption spectrum by UV analysis at 0 th day showed that the absorption maximum of synthesized nano-ZnO was at 364 nm (Fig. 6(a)) whereas for bulk at 380 nm,[38] and this blue shift is due to the quantum

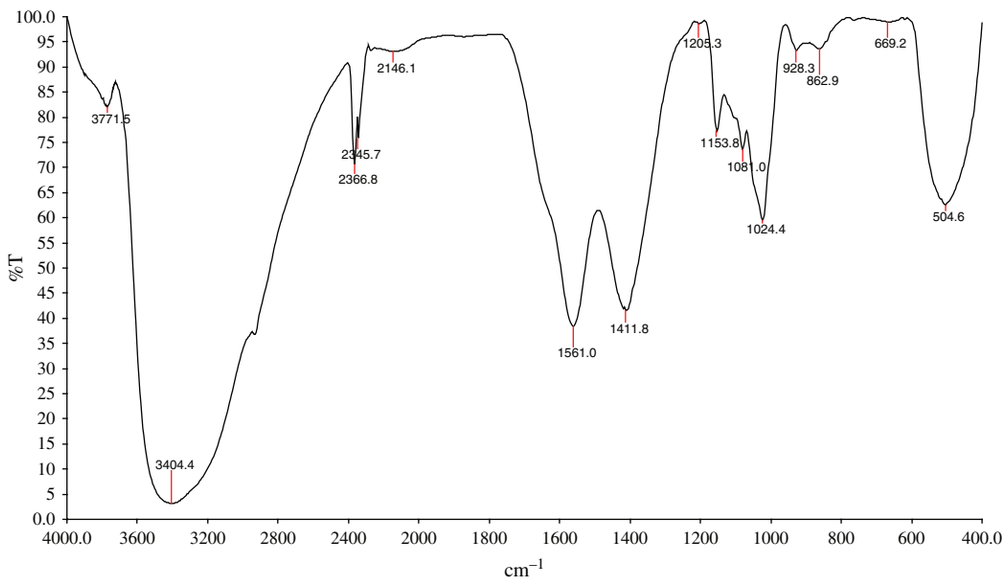

Figure 4. FTIR spectra of nano-ZnO synthesized with starch.

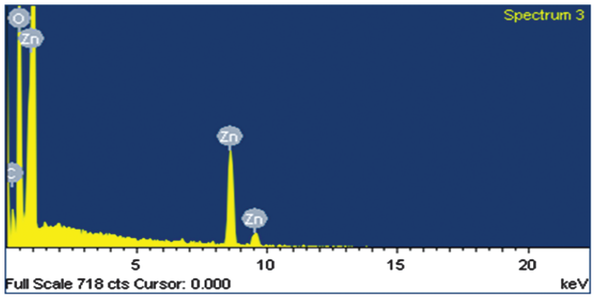

Figure 5. EDAX spectrum of nano-ZnO.

Table II. Elemental composition of nano-ZnO.

Elements	Zn	O	C
nZnO_1S (Wt%)	29.16	45.84	25.00
nZnO_0.5S (Wt%)	38.69	51.04	10.27
nZnO_0.1S (Wt%)	35.32	50.99	9.00

confinement effect because of the reduction in particle size.[39] This again confirms nano-ZnO formation. The stability of the prepared nano-ZnO was determined by studying the absorption spectrum after 30 days. It was observed that the shift remained stable with increase in storage time, which confirmed the stability of starch capped nano-ZnO (Fig. 6(b)).

