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Review

Potential impacts of silver nanoparticles on bacteria in the aquatic environment

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ABSTRACT

It is inevitable that nano-silver will be released into the environment. Therefore, there is an urgent need to better understand the effects of silver nanoparticles (Ag-NPs) on microbes in natural and engineered environments. The most remarkable gap in our knowledge on this lies on the low Ag-NPs dose side. This review summarized studies on the effects of Ag-NPs on bacteria from simple to complicated aquatic systems. A hormetic model with a narrow stimulatory zone has been proposed based on both experimental phenomenon and the potential mechanisms of the observed effects. Spectrum of the stimulating zone depends on Ag-NP properties, bacterial types and environmental conditions tested. This may become a concern in terms of Ag-NP disposal, and further research is required to build a sophisticated toxicity model for Ag-NPs.

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1. Introduction

Nano-silver has been the most commonly used nanomaterial in consumer products (Project-on-Emerging-Nanotechnologies, 2014) since the boom of nanotechnology in commercial products. It is inevitable that nano-silver will be released into the environment (Benn and Westerhoff, 2008; Hagendorfer et al., 2010). This raises a question: What are the effects of silver nanoparticles (Ag-NPs) on microbes in the environment and biological wastewater treatment processes? Considerable efforts have been made to

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answer this question and research has shown that the effects of Ag-NPs depend on the dose, the time period applied, the property of Ag-NPs (size, shape and coating, etc.), and the system to which Ag-NPs are applied. The system can vary from pure culture to complicated engineered ecosystems.

However, substantial controversy exists on how each of these parameters affects the impact of Ag-NPs, and a sophisticated toxicology model has not been built. Previous research covers only a tip of the iceberg of all possible combination of these parameters. Not to mention that the mechanisms behind the phenomena are poorly understood. In recent years, more and more studies tend to focus on long-term effects of Ag-NPs under real-world conditions, i.e. relatively low concentration of Ag-NPs and presence of all kinds of ligands.

While the antibacterial activity under sufficient concentration is the major application of Ag-NPs, the most noteworthy gap in our knowledge concerns the effects of Ag-NPs under sublethal concentration. Experiments testing the hormetic effects of many antibiotics under sublethal concentration date back to the late 1890s, although the concept has stayed marginalized so far (Calabrese, 2001, 2002; Hayes, 2007). Stimulatory bacterial response to low dose Ag-NP treatment has been detected occasionally but often overlooked. Therefore, it worth to think about the question now: In biological wastewater treatment, does the response of microbes to Ag-NPs confirm with the hormetic model as these antibiotics do?

This review summarizes studies on the effects of Ag-NPs on bacteria from simple to complicated systems. Based on previous research, a hypothesis about the effects of Ag-NPs under low dose is presented and a theoretical model is proposed. The conclusion is rationalized based on both experimental phenomenon and the potential mechanisms of the observed effects.

2. Tests based on bacteria single strains

2.1. Dosage

Most of the research on single strain bacteria was performed before 2012 and most Ag-NP concentrations tested in these studies are above 1 mg/L (all concentration are based on silver). Model single strains tested include: model gram-negative strain: *Escherichia coli* (*E. coli*), model gram-positive strain: *Staphylococcus aureus* (*S. aureus*), model ammonia-oxidizing bacterium: *Nitrosomonas europaea* (*N. europaea*), and model biofilm formation bacterium: *Pseudomonas fluorescens* (*P. fluorescens*). Table 1 summarizes the reported dose response of bacteria strains to Ag-NPs. Most experiments were carried out in aqueous suspension and the detection methods were often optical density (OD), plate counting, inhibition zone or activity observation. These results demonstrate that the effects of Ag-NPs are dose-dependent, and higher concentration usually lead to more severe adverse effects. It should be noted that microbial stimulatory response after Ag-NPs treatment has also been observed (Fabrega et al., 2009b, Xiu et al., 2012; Yang et al., 2013; Cui et al., 2015; Jin et al., 2015). Several studies demonstrated the over growth of microbes under sublethal Ag-NPs dosage conditions (concentration often below 1 mg/L). These stimulatory responses of Ag-NPs imply a hormetic model type response of bacteria towards Ag-NPs (Calabrese, 2002). The hormetic model, as shown in Fig. 1, is often used to describe the dose response of bacteria to antibiotics. This model may also be applied for both pure and mixed bacteria cultures to describe bacterial dose response to Ag-NPs in natural and engineering aquatic environment. The shift of the dose-response curve depends on the population types.

