



A Review on Application of Zinc Oxide Nanoparticles as Biocide, Problems of Administration, and Improved Delivery Techniques

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ABSTRACT

The incorporation of nanoparticles is trending in a wide range of applications for its antimicrobial properties. The antimicrobial properties, potency, and risk of ZnO nanoparticle have been assessed and justified for its efficacy in different applications. However, there is an existing gap between lab scale and on-site usage due to colloidal stability of the nanoparticles. Despite exhibiting antimicrobial properties under controlled laboratory condition, sensitivity of nanoparticles to ambient condition might reduce or negate its potency under different administered conditions. It is envisaged that encapsulation of zinc oxide nanoparticle shall improve its stability and enhance its performance as biocide. This review paper delves into the attack mechanism of ZnO nanoparticles against different bacteria, fungus, and viruses. In addition, dissolution properties and colloidal stability of nanoparticles, flaws with direct administration of nanoparticles in solid particles form and the current trend in delivery of ZnO nanoparticles were discussed to emphasize the importance of material preservation for performance maximization.

1. Introduction

Biocidal products, which consist of active ingredients, are often employed for the purpose of inactivation or inhibition of harmful organisms. The attack mechanisms of a biocide could be categorized in three interaction levels, which are interaction with the cell wall, the cell membrane and the cytoplasmic components. Cell membrane is the primary target site of most biocides. Attack on cell membrane diverges into three branches, by which the biocide causes physical disruption to cytoplasmic membrane, disturbance of membrane potential and inhibition of membrane enzymes [1]. From the general attack mechanism of biocides, it is deduced that a good biocide should be able to gain entry through cell wall of bacteria to interact with its target site on cytoplasmic membrane.

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An effective biocidal product should be able to attack multiple target sites to boost its bactericidal effect.

Bulk zinc oxide (ZnO) has been employed as a common ingredient in skin care and dermal pharmaceutical products until more recent research in exploring antibacterial function of ZnO nanoparticles [2-5]. At present, zinc oxide nanoparticles have been widely used as sun protection agent in cosmetic products, conductor in electronics and antimicrobial agent in food packaging [6]. ZnO nanoparticles were reported to possess highest antibacterial strength compared to other metal oxide nanoparticles examined [7].

The potential of zinc oxide nanoparticles to be employed in biocidal product could be justified through its biocidal activity. The determining factors of a biocide are concentration and phenotype of microorganism, compatibility with target microbes and ambient conditions which include temperature, pH and the presence of organic matter [8].

In this literature review, effectiveness of ZnO nanoparticles against different microorganisms is reviewed. The mechanisms of ZnO nanoparticles against bacterial cells, fungus and virus are discussed. Attention is directed towards the dissolution and colloidal stability of ZnO nanoparticles and the problems associated with direct application of the nanoparticles in solid particles form in different fields. Besides, improved delivery techniques for ZnO nanoparticles are elaborated in the last section of the paper.

2. Previous Applications of ZnO Nanoparticles against Microorganisms

The efficacy of ZnO nanoparticles (NPs) against a range of microorganisms, such as bacteria, fungus, viruses, and mycobacteria, is shown in Table 1. ZnO NPs (in a variety of forms and sizes) dramatically reduced the ability of enveloped viruses such as HSV-1, HSV-2, H1N1, TMV, Hepatitis E and C, and SARS-CoV-2 to enter and multiply. For gram-positive bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Streptococcus pyogenes*, ZnO NPs reduced bacterial cells effectively. Similarly, for gram-negative bacteria like *Campylobacter jejuni*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Escherichia coli*, ZnO NPs disrupted cell morphology and inhibited growth. Additionally, ZnO NPs showed substantial antifungal activity against fungi like *Candida albicans*, *Fusarium graminearum*, and *Sclerotinia homoeocarpa*, and inhibited the growth of mycobacteria such as *Mycobacterium tuberculosis*. Overall, ZnO NPs exhibit broad-spectrum antimicrobial properties across various pathogens.

From Table 1, it could be inferred that the working temperature of ZnO nanoparticles as antimicrobial agent ranges from 25 °C to 42 °C. Further increment in working temperature will result in agglomeration of nanoparticles, thus negating its antimicrobial properties. Heat energy assisted the coalescence of nanoparticles as the rapid collision of particles will lead to formation of agglomerates [9]. Agglomeration will result in reduction in dissociation of ZnO into Zn²⁺ and cellular internalization for the nanoparticles to exhibit maximum antimicrobial activities.

The existing gap between advancement in research of biocidal properties of ZnO nanoparticles and its commercialization is the lack of information integration for optimum working conditions against specific microorganism group. The hindrance for its application is attributed by sensitivity of nanoparticles against ambient condition where it loses the nanostructure due to agglomeration or dissociation upon contact with environment.