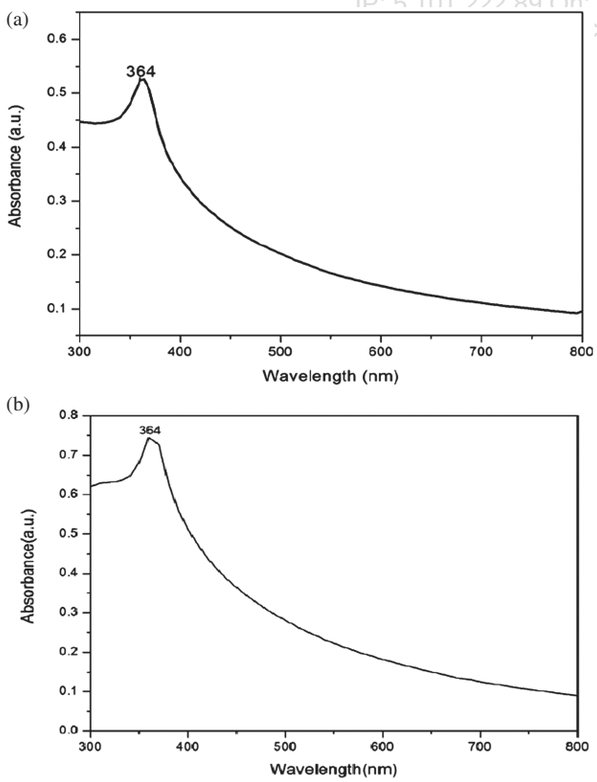

Figure 6. UV-Visible spectrum of nano-ZnO: (a) absorbance on 0th day, (b) absorbance on 30th day.

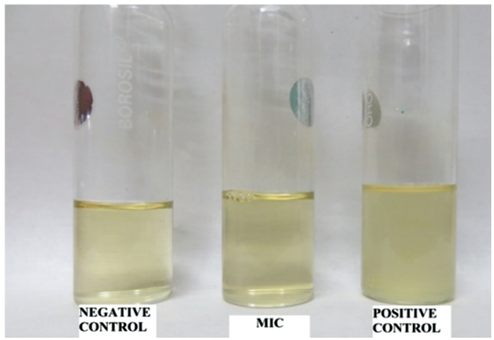

Figure 7. MIC of nano-ZnO against *V. anguillarum.*

3.2. Evaluation of Antibacterial Properties Determination of MIC and MBC

MIC study showed that there was complete absence of turbidity in negative control and at concentrations greater than or equal to MIC of the respective nano-ZnO samples (Fig. 7).

MBC study (Fig. 8) observed that lawn like growth of bacteria on solid medium at concentrations below MIC. At MIC, though there was absence of turbidity in liquid culture, few colonies appeared upon plating indicating that nano-ZnO at that concentration only inhibited bacterial growth (bacteriostatic), so, upon removing bacteria from nano-ZnO suspension and plating it on nutrient medium, bacteria exhibited growth. At MBC, no growth was observed on plating, as nano-ZnO nanoparticles killed the bacteria (bactericidal) at that concentration.[40]

The results of absorbance measurement and plating have been tabulated in Table III. nZnO_1S and nZnO_C had MIC of 60 μg/ml and MBC of 70 μg/ml. nZnO_0.5S had MIC of 30 μg/ml and MBC of 40 μg/ml. nZnO_0.1S had MIC of 40 μg/ml and MBC of 50 μg/ml. Among all

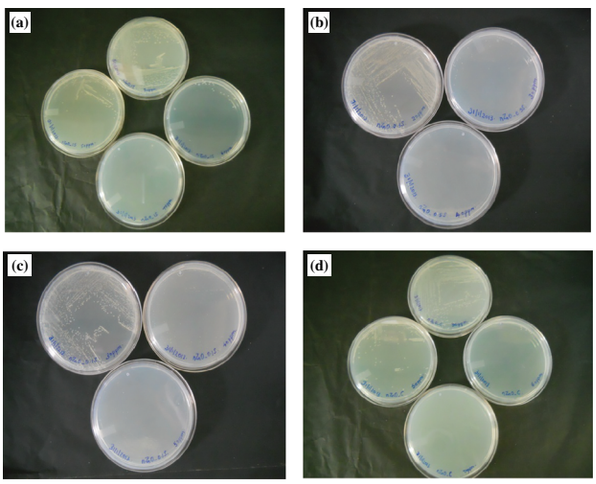

Figure 8. Bioactivity of nano-ZnO against *V. anguillarum:* (a) nZnO_1S, (b) nZnO_0.5S, (c) nZnO_0.1S, (d) nZnO_C.

