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Inhibition of an enriched culture of ammonia oxidizing bacteria by two different nanoparticles: Silver and magnetite

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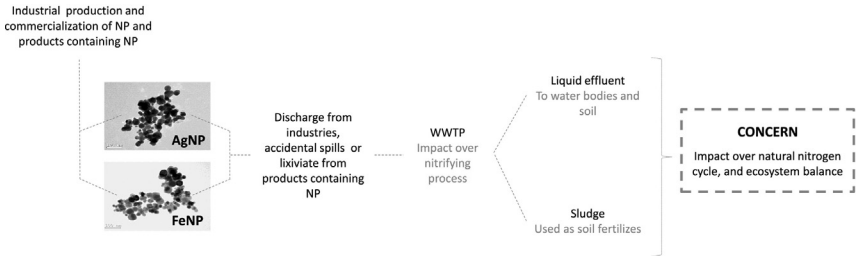
HIGHLIGHTS

- Silver nanoparticles and magnetite nanoparticles hinder nitrifying activity.
- Both nanoparticles attach to bacterial surface, reducing membrane permeability.
- If sludge containing nanoparticles is used as biofertilizer, it can affect soil quality and other ecosystems.

GRAPHICAL ABSTRACT



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ABSTRACT

Metal and metal oxide nanoparticles are getting attention over the past years. They can be used to several purposes, especially in commercial and medical applications. Undoubtedly, this lead to higher production and, consequently, increasing the risks of exposition, once they can be released into environment without a proper control. However, their impact over the bacteria present in wastewater treatment plants (WWTP), manly over nitrifying bacteria, which are the most susceptible to toxic compounds, are still not very well established. Herein it was investigated the impact of silver nanoparticles (AgNP) and magnetite nanoparticles (FeNP), separately, over an ammonia oxidizing bacteria (AOB), during short-term exposure tests and it was also verified their impact on bacterial surface. The concentrations assessed were from 0 to 30 mg AgNP L^{-1} and from 0 to 1000 mg FeNP L^{-1}. Results showed that AOB specific nitrite production rate reduced 90% when exposed to 30 mg AgNP L^{-1}, and in almost 71% in the presence of 1000 mg FeNP L^{-1}. The concentration necessary to reduce 50% of AOB activity was 10.75 mg AgNP L^{-1} and 483.01 mg FeNP L^{-1} highlighting that AgNP can be 45 times more toxic to AOB than FeNP. Both nanoparticles attached to bacterial surface, even in the lower concentration tested, hindering AOB activity due to changes in the membrane permeability. Once nanoparticles remain attached in the biological sludge, which is used as fertilizer to soil, they can affect not only WWTP performance but also hindering soil quality and the ecosystem balance.

1. Introduction

Engineered nanoparticles (NP) are known to have at least one dimension between 1 and 100 nm and, most of them show physical and chemical properties different from the corresponding bulk material (Buzea et al., 2007). This characteristic turns NP remarkable for many uses. They have a strong potential to improve air, water and soil quality and are being widely used in consumer products to improve their quality, and give different properties. However, nanoparticles production,

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use and disposal must be controlled, since they can also damage the environment (Biswas and Wu, 2005).

The most extensively studied nanomaterials include nanoparticles of metal, followed by metal oxides (i.e. zinc, titanium, cerium, silicon and iron) and carbon nanotubes. Among these, we emphasis two, because their considerable commercial interest: Silver and Magnetite nanoparticles. Silver is known as antimicrobial component since ancient Egypt and Rome, to treat wounds and to preserve water (Reidy et al., 2013). It was also used during the World War I in order to prevent wound infection, before antibiotics (El-Badawy et al., 2010). The antimicrobial property is conserved in other forms of silver, such as silver nanoparticles (AgNP), therefore, they can be added to several materials to avoid bacterial proliferation in different products, such as sports clothes, baby bottles, kitchen supplies, medical appliances, wall paints, etc. (Morones et al., 2005). Hence, almost 25% of all consumer products that contains NP, have AgNP in their composition (Vance et al., 2015). Magnetite nanoparticles (FeNP) are interesting due the superparamagnetic properties, high catalytic capacity and antimicrobial activity (Kádár et al., 2010; Klaine et al., 2008), enabling application in areas such as biomedicine and environmental remediation (Zhu et al., 2012).