Table 1
 Previous application of ZnO nanoparticles against different microorganisms' group

| Microorganism group | Studied microorganisms | Properties of ZnO nanoparticles | | | Study design and results | | Reference |
|---------------------|---|---|--------------------------------------|--|--|---|-----------|
| | | Size | Shape and surface modification | Dosage and microbial load | Analytical technique and treatment | Effect | |
| Enveloped virus | Herpes simplex virus type 1 (HSV-1); 52-55 nm | Length of spike: 2-8 μm ; thickness: 100-200 nm | Sea-urchin structure with nanospikes | 0.1 mgmL^{-1} ; UV illumination for 30 min; 2×10^4 cell each well) | Viral entry assay; 6 h (37 °C) | Virus attachment to ZnO micro-nano structures restricted its entry into human corneal fibroblasts | [11] |
| | Herpes simplex virus type 2 (HSV-2); 52-55 nm | Lateral diameter: 200-1000 nm; arm length: 5-30 μm | Tetrapod | | Viral entry assay for neutralization treatment; 6 h (37 °C) Viral entry assay for prophylactic treatment; 6 h (37 °C) Viral entry assay for therapeutic treatment; 6 h (37 °C) | Virus attachment to ZnO nanoparticles restricted its entry into HeLa and VK2/E6 cells | [12] |
| | H1N1; 80-120 nm | 20-50 nm | Spherical | *0.075 mgmL^{-1} (maximum non-cytotoxic concentration); 1×10^5 cellmL^{-1} *0.2 mgmL^{-1} (maximum non-cytotoxic concentration); 1×10^5 cellmL^{-1} | TCID50 assay for therapeutic treatment; 48 h (37 °C) | 1.2 \log_{10} TCID50 reduction (inhibition rate of 52.2 %) post-exposure of MDCK-SIAt1 cells within 48 h 0.7 \log_{10} TCID50 reduction (13.5 % inhibition rate) post-exposure of MDCK-SIAt1 cells within 48 h | [13] |
| | | | | | | | |
| | Tobacco mosaic virus, TMV; length: 300 nm, width: 18 nm | Average at 18 nm | Spherical | 0.1 mgmL^{-1} | Direct antiviral activity; 2 h (25 °C) | Aggregation of virus | [14] |

| | | | | | | | |
|------------------------|---|---|---|---|---|--|------|
| | | | | 5 mL foliar treatment at 0.1 mgmL ⁻¹ | Plant treatment: daily foliar spraying with ZnO; followed by quantitative polymerase chain reaction of viral cell | Significantly reduced viral multiplication | |
| | Hepatitis E (32 – 34 nm) and Hepatitis C virus (40 – 80 nm) | 5 – 50 nm | Spherical | 100 and 200 µM; 8 x 10 ⁶ genome copies of virus | 24 h incubation followed by quantitative polymerase chain reaction of viral cell; 24h | Significantly reduced viral multiplication | [15] |
| | SARS-CoV-2 (approximately 100 nm) | 50 – 1000 nm | Spherical, wire-like and rice-grain structures | 1 g/L and 10 g/L; 3634.9 ± 0.5 pg/mL antigen | 24 h incubation followed by SARS-CoV-2 Nucleocapsid protein antigen quantification | Significant reduction in antigen quantification | [16] |
| | | 30 – 82 nm | - | 5 – 20 mg/mL | Viral neutralization assays followed by quantitative polymerase chain reaction | Inactivation of viral cells by a factor of more than 10 ⁶ | [17] |
| Gram-positive bacteria | <i>Staphylococcus aureus</i> ; 500-1,500 nm | Length: 100 – 500 nm; diameter: 30-120 nm | Micro/nanorods and irregularly-shaped particles | 2.0 mM (0.163 mgmL ⁻¹); 1 x 10 ⁶ CFUmL ⁻¹ | Microdilution technique; 24 h (37 °C) | Attachment of ZnO np on cells; Bacterial cells reduction by 69 % after 8 h treatment | [18] |
| | | Length: 120-400 nm; width: 40-200 nm | Plates, slabs and irregularly-shaped particles | | | Attachment of ZnO np on cells; Bacterial cells reduction by 52 % after 8 h treatment | |
| | | - | Nanorods | Immobilized on 50 mgmL ⁻¹ LDPE film in | Plate count technique | 87.64 % bacterial reduction | [19] |
| | | - | Nanoflakes | 1.0 mgmL ⁻¹ | (Dynamic contact | 100 % bacterial reduction | |