Table III. MIC and MBC of nano-ZnO against *V. anguillarum*.

Code of ZnO	Concentration of ZnO (μg/ml)	Status of bioactivity (After 24 hours of incubation)
nZnO_1S	20	No activity
nZnO_1S	30	No activity
nZnO_1S	40	No activity
nZnO_1S	50	No activity
nZnO_1S	60	Bacteriostatic (MIC)
nZnO_1S	70	Bactericidal (MBC)
nZnO_1S	80	Bactericidal
nZnO_0.5S	20	No activity
nZnO_0.5S	30	Bacteriostatic (MIC)
nZnO_0.5S	40	Bactericidal (MBC)
nZnO_0.5S	50	Bactericidal
nZnO_0.5S	60	Bactericidal
nZnO_0.5S	70	Bactericidal
nZnO_0.5S	80	Bactericidal
nZnO_0.1S	20	No activity
nZnO_0.1S	30	No activity
nZnO_0.1S	40	Bacteriostatic (MIC)
nZnO_0.1S	50	Bactericidal (MBC)
nZnO_0.1S	60	Bactericidal
nZnO_0.1S	70	Bactericidal
nZnO_0.1S	80	Bactericidal
nZnO_C	20	No activity
nZnO_C	30	No activity
nZnO_C	40	No activity
nZnO_C	50	No activity
nZnO_C	60	Bacteriostatic (MIC)
nZnO_C	70	Bactericidal (MBC)
nZnO_C	80	Bactericidal

the nano-ZnO samples, nZnO_0.5S had the most effective antibacterial activity with the least MIC and MBC.

The antibacterial activity increased from nZnO_0.1S to nZnO_0.5S, whereas, nZnO_1S showed lesser antibacterial activity, which is in direct proportion to their respective particle sizes (Table I).[40] The maximum antibacterial activity of nZnO_0.5S when compared to nZnO_1S and nZnO_0.1S could also be attributed to the greatest yield of Zn (38.69)% as obtained through EDAX analysis (Table II). Hence, order of biocidal activity of nano-ZnO was: nZnO_0.5S > nZnO_0.1S > nZnO_1S, nZnO_C. As the particle size decreases, there is an increase in surface area to volume ratio, which contributed to more oxygen species on the surface and resulted in increased production of reactive oxygen species (ROS) viz. hydroxyl radicals and H_2O_2.[41] The ROS brought about bacterial membrane damage and leakage of intracellular contents and resulted in death of bacteria.[20] Also, exposure of bacteria to small nanometre scale ZnO particles increased cellular internalisation of the nanoparticles and bacterial cell damage.[42] So, there is an inverse relationship between particles size and antibacterial activity, which explains the efficacy of nano-ZnO against *V. anguillarum*.

Determination of Zone of Inhibition

Zone of inhibition quantitatively determines the antibacterial activity of an antibacterial agent (nano-ZnO) and it was measured by well diffusion method for concentrations

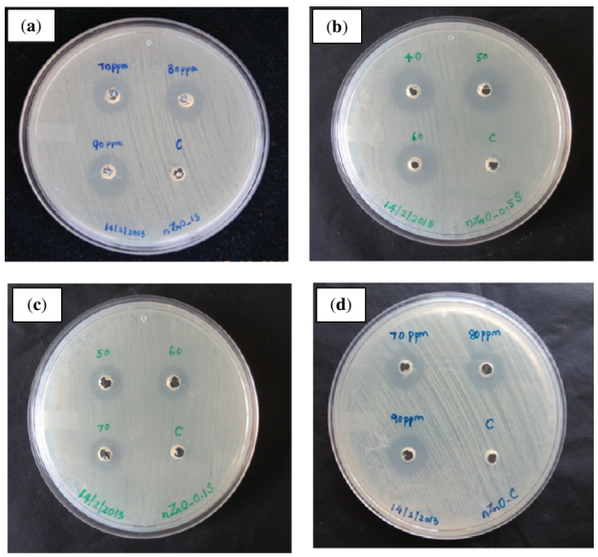

Figure 9. Zone of inhibition of nano-ZnO against *V. anguillarum*: (a) nZnO_1S, (b) nZnO_0.5S, (c) nZnO_0.1S, (d) nZnO_C.

