Mechanisms of oxide copper nanoparticles toxicity to microorganisms and ammonia-oxidizing bacteria and recommendations for future study

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Abstract. In recent years, the biological toxicity of oxide copper nanoparticles (CuO NPs) to microorganisms has received some attentions. The toxicity mechanism of CuO NPs can be concluded as follows: (1) CuO NPs facilitate more rapid dissolution of ions than equivalent bulk material, which potentially leading to increased toxicity of CuO NPs; (2) CuO NPs can generate reactive oxygen species (ROS), and potentially disturb the functioning of protein, enzymes and DNA; (3) CuO NPs have high capacity to adsorb biomolecules and interact with biological receptors, they can reach sub-cellular locations leading to potentially higher localized concentrations of ions once those particles start to dissolve or degrade in situ. Ammonia-oxidizing bacteria (AOB) are the main functional bacteria for biological nitrogen removal in the environment. It has been reported that CuO NPs can change the community structures of AOB, however, the toxicity mechanism of CuO NPs to AOB in cytological level is rarely reported. This review aims to evaluate the current understanding of CuO NPs toxicity to microorganisms, as well as to provide a set of pointers and guidelines for future studies to access the toxicity mechanism of CuO NPs to AOB.

1. Introduction

In the past two decades, the production and application of nanomaterial have developed rapidly. Meanwhile, the ecological safety of nanomaterial has been received increasing attention. Oxide copper nanoparticles (CuO NPs) are typical nanomaterials which are widely used in the fields of preparing photochemical catalysts, biosensors, and antibacterial agents due to their catalytic, optical, and bacteriostatic effects [1]. In recent years, global CuO NPs consumption has been increased gradually [2]. According to reports, nearly half of the copper in the semiconductor manufacturing industrial wastewater is in the form of CuO NPs, with a concentration of up to 50 mg / L [3]. A large amount of CuO NPs flow into the sewage treatment system and are finally discharged to the excess activated sludge, natural water bodies, farmlands and other places. Therefore, there are great exposure risks to environmental microorganisms.

Ammonia-oxidizing bacteria (AOB) which have multiple nitrogen metabolism pathways are the main functional bacteria of biological nitrogen removal in wastewater, marine and soil. Under aerobic conditions, AOB can gradually oxidize ammonia nitrogen (NH3-N) to hydroxylamine (NH2OH) and then nitrite nitrogen (NO2-N), which is the main step of nitrification-denitrification process and...
shortcut nitrification-denitrification process. Under anaerobic conditions, AOB can reduce NO$_2$-N to N$_2$O causing about 300 times stronger greenhouse effect than CO$_2$ [4-5]. Therefore, AOB play an important role in the environment. Researchers have been focusing on environmental conditions such as pH, dissolved oxygen concentration, and temperature on AOB activities [6-7], and found that AOB were extremely sensitive to environmental conditions. In recent years, the biological toxicity of CuO NPs to AOB has received some attentions.

In this paper, recent advances in the biological toxicity of CuO NPs to microorganisms especially AOB are reviewed. The purpose of this study is to supplement and enrich the theory of the biological toxicity mechanism of CuO NPs to microorganisms, and further provide the theoretical basis for promoting biological nitrogen removal under nanomaterial pollution.

2. The advances of CuO NPs toxicity to microorganisms

2.1 The mechanism of CuO NPs toxicity to microorganisms

At present, there is no unified conclusion about the toxicity mechanism of CuO NPs to organisms, and there are even contradictions.

Baeck et al [8] found that the toxicity of CuO NPs to Escherichia coli and Bacillus subtilis increased with the increase of CuO NPs concentration in the culture medium, while the concentration of dissolved Cu$^{2+}$ decreased gradually. Thus, it was inferred that the toxicity of CuO NPs to microorganisms was mainly caused by the CuO NPs aggregates. Moreover, the stability of aggregates (mainly the dispersion and aggregation of particles), the binding force between aggregates and cell membrane will affect the toxicity.

Clar et al [9] pointed out that Cu$^{2+}$ and Cu$^+$ releasing from CuO NPs dissolution undergo fenton-like chemical reactions, producing a large number of oxidative free radicals (ROS) which can damage the cell respiratory chain and membrane structure, and cause DNA cleavage. Therefore, it was considered that Cu$^{2+}$ dissolved by CuO NPs is the main factor causing the cytotoxicity of CuO NP.

Some researchers state that the nanoscale effect of CuO NPs is the main cause of CuO NPs cytotoxicity [10-11]. Because CuO NPs have high capacity to adsorb biomolecules and interact with biological receptors, they can reach sub-cellular locations leading to potentially higher localized concentrations of ions once those particles start to dissolve or degrade in situ. This process is also called “Trojan horse mechanism”.

Furthermore, the chemical morphology of copper released from CuO NPs was also reported to have a significant effect on the cytotoxicity of CuO NPs. Copper ions can be transformed into different chemical forms through methylation, adsorption, and complexation, and the conversion products will directly affect the bioavailability and conversion rate of copper [11]. Studies have found that free copper ions and unstable copper complexes are more toxic than strongly bound copper complexes [13]. However, there are few reports on the biotoxicity of copper in different chemical forms.