| | | | | | | | |
|------------------------|--|--------------------------------------|---|--|---|---|------|
| | | - | Nanospheres | | condition; 1 h; 37 °C) | 100 % bacterial reduction | |
| | <i>Listeria monocytogenes</i> ; | 25-204 nm | Sphere | 1.0 mgmL ⁻¹ | Agar disk diffusion technique (24 h; 37 °C) | 10 mm zone inhibition after 24 h; growth inhibition | [20] |
| | diameter: 500-4,000 nm, length: 500-2,000 nm | 21-243 nm | Sphere | | | | |
| | <i>Streptococcus pyogenes</i> ; | Length: 100 – 500 nm; | Micro/nanorods and irregularly-shaped particles | 0.042 mgmL ⁻¹ ; 1 x 10 ⁶ CFUmL ⁻¹ | Microdilution technique; 24 h (37 °C) | Attachement of ZnO np on cells; Bacterial reduction by 84 % after 10 h treatment | [18] |
| | 500-2,000 nm | diameter: 30-120 nm | | | | Attachement of ZnO np on cells; Bacterial reduction by 84 % after 10 h treatment | |
| | | Length: 120-400 nm; width: 40-200 nm | Plates, slabs and irregularly-shaped particles | | | | |
| Gram-negative bacteria | <i>Campylobacter jejuni</i> ; | Average at 30 nm | - | 0.1 mgmL ⁻¹ | MIC technique; 24 h (42 °C) | Cell morphology destruction; 100 % killing of 10 ⁸ CFUmL ⁻¹ bacterial cell in 3 h | [21] |
| | length: 500-5000 nm, width: 200-900 nm | | | | | | |
| | <i>Klebsiella pneumonia</i> ; | Average at 159 nm | Irregular | *0.04 mgmL ⁻¹ | MIC technique; 24 h (37 °C) | Growth inhibition | [22] |
| | length: 300-1000 nm, width: 600-6,000 nm | Average at 88.7 nm | Irregular; capping agent present | | | | |
| | <i>Pseudomonas aeruginosa</i> ; | Length: 100 – 500 nm; | Micro/nanorods and irregularly-shaped particles | 0.081 mgmL ⁻¹ ; 1 x 10 ⁶ CFUmL ⁻¹ | Microdilution technique; 24 h (37 °C) | Membrane damage and cell shrinkage; Bacterial reduction by 72 % after 8 h treatment | [18] |
| | diameter: 400 nm, length: 1,200 nm | diameter: 30-120 nm | | | | | |
| | | Length: 120-400 nm; width: 40-200 nm | Plates, slabs and irregularly-shaped particles | | | Membrane damage and cell shrinkage; Bacterial reduction by 66 % after 8 h treatment | |
| | <i>Salmonella thyphimurium</i> | 25-204 nm | Sphere | 1.0 mgmL ⁻¹ | Agar disk diffusion technique (24h; 37°C) | No inhibitory effect | [20] |
| | | 21-243 nm | Sphere | | | | |

| | | | | | | | |
|--------------|--|---------------------------------------|---|---|--|--|------------------|
| | <i>Escherichia coli</i> ; width: 500 nm, length: 2,000 nm | 48.3 ± 3.5 nm | Sphere | 0.05 mgmL ⁻¹ with dual UV irradiation; 2.0 x 10 ⁴ CFUmL ⁻¹ 0.05 mgmL ⁻¹ Immobilization of dual UV-irradiated ZnO np on silicon wafer; 2.0 x 10 ⁴ CFUmL ⁻¹ | Plate count technique (24h; 37 °C) Plate count technique (24h; 37 °C) | Growth inhibition by membrane disruption and ROS generation | [23] |
| Fungi | <i>Candida albicans</i> (Yeast form: 1,000- 1,200 nm <i>Fusarium graminearum</i> | Average at 11.6 nm < 100 nm | Sphere - | *0.1 mgmL ⁻¹ 500 mgL ⁻¹ | MIC (24 h) Mycelial radial growth (7 d) | Over 95 % growth inhibition Significantly more effective in growth inhibition compared to microparticles counterpart | [24] [25] |
| | <i>Sclerotinia homoeocarpa</i> | Average at 30 nm | - | 200 µgmL ⁻¹ | Mycelial radial growth (48 h; room temperature) | Growth inhibition after 2 d of treatment | [26] |
| | <i>Erythricium salmonicolor</i> | 20-35 nm | Spherical and acicular | 9 mmolL ⁻¹ from 0.732 mgmL ⁻¹ ZnO np | Mycelial radial growth | Growth inhibition after 16 d of treatment | [27] |
| | <i>Colletotrichum gloeosporioides</i> | 53 ± 17 nm 77 ± 31 nm | Hexagonal bars Prisms with pyramidal ends | *0.156 mgmL ⁻¹ *0.312 mgmL ⁻¹ | Mycelial radial growth (28 °C) | Growth inhibition within 48 h Growth inhibition within 48 h | [28] |
| Mycobacteria | XDR stains of <i>Mycobacterium tuberculosis</i> ; length: 2,000-4,000 nm, width: 200-500 nm | 9.3 ± 3.9 nm | Sphere | *1 µgmL ⁻¹ ; 3 x 10 ⁸ CFUmL ⁻¹ | MIC (5 d; 37 °C) | Growth inhibition | [29] |

*Minimum inhibition concentration (MIC)

3. Mechanism of ZnO Nanoparticles

3.1 Antibacterial

ZnO nanoparticles disrupt the cell wall, gain entry into the cell and consequently accumulating in cell membrane to deliver their antibacterial effect [7]. Antibacterial mechanisms of ZnO nanoparticles are through the production of Reactive Oxygen Species (ROS), charge alteration of cell membrane and the dissociation of ZnO into Zn^{2+} ions [29], as shown in Figure 1.