of respective MBCs and two concentrations above MBC. Figure 9 shows the appearance of clear zone of inhibition around wells loaded with nano-ZnO. From the ZOI values in Table IV, the bactericidal activity was not significantly different with increase in concentration of nano-ZnO beyond respective MBCs.

3.3. Mechanism of Action of Nano-ZnO Against *Vibrio anguillarum*

SEM analysis of bacteria was done to determine the mechanism of action of nano-ZnO against *V. anguillarum* (Fig. 10). *V. anguillarum* treated by nano-ZnO (Fig. 10(b)) showed disruption and damage of bacterial cell membrane, resulting in a leakage of intracellular contents and eventually the death of bacterial cells. Similar results were observed by Brayner et al.[43] Zhang et al.[44] and Liu et al.[20] with E. coli.

Membrane disorganisation by nano-ZnO could be because of direct interaction between ZnO particles

Table IV. Zone of inhibition of nano-ZnO against *V. anguillarum*.

Code of ZnO	Concentration of ZnO (ppm)	ZOI (mm)
nZnO_1S	70	14
nZnO_1S	80	14.5
nZnO_1S	90	14.5
nZnO_0.5S	40	15
nZnO_0.5S	50	15
nZnO_0.5S	60	15
nZnO_0.1S	50	11.5
nZnO_0.1S	60	11.5
nZnO_0.1S	70	11.5
nZnO_C	70	12.5
nZnO_C	80	13
nZnO_C	90	13

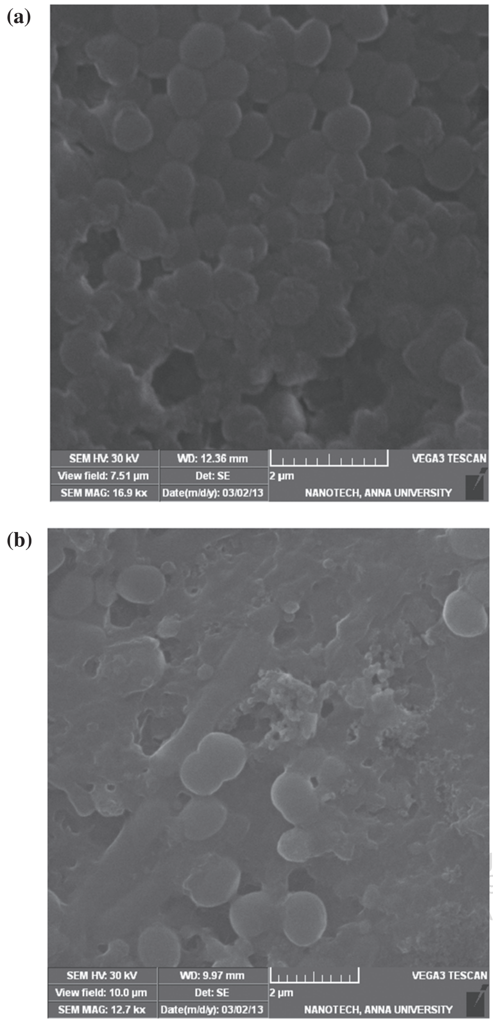

Figure 10. SEM image of *V. anguillarum*: (a) without nano-ZnO (b) with nano-ZnO.