2.2. Nanoparticle properties

Over 1000 articles have been published on the effects of nanoparticles on bacteria. Over 90% of these studies were done after 2008 when the methods to synthesize silver nanoparticle became mature and various methods and reagents were applied to generate Ag-NPs, producing Ag-NPs with various shapes, sizes and coating. In 2015, over 60% of papers in this area are about biosynthesized Ag-NPs and significant antibacterial activity were usually detected at concentration higher than 10 mg/L, which may indicates that biogenic coating reduces the toxicity of Ag-NPs (Mittal et al., 2015; Bose and Chatterjee, 2015; Ramesh et al., 2015). Pal et al. reported that testing with *E. coli* the Ag-NPs antibacterial effects from the strongest to the weakest followed the order truncated triangular > spherical > elongated (rod-shaped) > Ag^+ ion (Pal et al., 2007). El-Zahry et al. reported a different order: hexagonal > spherical > triangular. However in this study, different shapes corresponded with different surface coatings in their study, thus it is hard to tell which factor played a more important role (El-Zahry et al., 2015). However, it should be noted that many other research claimed that Ag^+ ion has stronger antibacterial effects than Ag-NPs at the same concentration, which will be discussed later in this review.

It is widely accepted that smaller Ag-NPs are more toxic (Morones et al., 2005; Lok et al., 2007; Zhang et al., 2008; Choi and Hu, 2008; Ivask et al., 2014; Matzke et al., 2014). Several studies done with *N. europaea* showed that smaller Ag-NPs released more Ag^+ ion and thus were more toxic and the smaller size came from better dispersity (Radniecki et al., 2011; Yuan et al., 2013). Surface coating affects the toxicity of Ag-NPs because it plays an important role in determining the dispersity and size of Ag-NPs and Ag^+ ion dissolution. Jin et al. tested four types of fluorescent carbon dots (CDs) as reducing and stabilizing agents to synthesize Ag-NPs. They found that CDs doped with sulfur could result in smaller Ag-NPs and higher bactericidal activity (Jin et al., 2015). Arnaout and Gunsch compared three types of coating: citrate, gum arabic (GA), and polyvinylpyrrolidone (PVP). At a concentration of 2 mg/L, citrate resulted in the highest Ag^+ ion dissolution and Ag-NPs with GA coating had the smallest size. Citrate and GA coated Ag-NPs caused 67.9 ± 3.6% and 91.4 ± 0.2% inhibition of nitrification, respectively (Arnaout and Gunsch, 2012). Kvítek et al. tested several kinds of coating and found out that sodium dodecyl sulfate-SDS, polyoxyethylenesorbitane monooleate-Tween 80 and PVP 360 had superior Ag-NP stabilization ability which resulted in an minimum inhibitory concentration (MIC) under 1 mg/L (Kvítek et al., 2008). Combination of Ag-NP with antibacterial coating can also lower MIC. Myramistin® stabilized Ag-NPs have a lower MIC than citrate capped ones (Vertelov et al., 2008). Jain et al. showed that bare Ag-NPs were more toxic than Ag-NPs with protein coating (Jain et al., 2015). Given similar sizes, surface charges play a role in the bactericidal activity of Ag-NPs, from the highest to the lowest following the order positively-charged > neutral > negatively charged (Abbaszadegan et al., 2015).

2.3. Treatment conditions

Similar to other disinfectants, the contact time adopted affects the antibacterial effects of Ag-NPs. Longer time results in more inhibition/killing (Smetana et al., 2008; Kumar et al., 2014). pH and ligands can also affect the toxicity of Ag-NPs. pH can affect Ag^+ ion dissolution; although within the bacterial exposure range (typically pH 6–9), this effect is moderate and does not affect toxicity significantly (Fabrega et al., 2009a). Inorganic ligands such as Cl^- could function as scavengers and increase bacteria survival (Smetana et al., 2008). Tested with *N. europaea*, Mg^{2+} and Ca^{2+}