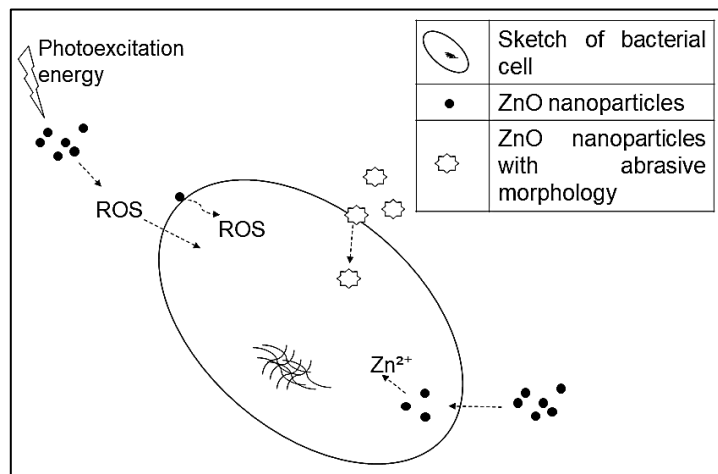


Fig. 1. Schematic representation of antibacterial mechanism of ZnO nanoparticles

Efficient photocatalytic properties of ZnO makes it sensitive to photo-induced oxidation process [30]. The chain reaction from activation of ZnO nanoparticles by ultraviolet and visible light produces radicals and hydrogen peroxide (H_2O_2) which lead to membrane destruction and cell inflammation respectively [31]. The second attack mechanism relies on the abrasiveness of ZnO nanoparticles which changes membrane permeability for the uptake of nanoparticles [32]. Bacterial biomolecules were observed to have adsorbed on surface of ZnO nanoparticles [33,34]. Morphology, crystallinity, and surface defects of nanoparticles are the determining factor of their antibacterial properties. Surface defect of ZnO nanoparticles is contributed by oxygen defect site. Previous studies indicated that oxygen vacancy sites of ZnO nanoparticles lead to generation of oxygen radicals in the dark [35,36].

Third established attack mechanism of ZnO is through release of Zn^{2+} ions which will subsequently be absorbed by the microbial cell. The amphoteric nature of zinc oxide allows it to dissolve in wide range of pH to produce Zn^{2+} [7]. Despite being a micronutrient essential for microbial growth, upon reaching its threshold concentration, zinc becomes a cytotoxic to the cells. Joe *et al.*, [37] demonstrated that the diffusion of Zn^{2+} ion into microbial cytoplasm is responsible for the antibacterial effect of both ZnO nanoparticles and $ZnCl_2$ regardless of its morphology. Subsequently, the ionized Zn^{2+} will travel into the cytoplasm and exhibit its concentration-dependent cytotoxic properties [37]. The Zn^{2+} produced from the dissolution of ZnO can diffuse through the cell membrane leading to metabolism disruption and altering charges on ion channels and active sites of enzymes [2].

3.2 Antifungal

In the recent decades, application of ZnO nanoparticles in agriculture as antifungal agent had been explored through lab scale and field application [24,26,27]. When extracellular ROS enters the living cells, it will result in an imbalance of oxidative stress, triggering series of metabolic activities and defense mechanism, and ultimately apoptosis [38]. The antifungal mechanism of ZnO nanoparticles through ROS generation had been justified by Lipovsky *et al.*, [23] where the blue light illumination enhanced antifungal effect. Meanwhile, growth inhibition of *Erythricum salmonicolor* was observed with few changes in the structure of the fungi when they are treated with ZnO nanoparticles [25]. The authors observed thinning of the hyphal fibre and a tendency for the fungal to clump besides the liquefaction of cytoplasmic content. Arciniegas-Grijalba *et al.*, [25] indicated that the uptake of ZnO nanoparticles causes *E. salmonicolor* to activate its defense mechanism by inflammatory reaction signifying disturbance to metabolic activities. The generation of ROS from inflammatory reaction accelerates fungal tissue damage. Besides ROS generation, the release of Zn²⁺ ions from ZnO nanoparticles also contributed to its toxicity [39].

From previous studies [7,23,25,37], it is observed that action mechanisms of ZnO nanoparticles against bacterial cells and fungi are similar. The antifungal mechanism of ZnO nanoparticles is mainly through the disruption of cellular structures, protein regulating macromolecular activity and deoxyribonucleic acid (DNA) replication and anti-oxidative system of the cell [39].

In addition to being a potential antifungal agent for the agricultural industry, ZnO nanoparticles had also been explored for its function as soil regenerative agent [40], fertilizer [41,42] and a potential phytotoxic agent [43]. This calls for a need to design a deployment method with dosage control to achieve desired functionality of ZnO nanoparticle as biocide.

3.3 Antiviral

The antiviral mechanisms are more complex in comparisons with other antimicrobial mechanisms due to the living characteristics of virus. The antiviral drugs could act directly on the viral cells, alternate the proteins or metabolic pathways of host cells, or a combination of both [44]. Research on antiviral mechanism of ZnO nanoparticles are relatively fewer as compared to its antibacterial and antifungal activity. To our best understanding, ZnO nanoparticles were mainly tested for their attack mechanisms against the viruses with the aim of future development as disinfectants rather than antiviral drug. In the form of nano-seaurchin and tetrapods, ZnO nanoparticles was observed to be blocking entry of herpes simplex virus type 1 (HSV-1) into cells by trapping the viruses on its surface; the effectiveness of virostatic activity was significantly enhanced with UV irradiation for the generation of oxygen vacancies on surface of nanoparticles [10]. Antoine *et al.*, [11] demonstrated that the presence of oxygen vacancies on ZnO tetrapods had led to the attachment of herpes simplex virus type 2 (HSV-2) onto the nanoparticles, thus preventing viral entry into cells. Additionally, when ZnO nanoparticles was deployed as antiviral drug against H1N1 virus, the effect was only prominent during post cellular exposure to the virus where it interfered with life cycle of virus after viral adsorption and cell internalization [12]. Ghaffari *et al.*, [12] proposed that even though the use of capping agent can reduce particle size, it can lead to significant decrement in antiviral effectiveness of the nanoparticles.