Nevertheless, despite the increasing use of nanoparticles, its release to the environment cannot be fully controlled. AgNP was detected in effluents from washing textiles (Benn and Westerhoff, 2008) and runoff from facade paints (Kaegi et al., 2010). The toxicity of silver compounds (AgNP and Ag^{+}) have been widely investigated by others authors, however, most of them considered only the impact of AgNP over pure cultures (Chairuangkitti et al., 2013; Lok et al., 2007; Michels et al., 2015; Xiu et al., 2011). They considered different aspects of nanoparticles, such as: coating (Arnaout and Gunsch, 2012), shape (Pal et al., 2007), size (Carlson et al., 2008) and different silver species (Choi et al., 2008). Recently, a few studies have been considering AgNP toxicity over activated sludge. Alito and Gunsch (2014) have shown that AgNP can reduce ammonia oxidation in activated sludge, during a short term experiment, in 30%, however, the culture was able to recover after three hydraulic retention times. The COD removal was less impacted by silver. Similar behavior was detected by Liang et al. (2010) where the nitrification was highly impacted by silver nanoparticles, and the organic matter removal was not significantly affected by it.

Nonetheless, both NP (Ag and Fe) can eventually get to WWTP and affect the bacterial community. Once the NP reaches WWTP they can impact nitrogen removal efficiency, mostly nitrification. Ammonia oxidizing bacteria (AOB), which is responsible for convert ammonia to nitrite, are used as biosensors due to their high sensitivity to environmental changes and to toxic compounds (Carucci et al., 2006). They can have their activity impacted by pharmaceutically active compounds (Bressan et al., 2013; Wang and Gunsch, 2011), by different pHs (Cytryn et al., 2012), low oxygen dissolved (Stenstrom and Poduska, 1980) and nanoparticles (Hou et al., 2015; Yang et al., 2013). Therefore, it is imperative to understand how NP can affect those systems, and develop mechanisms to prevent future impact in WWTP. Thus, this study aimed to compare the inhibition and understand the effects of AgNP and FeNP over an enriched nitrifying community composed manly by AOB, through a short term exposure test and establish the NP concentration needed to inhibit 50% of AOB activity.

2. Material and methods

2.1. Nanoparticles characterization

Silver nanoparticles were purchased from Novacentrix (TX, USA) and magnetite nanoparticles, from SkySpring Nanomaterials (TX, USA). Stock suspensions of AgNP (2.5 g AgNP L^{-1}) and FeNP (4.0 g FeNP L^{-1}) were prepared, by dispersing nanoparticles in ultrapure water (oxygen free), and the both solutions were sonicated for 20 to 30 min to ensure that NP were dispersed. The AgNPs and FeNPs stock solution were added as needed to the test media to achieve the desired concentration. Size distribution was estimated by Transmission Electron Microscopy (JEOL JEM-1011 TEM 100K) at Central Laboratory of Electronic Microscopy (UFSC, Brazil). Samples were diluted with isopropanol, sonicated for 5 min, evaporated at room temperature on a carbon-coated copper grid (Mesh200-CF200Cu, Electron Microscopy Sciences Company, USA) and visualized by TEM.