and bacterial cells caused by electrostatic forces.[13] ZnO nanoparticles have abrasive surface texture when compared to bulk. This surface roughness could cause mechanical damage of bacterial cell membrane surface.[10] Another mechanism is aqueous suspensions of small nanoparticles of ZnO produced increased levels of reactive oxygen species. Moreover, an exposure of bacteria to the small ZnO nanoparticles could have resulted in an increased cellular internalization of the nanoparticles and subsequent bacterial cell damage.[42]

4. CONCLUSION

Zinc oxide nanoparticles prepared using varying concentrations of starch as stabilising agent, were characterised by XRD, FE-SEM, FTIR, EDAX and UV-Vis. The characterisations confirmed that ZnO nanoparticles were well formed, pure, stable and homogeneous with average size around 50 nm and hexagonal morphology. Prepared nano-ZnO were tested for its antibacterial activity against *V. anguillarum* and the results showed that nano-ZnO prepared using 0.5% starch had maximum antibacterial activity with MIC of 30 μg/ml; MBC of 40 μg/ml and zone of inhibition of 15 mm.

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References and Notes

1. D. V. Lightner, Diseases of cultured penaeid shrimp, CRC Handbook of Mariculture Crustacean Aquaculture, edited by J. P. McVey, CRC Press Boca Raton, FL **(1993)**, pp. 393–486.
2. B. Austin and D. A. Austin, Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, Springer, London **(1999)**, p. 457.
3. G. Rheinheimer, Aquatic Microbiology, Wiley, London **(1992)**, p. 363.
4. I. S. Azad, A. R. Thirunavukkarasu, M. Kailasam, and J. J. S. Rajan, *Asian Fish. Sci.* 17, 101 **(2004)**.
5. C. B. Munn, *Aquac. Res.* 8, 11 **(1977)**.
6. O. Samuelsen, *Aquaculture* 255, 55 **(2006)**.
7. C. Tafalla, B. Novoa, J. M. Alvarez, and A. Figueras, *J. Fish Dis.* 22, 271 **(2002)**.
8. C. C. Felipe, *Environ. Microbiol.* 8, 1137 **(2006)**.
9. J. Sawai, *J. Microbiol. Methods* 54, 177 **(2003)**.
10. K. S. Peter, L. K. Rosalyn, L. M. George, and J. K. Kenneth, *Langmuir* 18, 6679 **(2002)**.
11. J. H. Li, R. H. Hong, M. Y. Li, H. Z. Li, Y. Zheng, and J. Ding, *Prog. Org. Coat.* 64, 504 **(2009)**.
12. A. Yadav, V. Prasad, A. A. Kathe, Sheela Raj, Deepti Yadav, C. Sundaramoorthy, and N. Vigneshwaran, *Bull. Mater. Sci.* 29, 641 **(2006)**.
13. Z. Lingling, D. Yulong, P. Malcolm, and Y. David, *Prog. Nat. Sci.* 18, 939 **(2008)**.
14. F. Fang, J. Kennedy, D. A. Carder, J. Futter, P. Murmu, and A. Markwitz, *J. Nanosci. Nanotechnol.* 10, 8239 **(2010)**.
15. F. Fang, J. Futter, A. Markwitz, and J. Kennedy, *Mater. Sci. Forum* 700, 150 **(2012)**.
16. K. Kaviyarasu, X. Fuku, G. T. Mola, E. Manikandan, J. Kennedy, and M. Maaza, *Mater. Lett.* 183, 351 **(2016)**.
17. L. R. Nathaniel and A. M. Chad, *Chem. Rev.* 105, 1547 **(2005)**.
18. R. Saraswathy, M. Muralidhar, D. Thulasi, N. Lalitha, A. Nagavel, and A. G. Ponniah, *Proc. of International Conference on Biotechnology: Emerging Trends*, Haryana, India **(2012)**, pp. 179–180.
19. H. Zhongbing, Z. Xu, Y. Danhong, Y. Guangfu, L. Xiaoming, K. Yunqing, Y. Yadong, H. Di, and H. Baoqing, *Langmuir* 24, 4140 **(2008)**.
20. Y. Liu, L. He, A. Mustapha, H. Li, Z. Q. Hu, and M. Lin, *J. Appl. Microbiol.* 107, 1193 **(2009)**.
21. T. Jin, D. Sun, J. Y. Su, H. Zhang, and H. J. Sue, *J. Food Sci.* 74, M46 **(2009)**.
22. R. R. Krishna, T. K. Ranjit, and C. M. Adhar, *Langmuir* 27, 4020 **(2011)**.
23. A. T. Ahmed, F. E. Wael, M. Shaaban, F. E. Ashraf, M. Hoda, F. S. Mohammed, and B. B. Leon, *J. Food Safety* 31, 211 **(2011)**.
24. H. Lili, L. Yang, M. Azlin, and L. Mengshi, *Microbiol. Res.* 166, 3207 **(2011)**.
25. C. Tanaya, C. Soumyananda, J. Prachi, P. S. Surinder, G. Vinay, and C. Pinak, *FEBS J.* 277, 4184 **(2010)**.
26. H. Margit, I. Angela, B. Irina, C. D. Henri, and K. Anne, *Chemosphere* 71, 1308 **(2008)**.