Gupta *et al.*, [14] demonstrated that ZnO nanoparticles and tetrapods could inhibit entry and replication of Hepatitis E and Hepatitis C viruses. Antiviral activity of ZnO nanoparticles with effectiveness up to 70 % against SARS-CoV-2 was reported by Sportelli *et al.*, [15]. The morphology of ZnO nanoparticles, which could affect the dissolution of the nanoparticles, was postulated to be one of the determinants for their effectiveness against SARS-CoV [15]. Wolfgruber *et al.*, [16] reported the remarkable antiviral properties of ZnO nanoparticles against the Delta and Omicron

SARS-CoV-2 variants, where the inhibition effect was substantially higher than the regulated log reduction value for virus disinfection. However, the antiviral mechanism of ZnO nanoparticles against the Delta and Omicron strains of coronavirus was not elucidated in the study.

The antiviral effect of ZnO nanoparticles was also tested against plant virus. When treated with ZnO nanoparticles suspension, the attachment of ZnO nanoparticles to the envelope glycoprotein of Tobacco Mosaic Virus (TMV) resulted in increment in particle size thus blocking entry of virus into plant cells [13]. Besides, it was also postulated that ZnO nanoparticles exhibit antiviral activity through damaging the shell protein of TMV leading to viral cell disaggregation and altering of electrostatic charge by attaching to TMV resulting to aggregation.

To summarize, the antiviral mechanism of ZnO nanoparticles is activated by attaching to the surface of virus which results in coagulation of viruses, charge alteration and damage of viral envelope causing loss of integrity.

4. Dissolution and Colloidal Stability of ZnO Nanoparticles

Nanoparticles form dispersion instead of solution when being introduced to liquid system. A zinc oxide nanoparticles dispersion is depicted as co-existence of both the particles and liquid phase as opposed to zinc oxide bulk counterpart solution which is made up by ZnO molecules dissolved from the solids. The correlation between reduction of particle size and increment in solubility is predicted by Ostwald-Freundlich equation. Thus, the dissolution rate of ZnO nanoparticles plays important role in determining its toxicity. Meanwhile, Misra *et al.*, [44] speculated that a complex suspension, composed of nanoparticles, ions derived from dissolution of nanoparticles and complexes formed as a result of ion adsorption by nanoparticles, would be form when nanoparticles are introduced into a liquid medium. The dissolution properties of nanoparticles are controlled by three major factors, which are physical properties, chemical properties and administered environment.

There is no definite trend between dissolution rate of ZnO nanoparticles and its size when surface chemistry of nanoparticles is concerned [45]. The tendency of nanoparticles to form agglomerates is the major hurdle for its dissolution. Upon dispersing into liquid medium, the nanoparticles will experience maximum dissolution rate which gradually decreases with time [46]. Vogelsberger *et al.*, [46] reported the reduction in dissolution rate was due to the secondary growth of dissolved nanoparticles into agglomerates.

It is interesting to be noted that though it is presumed that the aggregation of nanoparticles shall render its loss of nano effects, study had shown that these aggregate (flocs with size ranging from hundreds of nanometers to several microns) can exert significant toxic effect [47,48]. Zhu *et al.*, [47] highlighted that even though significant amount of Zn^{2+} was dissolved from the ZnO nanoparticles aggregates, it was not the source of toxicity effect against the zebrafish embryos. The underlying toxicity effect could be attributed to the compromised cellular oxidative stress response when it encountered the aggregates.

Surface chemistry of nanoparticles and administered water chemistry affect their colloidal stability. According to Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, colloidal stability of nanoparticles is the interplay of attractive and repulsive forces. Surface modification through electrostatic and steric stabilization had been reported to improve colloidal stability of nanoparticles [49]. In addition, particle size and chemistry of capping agent would alter point of zero charge (pzc) of ZnO nanoparticles. The pzc for ZnO nanoparticles is associated with its crystal size where smaller nanoparticles exhibited lower pzc [50]. The reduction of particle size will result to a more basic pzc [51]. Considering the effect surface chemistry had on colloidal stability, it is of great importance to

identify water chemistry of potential deployed condition to optimize dissolution rate of nanoparticles.

The effect of water chemistry as contributed by pH, ionic strength, and concentration of dissolved organic matter (DOM) on dissolution of ZnO nanoparticles were extensively studied. The water pH contributes to agglomeration of nanoparticles where the general observed pH trend was that the solubility reduced with increasing pH due to the inhibition effect of hydroxide ions on surface of ZnO nanoparticles [52-55]. However, studies had shown ZnO nanoparticles, and their agglomerates retained its toxicity when discharged into wastewater medium of different pH and conductivities [48,56,57]. In synthetic wastewater (pH 7.5), deployment of ZnO nanoparticles at 10 and 50 mgL⁻¹ exhibited inhibitory effect against activated sludge via attack mechanism of Zn²⁺ release and ROS production [56]. Zhou *et al.*, [48] reported the antibacterial activity of ZnO nanoparticles introduced into wastewater (pH 7.82 and 8.15 and conductivity at 1.503 mScm⁻¹ and 1.439 mScm⁻¹ respectively) reflected by significant reduced oxygen uptake by activated sludge for a period up to 4.5 hours. When various concentrations of nanoparticles were introduced to wastewater at initial pH of 7.2 and initial conductivity at 5.25 mScm⁻¹, toxicity of ZnO nanoparticles (6-60 nm) against bacterial microbiome was reported at the lowest tested concentration (5 mgL⁻¹) [57]. These observations justified toxicity of ZnO aggregates to the consortium of microorganisms in treatment plant.

