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Detrimental effects of commercial zinc oxide and silver nanomaterials on bacterial populations and performance of wastewater systems

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ABSTRACT

The widespread use of commercial nanomaterials (NMs) in consumer products has raised environmental concerns as they can enter and affect the efficiency of the wastewater treatment plants. In this study the effect of various concentrations of zinc oxide NMs (nZnO) and silver NMs (nAg) on the selected wastewater bacterial species (*Bacillus licheniformis, Brevibacillus laterosporus* and *Pseudomonas putida*) was ascertained at different pH levels (pH 2, 7 and 10). Lethal concentrations (LC) of NMs and parameters such as chemical oxygen demand (COD) and dissolved oxygen (DO) were taken into consideration to assess the performance of a wastewater batch reactor. Bacterial isolates were susceptible to varying concentrations of both nZnO and nAg at pH 2, 7 and 10. It was found that a change in pH did not significantly affect the toxicity of test NMs towards target bacterial isolates. All bacterial species were significantly inhibited ($p < 0.05$) in the presence of 0.65 g/L of nZnO and nAg. In contrast, there was no significant difference ($p > 0.05$) in COD removal in the presence of increasing concentrations of NMs, which resulted in increasing releases of COD. Noticeably, there was no significant difference ($p > 0.05$) in the decrease in DO uptake in the presence of increasing NM concentrations for all bacterial isolates. The toxic effects of the target NMs on bacterial populations in wastewater may negatively impact the performance of biological treatment processes and may thus affect the efficiency of wastewater treatment plants in producing effluent of high quality.

1. Introduction

Water is an essential, sustainable and renewable substance for all life on earth. However, due to the alarming contamination of this precious resource and degradation of its quality and quantity, many people lack access to safe clean water. In developing countries, this situation is exacerbated by the lack of adequate sanitation facilities and consequently results in the death of 1.8 million people every year from diarrhoeal diseases (including cholera) where 90% are children under 5 years of age (Schwarzenbach et al., 2006). Noticeably, anthropogenic contaminants are continuously entering water systems, ranging from heavy metals to emerging micro-pollutants (endocrine disrupters and nitrosamines). Recently, the emergence of nano-enabled materials has inevitably raised public concerns over human health and the environment (Schwarzenbach et al., 2006; Snyder et al., 2003). There have been rapid advances and emerging technologies in nanoscale systems, particularly nanomaterials, and the incorporation of nanomaterials (NMs) in products utilised for environmental remediation, water purification, paints, consumer products, food packaging and medical purposes, is expected to proliferate over time (Kaegi et al., 2013).

Nanomaterials with at least one dimension in the size range ≤100 nm, as defined by the Royal Society, are increasingly being used in commercial applications for various purposes due to their unique physicochemical properties that are useful in many applications in comparison to their larger counterparts (Savolainen et al., 2010). While many NMs are still reportedly in their development stages, some commercial dry powder NM suspensions such as silver (Ag), titanium dioxide (TiO_2), carbon nanotubes (C_{60}) and zinc oxide (ZnO) (Zhang et al., 2007) are actually applied in a wide spectrum of consumer products and health care (Yuan et al., 2013). Implemented fully, these nano-enabled technologies have the ability to assist in issues such as water sanitation, treatment of disease, information technology, energy, environmental science, medicine, homeland security, food safety, and transportation (Tedesco and Sheehan, 2010; Petersen et al., 2014; Westerhoff et al.,

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2013). Consequently, they have attracted a great deal of attention to large technological and economic growth, and investment currently at billions of US$ is projected to reach trillions of US$ (Savolainen et al., 2010).

In spite of numerous advantages cited, the question remains as to whether the benefits of this technology outweigh its risks (Colvin, 2003) towards human health and safety (Baalousha et al., 2010). It has been reported that one of the concerns of NMs application at a cellular level is their potential toxicity, especially if they build up in the water supply or enter the food chain (Tedesco and Sheehan, 2010; Colvin, 2003; Neal, 2008). These concerns are unquestionably raised due to the inherent properties of NMs, which can lead to a higher possibility of interacting with biological surroundings (Garcia et al., 2012) and having toxic effects such as inflammation, genotoxicity, autophagy, neurotoxicity, and ultimately cell death (Zhang et al., 2007).

Previous studies have been carried out on the application of NMs, risk assessment and toxicity research and they have gained great momentum to understand the health implications of these NMs (Schulte et al., 2008; Savolainen et al., 2010). The toxicity of several metallic NMs has been attributed to both their size and to the released metallic ion (Thwala et al., 2013). However, the dispersed dry powder NMs could not be kept at a scale within the nanometre range as aggregation plays a significant role, including through their surface properties, size, presence of other constituents and environmental conditions (pH and ionic strength), (Kaegi et al., 2013; Thwala et al., 2013; Tso et al., 2010). Therefore, an understanding of the stability of nZnO and nAg especially in an aquatic environment is essential as aggregation of NMs can decrease their toxicity. According to Labille and Brant (2010), toxicity of NMs is not only influenced by their physicochemical properties and their chemical stability, but their dispersion and mobility in a given medium to determining their fate and behaviour. It was shown that particles tend to agglomerate with increased ionic strength and change of pH (Tso et al., 2010; Bian et al., 2011).