2.2 The resistance mechanism of microorganisms to metal nanoparticles

At present, the resistance mechanism of microorganisms to metal nanoparticles is mainly divided into extracellular and intracellular mechanism. For the extracellular mechanism, the extracellular polymers (EPS) can block the metal nanoparticles outside the cell through complexion, precipitation, ion exchange and electrostatic adsorption with metal nanoparticles. It is reported that the EPS secreted by the cell membrane has a strong adsorption effect on the nanoparticles and can reduce the toxicity of nanoparticles by changing the agglomeration effect of nanoparticles [14]. As for the intracellular mechanism, firstly, there are various negative charge functional groups on the cell membrane, such as the carboxyl group and phosphoric acid group. These groups have a strong affinity for metals and can bind some metal nanoparticles in their transmembrane process, thus reducing the amount of metal nanoparticles invading into the cell [15]. Secondly, some proteins on the cell membrane act as efflux pump, which can drive the metal ions released by the nanoparticles to the outside of the cell [16]. It
has been reported that there are two types of efflux pump, one is the P-type ATP pump which can generate energy by ATP hydrolysis to output the intracellular metal ions to the cell periplasmic space [17]. The other is the resistance-nodulation division (RND) pump, which can discharge the metal ions outside of the cell from the periplasmic space.

2.3 The toxicity mechanisms of CuO NPs to AOB and recommendations for future study

Currently, scientists mainly focus on CuO NPs impacts to AOB community structure in the activated sludge system in wastewater treatment plant [2]. It is reported that Nitrosomonas is the main AOB functional species in the activated sludge process, such as the short-cut nitrification and denitrification process and anaerobic ammonia oxidation process. The abundance of Nitrosomonas would be increased when CuO NPs was low, while be decreased with the increase of CuO NPs concentration [18].

Actually, due to the extremely complex microbial composition in the environmental samples, it is difficult to describe the mechanism involved in analysing the toxicity of CuO NPs to AOB based on the environmental samples. However, there are few reports about the toxicity mechanism of CuO NPs to AOB on cytological level.

Fortunately, the cytotoxicity mechanism of oxide zinc nanoparticles (ZnO NPs), oxide silver nanoparticles (AgO NPs), oxide cerium nanoparticles (CeO$_2$ NPs) and dioxide titanium nanoparticles (TiO$_2$ NPs) to AOB in pure culture system were studied, and the model strains were also mostly used Nitrosomonas europaea (N.europaea) [19]. This provides a theoretical basis for the study on the cytotoxic of CuO NPs to AOB. Wu et al [20] studied the cytotoxicity of ZnO NPs on N.europaea, and found that ZnO NPs destroyed the structure of functional protease in the membrane of N.europaea by releasing Zn$^{2+}$, thus affecting the electron transfer and energy metabolism of cellular respiratory chain. Choi et al [17] reported that AgO NPs destroyed the membrane permeability of N.europaea through the agglomeration of nanoparticles, thereby damaging cell function. Wu et al [21] found that TiO$_2$ NPs without UV irradiation, showed low toxicity impacts on N.europaea, however, when under UV irradiation, TiO$_2$ NPs could release an order of magnitude higher content of ROS, resulted in the destroy of membrane structure of N.europaea. Then it comes to a conclusion: the cytotoxicity of TiO$_2$ NPs to AOB is mainly caused by ROS produced by catalytic conditions. Through the above analysis, it can be seen that the toxicity of different nanoparticles to N.europaea depends on the nanoparticles themselves, while the destruction of cell membrane function is a common cytotoxic pathway of all nanoparticles to N.europaea. So, whether CuO NPs can generate cytotoxicity by destroying the cell membrane functions and structure of N.europaea? How does the distribution of CuO NPs work? These questions remain unanswered and need further exploration.

As described in part 2.2, efflux pump is an important barrier for cells to block external metal pollution. Figure 1 is a newly report on how cell membrane efflux pump to exclude copper ions out of gram-negative bacteria [22].

It has been demonstrated that CuO NPs could inhibit the cell membrane efflux pump effect of bacteria [23], whereas the mechanism of inhibition is also lacking in-depth discussion. As AOB are typical gram-negative bacteria, basing on the existing researches to investigate the mechanism of CuO NPs toxicity on AOB cell membrane will be a supplement and development to the study of ecotoxicity of CuO NPs. Despite this, the potential for AOB resistance to CuO NPs should be taken into account. The mechanism of the appearance and spread of AOB resistance to copper (ions and particles) is an urgent need.
Figure 1. The mechanism of copper resistance by membrane efflux pump of Gram-negative bacteria [22]. When copper enters the cell stroma, it will be complexed by CopZ protein, meanwhile induce the expression of CopA ATP pump to exclude the copper ion to the periplasm space. Then, with the help of CusF protein, the CusCFBA protein will further discharge the copper ion outside of the cell. Furthermore, CueO protein can convert Cu$^{+}$ into low toxic Cu$^{2+}$ in the periplasm space, Cu$^{2+}$ can pass through the outer membrane pore non-specifically to the outside of the cell.

3. Conclusion
The ecological toxicity of CuO NPs has received more and more attention as its wide application in industrial fields. Yet, the toxicity mechanism of CuO NPs to microorganisms didn’t be fully elucidated. There is no unified conclusion on the biological toxicity of CuO NPs to microorganisms. An on-going challenge is the need to distinguish effects between dissolved ionic copper and nanoparticulate oxide copper. Ionic copper diffuses across cell membrane and reaches equilibrium concentrations throughout cells whereas nanoscale oxide copper particles entered the cells for high accumulation in specifically cellular/sub-cellular locations, whereupon subsequent release high dissolved ionic copper locally. Furthermore, the lack of cellular uptake mechanism and location makes the distinguishing work even harder. AOB as one of the most important nitrogen removal bacteria, are sensitive to environment conditions including CuO NPs. The toxicity mechanism of CuO NPs to AOB received some concern. However, information on cellular and bimolecular level is lacking. Some researchers focused on the function of cell membrane, such as efflux pump, to defend external metal invasion. This helps to understand AOB resistance to CuO NPs. Further study is required to investigate the characterization of the CuO NPs, including particle size, stability, dissolved copper ionic species. The mechanism of the appearance and spread of AOB resistance to copper (ions and particles) is also an urgent need.

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