27. R. Saraswathy, K. Pandiyan, M. Muralidhar, D. Thulasi, M. Jeba, N. Lalitha, A. Nagavel, and A. G. Ponniah, *Indian J. Fish.* 107 **(2013)**.

28. N. Vigneshwaran, R. P. Nachane, R. H. Balasubramanya, and P. V. Varadarajan, *Carbohyd. Res.* 341, 2012 **(2006)**.

29. S. A. El and O. A. Abou, *Carbohyd. Polym.* 83, 920 **(2011)**.

30. R. Wahab, S. G. Ansari, Y. S. Kim, M. Song, and H. S. Shin, *Appl. Surf. Sci.* 255, 4891 **(2009)**.

31. Clinical and Laboratory Standard Institute (CLSI), Eighth edition, M07-A8 **(2009)**.

32. C. Perez, M. Paul, and P. Bazerque, *Acta Biologica Et Medica Experimentalis Exp.* 15, 113 **(1990)**.

33. T. Andreas and W. Gerhard, *J. Mater. Chem.* 12, 805 **(2002)**.

34. M. Xiaofei, R. C. Peter, Y. Jingwen, and Y. Jiugao, *Carbohyd. Polym.* 75, 472 **(2009)**.

35. P. Raveendran, J. Fu, and S. L. Wallen, *J. Am. Chem. Soc.* 125, 13940 **(2003)**.

36. X. F. Ma, P. R. Chang, J. W. Yang, and J. G. Yu, *Carbohyd. Polym.* 75, 472 **(2009)**.

37. J. M. Fang, P. A. Fowler, and J. Tomkinson, *Carbohyd. Polym.* 47, 245 **(2002)**.

38. V. Prasad, C. D. Souza, D. Yadav, A. J. Shaikh, and N. Vigneshwaran, *Spectrochim. Acta A* 65, 173 **(2006)**.

39. M. Haase, H. Weller, and A. Henglein, *J. Phys. Chem.* 92, 482 **(1988)**.

40. P. Nagarajan and V. Rajagopalan, *Sci. Technol. Adv. Mat.* 9, 5004 **(2008)**.

41. Y. Osamu, K. Miyako, S. Jun, and N. Zenbe-e, *J. Mater. Sci.-Mater. M* 15, 847 **(2004)**.

42. A. Guy, L. Anat, D. Rachel, P. Nina, N. Yeshayahu, L. Rachel, and G. Aharon, *Adv. Funct. Mater.* 19, 842 **(2009)**.

43. R. Brayner, R. Ferrari-Iliou, N. Brivois, S. Djediat, M. F. Benedetti, and F. Fiévet, *Nano Lett.* 6, 866 **(2006)**.

44. Z. Lingling, J. Yunhong, D. Yulong, P. Malcolm, and Y. David, *J. Nanopart. Res.* 9, 479 **(2007)**.

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