It has been reported that highly toxic NMs are intentionally or unintentionally finding their way every day into wastewater treatment facilities and end up in the aquatic environment (Brar et al., 2010). Based on their potential environmental toxicity, their presence in the wastewater treatment system is a matter of great concern as their impact in such systems is largely unknown and yet to be understood. Therefore, understanding how NMs such as zinc oxide (nZnO) and silver (nAg) affect vital microorganisms in biological wastewater treatment plants is thus essential to properly evaluate their risks (Tedesco and Sheehan, 2010; Brar et al., 2010). During the biological wastewater treatment, microorganisms are essential as they are known to participate in the detoxification process to produce a high-quality effluent (Wagner et al., 2002). However, at high NM concentrations, single-cell microorganisms are the first to be affected and this has noxious effects on these tiny organisms by inhibiting their growth, and reducing their cell densities and species richness (Colman et al., 2013; Yang et al., 2013).

To date, several studies have reported the antibacterial activities of nZnO and nAg toward *Escherichia coli* (Zhang et al., 2007; Adams et al., 2006), *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Bacillus subtilis* and *Staphylococcus aureus* (Adams et al., 2006). At the laboratory-scale bioreactor level, it has been further shown that the silver nanomaterials can change the microbial population structure, effectively inhibiting nitrification activity and causing damage of the cell wall of *Nitrosomonas europaea* (*N. europaea)* and DNA condensation and disintegration of the nuclear membrane and nucleoids (Choi and Hu, 2008; Yuan et al., 2013). These results indicate that nitrifying bacteria are more sensitive compared to the heterotrophic bacteria, resulting in a decrease in the removal of nitrogen and other nutrients (Yuan et al., 2013).

Bacterial species such as *Bacillus licheniformis, Brevibacillus laterosporus* and *Pseudomonas putida* are commonly found in soil, water, plants and elsewhere in the human environment and have shown tolerance to harsh environmental conditions. For instance, *Brevibacillus laterosporus* species were selected due to their ability to biodegrade and decolour commercial textile dyes in wastewater prior to its disposal (Gomare and Govindwar, 2009). The *Bacillus licheniformis* have been shown to recover and concentrate the alkaline protease activity of fermented wastewater sludge with maximum extracellular activity to reduce the cost of enzyme (Bezawada et al., 2010, 2011). Similarly *Pseudomonas putida* have been used as a biosensor to assess acute toxic in wastewater and to reduce industrial compounds such as chromate (Ishibashi et al., 1990). Therefore, the increasing concentration of nZnO and nAg could reduce the effectiveness of biological processes in wastewater treatment plants (Madoni et al., 1996). Given the potentially serious health and environmental risks and social implications associated with the use of nZnO and nAg, the aim of this laboratory-scale batch study was to evaluate the accumulation toxic effects associated with nZnO and nAg suspensions towards selected bacterial species (*Bacillus licheniformis, Brevibacillus laterosporus* and *Pseudomonas putida*) at different pH values using modified mixed liquor wastewater samples.

2. Materials and methods

2.1. Selection of test organisms

Three bacterial isolates commonly found in surface water, soil and wastewater, namely *Bacillus licheniformis*-ATCC12759, *Brevibacillus laterosporus*-ATCC64 and *Pseudomonas putida*-ATCC31483 (Quantum Biotechnologies, Strydompark, Randburg, South Africa) were prepared as outlined in standard methods (APHA, 2001). For experimental purposes, separate flasks containing 100 mL of sterile nutrient broth (NB) were aseptically inoculated with a 100 μL aliquot of each bacterial culture and thereafter aerobically incubated in a shaking incubator (Scientific Model 353, Lasec South Africa) at 30 °C for *Brevibacillus laterosporus* and *Pseudomonas putida* and at 50 °C for *Bacillus licheniformis* at 120 r/min for 24 h prior to being exposed to various concentrations of test NMs.

2.2. Characterization of commercial nanopowders

Zinc oxide nanopowder (544906, Lot # MKBD9523V) and silver nanopowder (576832, Lot # MKBN3581V) were purchased from Sigma-Aldrich (Johannesburg, South Africa). To facilitate an understanding of their behaviour, NMs were characterized prior to their usage for experimental purposes, in terms of their shape, size and actual primary surface area using JEOL JEM-2100 high-resolution transmission electron microscope (TEM) and JEOL JSM-6480 LV scanning electron microscope (SEM) images. Actual surface areas were determined using the Brunauer-Emmett-Teller (BET) method.

2.3. Wastewater profile and preparation of modified mixed liquor media

Wastewater samples were collected from the aerated tanks of the Daspoort Wastewater Treatment Works (WWTW) (Pretoria, South Africa). To remove biomass and suspended solids, samples were allowed to settle for 2 h and thereafter filtered using 0.45 μm pore size Whatman No.1 filter papers. The water sample profile was determined by detecting the presence of other trace metals using inductively coupled plasma/optical emission spectrometer (ICP/

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