Additionally, the stability of ZnO nanoparticles is highly dependent on the type and concentration of electrolyte in the liquid medium [55,58]. Surface charge of nanoparticles varies with its particle size and coating. In condition where ZnO nanoparticles is negatively charged, the aqueous anions play the role as co-ions and compress the electric double layers (EDL) of ZnO nanoparticles; in opposed condition, the aqueous cations would be the counter-ions which in turn neutralize the surface charge of nanoparticles while simultaneously compress its surface charge. Both aforementioned conditions assist nanoparticles aggregation [55].

Another general trend observed was the increment of dissolved organic matter would result in lower dissolution and aggregation due to the coating of organic matter on surface of ZnO nanoparticles which in turn generates bigger steric hindrance [50,53,55,59,60]. The adsorption of organic matter would impart negative charge to surface of nanoparticles; this significantly increased its EDLs which in turn reducing aggregation rate due to the energy barrier between nanoparticles [59]. Even though DOM alters surface chemistry and improves colloidal stability of nanoparticles, it will reduce antimicrobial activity of ZnO nanoparticles as reported by Prasanna and Vijayaraghavan [36] on the effect of coating agent against ZnO nanoparticles antibacterial properties.

In addition, Yung *et al.*, [51] reported that temperature and salinity could significantly alter toxicity of ZnO nanoparticles against marine diatom *Thalassiosira pseudonana*. Focusing on toxicity of Zn²⁺ released from ZnO nanoparticles, the authors demonstrated that size of ZnO aggregates increased with the increment of temperature and salinity, which was inversely proportional to the concentration of Zn²⁺ released [51]. The two reasons driving aggregation of nanoparticles under higher temperature are the reduction of solution's viscosity and the decrement in interaction energies between nanoparticles [61]. In addition, salinity leads to compression of EDLs and consequently contributed to nanoparticles aggregation [51].

5. Problems of Administration of ZnO Nanoparticles in Different Industries

In recent years, the trend of incorporation of ZnO nanoparticles in the field of agriculture and water treatment is observed. Despite its wondrous nano effects, it is undeniable that the efficacy of ZnO nanoparticles is highly dependent on its physical properties and the medium.

The investigation on the effects of chemistry of administered conditions and efficiency of ZnO nanoparticles are discussed to highlight significance of matching the material specification for maximizing its purpose. Though reputed as potential antimicrobial and phytotoxic agent, ZnO nanoparticles exhibits growth enhanced ability besides acting as micronutrient for the plants [62,63]. At a concentration lower than 50 mgkg^{-1} , ZnO nanoparticles incorporation is beneficial for the plants [63]. The application of nano fertilizers encounters several problems, namely material loss into atmosphere due to its light weight from direct application of dry powders, obstruction to efficient delivery due to particle adhesion from deep placement into soil and negation of nano effect due to dissolution and aggregation from application in aqueous form [64]. The mobility and transformation of ZnO nanoparticles depends on the soil chemistry. Soil chemistry will affect dissolution, transformation, and spatial distribution of ZnO nanoparticles [63,65,66]. When employed as nanofertilizer, ZnO nanoparticles is expected to exhibit higher solubility and release of Zn^{2+} as fertilizer as opposed to other microstructure Zn sources which are prone to transforming into less available precipitates upon administration to the soil [66].

Milani *et al.*, [67] reported incorporation of ZnO nanoparticles (1.5 %) in granular macronutrient fertilizers of different acidity will result in significant difference in dissolution rate, where solubility is higher in acidic medium (pH 4.8) than alkaline medium (pH 7.6). With the zeta potential of ZnO nanoparticles reported at +15.7 mV at pH 8, it was deciphered that the increment in solubility at pH 4.8 was due to the greater effect on electrostatic stability of nanoparticles as affected by ionic strength in surrounding medium. When ZnO nanoparticles ($67 \pm 2.0 \text{ nm}$; $46.1 \pm 1.5 \text{ mV}$) was deployed in acidic soil (pH 5.0), complete dissolution was observed with no nanospecific effect to cowpea plant [52].

Dimkpa *et al.*, [24] highlighted the problems with deployment of ZnO nanoparticles as antifungal agent (in vitro) where the aggregation rate of nanoparticles differed significantly when dispersed in mung bean broth and in water. The cations and organic substances present in mung bean broth modified dissolution and aggregation state of the nanoparticles [24]. The pH and ionic strength of soil would manipulate dissolution of ZnO nanoparticles when employed as antifungal agent against *F. graminearum* [24,40]. Moghaddasi *et al.*, [68] reported higher ZnO nanoparticles availability ($+3.1 \text{ mV}$) in cow manure-treated soil (pH 5.6 ± 0.3) than untreated soil (pH 6.8 ± 0.2). The research enlightened on the importance in pH manipulation of surrounding medium with reference to pzc of nanoparticles to achieve optimum dissolution.

Alteration of surface chemistry by coating agent would change the pzc of nanoparticles. The acidity of soil affects solubility of ZnO nanoparticles where humic acid-like materials in acid soil electrostatically stabilize ZnO nanoparticles and prevent the aggregation [40]. Urea is known to be a polar molecule; when deployed as coating material, it improves steric stability of nanoparticles. When employed in alkaline calcareous soil, urea coated ZnO nanoparticles could be retained in its zincite form, however, Zn availability and solubility was not improved [66]. Similarly, Dimkpa *et al.*, [64] evaluated urea-coated ZnO nanoparticles could eliminate the problem of particle-size dependent segregation for the application of nanoparticles fortified fertilizer, however, the coating method was insufficient to improve efficacy of ZnO nanoparticles. Similarly, when coated with triethoxyoctylsilane, a non-polar stabilizing agent, the solubility of ZnO nanoparticles in loamy sand soil ($\text{pH}_{\text{CaCl}_2} 5.5$) was significantly reduced [69].

Additionally, under simulated drought condition (40 % field moisture capacity), fertilization with ZnO nanoparticles (2.17 mgkg^{-1} ; employed at half the dosage of bulk ZnO) was observed to alleviate drought stress, increase chlorophyll content, and accelerate panicle emergence of winter wheat [42,64]. Previously, Milani *et al.*, [66] reported that the hygroscopicity of fertilizer would result in mass flow of water in the direction towards the fertilizer thus restricting the diffusion of Zn for plant

uptake. Under simulated drought condition, it was postulated that despite the mass flow of water towards soil, higher dissolution rate of ZnO nanoparticles as opposed to its bulk counterpart would improve yield of agriculture product.

The advantages of nanoparticles deployment in water remediation against conventional physical and chemical treatments, namely chlorination, ozonation and UV treatment, are the ability to remove finest contaminants (<300 nm) from water and the removal, transformation and immobilization of environmental pollutants and pathogenic microorganisms through modification of nanomaterials [70]. The photocatalytic activity of ZnO nanoparticles, known as advanced oxidation process (AOP), facilitates pollutant degradation in wastewater treatment through generation of reactive hydroxyl radicals [71,72]. Other than its redox reaction, nanoparticles are also employed for adsorption process of pollutants [73]. Decentralized and point-of-use water treatment system was envisioned with the application of antimicrobial nanomaterials [74].

When deployed for contaminant degradation, nanoparticles shall exhibit high colloidal stability and remain in nanostructure throughout the reaction. Owing to lower reactivity of ZnO in visible light range, its application in water remediation is improved with modification by doping. Doping on ZnO surface could prevent the recombination of photogenerated hole and electron for sustainable photocatalytic reaction [70,71].

To our best understanding, present studies focus on the effect of ambient condition on ROS generation by nanoparticles with lesser concern on its colloidal stability. It was known the ROS generation efficiency of ZnO nanoparticles in water treatment is relied on substrate concentration, pH and temperature [71]. The absorption of organic matter on ZnO surface would reduce the active site for the adsorption of hydroxyl ions for the generation of hydroxyl radical [71,75]. In addition, there shall be increment in photodegradation efficiency in alkaline solution due to greater availability of OH⁻ ion for the generation of hydroxyl radicals; however, there is possibility that the electric repulsion between O polar of ZnO and OH⁻ will reduce their contact thus hindering ROS generation [75]. Besides its photocatalytic properties, nanoparticles are deployed as adsorbent in water treatment as well. Function of nanoparticles as chemical adsorbent depends on its surface charge; ionic bonding formed between ZnO nanoparticles and dye leads to dye removal [76,77].

The identified issue with direct deployment of nanoparticles in water treatment is the difficulty for its removal after application and its colloidal stability against aggregation [70,72]. Fragalà *et al.*, [72] reported the efficacy of photodegradation of methylene blue through growing of ZnO nanorods on ultrathin ZnO seed layers – atomic layer deposition method [72]. Even though the deposition method was promising for recovery and reuse in addition to the increment in colloidal stability of ZnO, water dispersion effect to ZnO nanoparticles was not tested.

Although current research in the field of photocatalytic degradation for water treatment primarily focus on nanoparticles efficiency by tuning its band gap [78,79], consideration of pH and ionic strength of administered condition is essential to maximize its colloidal stability for optimum function.

6. Improved Delivery Techniques for ZnO Nanoparticles

Metal oxide nanoparticles might dissociate into its metallic ions in event where colloidal stability is disturbed, resulting to the negation of its nano effects. Encapsulation of nanoparticles is explored to enhance delivery of nanoparticles. With encapsulation, the core material could be immobilized thus minimizing the interaction between nanomaterial and the administered environment.

In recent years, immobilization of nanoparticles in a polymeric network had been attempted to resolve problems associated with agglomeration and settling of nanoparticles when deployed in

liquid medium. In addition, it will improve delivery efficiency, ease deployment, and reduce environmental risk [80,81]. ZnO nanoparticles had been encapsulated in calcium alginate beads for the purpose of pollutant adsorption and inactivation of microorganisms in water treatment [82-85].

The major challenge with immobilization of metal oxide nanoparticles in calcium alginate is its photocatalytic nature. There is a contradiction with the use of calcium alginate in nanocomposite since dye removal by photocatalytic metal oxide nanoparticles is enhanced by UV light, however, exposure to UV might lead to degradation of calcium alginate polymer [82,83]. However, this condition was not observed in the studies reported by Isik *et al.*, [84] and Balici *et al.*, [85] where reusability of nanocomposites was vouched. The contradicting observations were due to the different photocatalytic approaches undertaken by the researchers, where Sirtori *et al.*, [82] and Lam *et al.*, [83] employed stationary UV irradiation for 60 minutes and 24 hours respectively while Isik *et al.*, [84] and Balici *et al.*, [85] irradiated the nanocomposites at wavelength of 365 nm with flow rate of 150 mLmin⁻¹. Even though alginate matrix is sensitive to photodegradation, the degradation tendency depends on various factors, which are wavelength of UV radiation and pH of ambient condition. It is worth noting that Lam *et al.*, [83] reported that incorporation of nanoparticles had significantly slowed degradation process of alginate beads as opposed to the blank counterparts.

Encapsulation of ZnO nanoparticles in alginate beads for water disinfection had been explored in the application of alginate nanocomposites [76,86,87]. Zhang *et al.*, [76] reported the beneficial effect of ZnO alginate nanocomposite in preventing contamination in biological wastewater treatment plant. The nanocomposite was deduced to play dual function both as biomass carrier, which serves as growth template for the treatment microbes (*Mycelium purpureus*), and as antibacterial agent against *E. coli* and *S. aureus* [76]. Motshekga *et al.*, [86] showed that alginate beads of ZnO nanoparticles could effectively inactivate *S. aureus* with minor leaching of Zn²⁺ into the medium. Motshekga *et al.*, [86] deduced that the alginate nanocomposite acted both by releasing Zn²⁺ and as attachment surface for bacterial disinfection. Baek *et al.*, [86] suggested promising industrial use of the alginate nanocomposite as disinfectant for water treatment for its reusability and antimicrobial property with low leaching of ZnO nanoparticles into liquid. The carboxylic functional group of alginate polymer serves both as adsorption site for the dissociated Zn²⁺ ions and attachment site for ZnO nanoparticles as it exerts electro-steric effect to prevent aggregation of nanoparticles. The pH and viscosity of sodium alginate solution was postulated to be the manipulating factors for colloidal stability of nanoparticles [87].

In addition to serving as contaminant removal and antimicrobial agent, Gautam *et al.*, [88] devised an innovative application of ZnO incorporated alginate beads in the field of animal husbandry where it was used to reduce gaseous emission from swine manure. Physical sorption and chemical conversion of hydrogen sulfide (H₂S) was proven through formation of zinc sulfide in the beads because of chemical reaction between the emitted gas and ZnO nanoparticles [88]. ZnO nanocomposite was tested to be a more effective agent for the treatment against gaseous pollutant when compared to silver nanocomposite [89]. In addition, Immobilization of ZnO nanoparticles in polymer matrix is applicable in the field of biomedical science. Halanayake *et al.*, [90] reported the feasibility of microencapsulation of ZnO nanoparticles in chitosan and cellulose for the use in pharmaceutical industry for its improved controlled release properties. The studies explored possibility of expanding the application of ZnO nanocomposites in various fields.

Hassan *et al.*, [91] reported the deployment of alginate-polyvinyl alcohol polymer blend as the immobilization carrier for ZnO nanopowder for the application as adsorbent of a mono-azo-basic dye. The polymeric beads possessed strong mechanical strength, chemical stability in acidic media and efficacy in decolorization of aqueous solution tainted with C.I. basic blue 41. As observed, there was slightly different in crystalline profile and chemical profile of immobilized ZnO nanoparticles

compared to its unbounded nanopowder [91]. This might be due to considerable interaction between the employed polymers and the nanoparticles. Pulit-Porciak *et al.*, [92] reported significant antifungal inhibition activity of ZnO nanocomposite prepared with polyvinyl alcohol with addition of different stabilizers – gelatine, guar gum and hydroxycellulose, using one-step gelation method. Even though the authors reported agglomeration of ZnO nanoparticles, from range between 150 and 500 nm to range varied from 232 to 692 nm, the nanocomposite retained its antimicrobial properties after its incorporation [92].

7. Conclusions

ZnO nanoparticles shows promising traits to be employed as active ingredient in biocidal products. To date, studies have shown its supreme antimicrobial properties with wide spectrum effectiveness against Gram positive and Gram-negative bacteria, fungus and viruses. Antimicrobial strength of ZnO nanoparticles depends on the intricate relationship between physical and chemical properties of the nanoparticles, the physical state it is administered and the chemistry of ambient condition to which it is deployed.

Processing parameters have been manipulated during nanosynthesis to maximize antimicrobial strength of ZnO nanoparticles. The nanostructure of ZnO nanoparticles could be modified with structure-directing agent during wet chemical synthesis. In addition, oxygen vacancy sites which enhances antibacterial properties of ZnO nanoparticles could be created during calcination of intermediate nanosynthesis product.

The administered condition has to be emphasized to enhance performance of ZnO nanoparticles. Material loss due to the light weight of ZnO nanoparticles would happen when it is administered in powder form. Moreover, when deployed in liquid environment in its powder form, agglomeration of ZnO nanoparticles negates the nano effects, thus reducing antimicrobial strength.

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