2.2. Bacterial culture and chemicals

To enrich the bacterial culture in ammonia oxidizing bacteria (AOB) two bench scale reactors were operated as sequential batch reactors (reactor A and reactor B, as biomass source to AgNP and FeNP tests, respectively). Both were made of poly methyl methacrylate. The feed solutions and effluents were added and removed with peristaltic pumps, the temperature was controlled to 32 ± 1 °C, and both had different mechanic agitation system. It is worth mentioning that two different reactors were performed due to the fact that experiments with silver nanoparticles require a specific nutrient medium, specifically without ions that could complex with Ag^{+} and that are occasionally released from AgNP (Choi et al., 2008). In case of the experiments with FeNP, they were assessed with the nutrient media suggested by Campos et al. (1999), as specified below. Once the impact of nanoparticles over enriched nitrifying cultures was investigated, results were compared to better understand the impact of FeNP and AgNP over AOB.

Reactor A, with effective volume of 8.0 L, was used as a biomass source to AgNP tests. It was inoculated with sludge from the local urban sewage treatment system, and it was operated in 8 h cycles, with 7 h of reaction and 1 h of settling and discards of the effluent. Each hour of reaction had intervals of 15 min aerated and 45 min of anoxic reaction in order to enrich AOB and reduce the presence of nitrite oxidizing bacteria (Bressan et al., 2013; Zdradek, 2005). The nutrient medium was adapted from Campos et al. (1999). However, ions such as Cl^{-}, PO_4^{-3} and S^{-2}, that are known for their complexation with silver (Choi et al., 2008), were replaced to nitrate salts, keeping the nutrient concentration. Ammonia concentration was kept in 800 mg NH_4^{+}-N L^{-1}. The synthetic feed contained the following macronutrients (g L^{-1}): 4.7193 $(NH_4)_2SO_4$, 0.222 KH_2PO_4, 0.0321 $Mg(NO_3)_2$, 11.88 $NaHCO_3$, 1.2914 $NaNO_3$ and 0.44 mL L^{-1} of the micronutrient solution, which was composed by (g L^{-1}): 1.79 $Ca(NO_3)_2$, 3.57 $FeCl_2 \cdot 4H_2O$, 4.32 $MnSO_4 \cdot H_2O$, 1.87 $(NH_4)_6Mo_7O_{24}.4H_2O$, 1.53 $CuSO_4$, 25.35 $Zn(NO_3)_2 \cdot 6H_2O$, 0.40 $Co(NO_3)_2 \cdot 6H_2O$.

Reactor B, with an effective volume of 5.0 L, was used as a biomass source to FeNP tests. It was inoculated with the same biomass from Reactor A. It was operated in 6 h cycles, with 5 h of reaction and 1 h of settling and discard of the effluent, in the same aeration/anoxic periods as Reactor A. The nutrient medium was also adapted from Campos et al. (1999). Ammonia concentration was kept in 900 mg NH_4^{+}-N L^{-1}. The synthetic feed contained the following macronutrients (g L^{-1}): 1.53 NH_4Cl, 2.46 $(NH_4)_2SO_4$, 0.25 KH_2PO_4, 0.05 $MgSO4.7H_2O$, 0.5 NaCl, 11.96 $NaHCO_3$ and 1.0 mL L^{-1} of the micronutrient solution, which was composed by (g L^{-1}): 5.54 $CaCl_2$, 2.73 $FeSO_4$, 3.22 $MnCl_2$, 1.04 $(NH_4)_6Mo_7O_{24}.4H_2O$, 1.0 $CuSO_4$, 12.35 $ZnSO_4$, 0.88 $CoCl_2$ and 50.00 EDTA.

Both reactors were monitored. Three times a week samples were taken from the effluent to determine nitrogen compounds (ammonia, nitrite and nitrate), pH and dissolved oxygen (DO) (data not shown). When the steady state was achieved, biomass was collected to perform short term exposure tests.

2.3. Short term exposure tests

In order to perform short term exposure tests, batch glass reactors (200 mL) were inoculated with an aliquot of washed biomass (~0.5 g VSS L^{-1}) from the reactors (A to AgNP and B to FeNP). The "washing process" intends to remove substrate (ammonia) or the reaction products (nitrite and nitrate) from the biomass (Bressan, 2012). Therefore, it was washed at least three times with a solution with similar composition of the feed solution, removing only $(NH_4)_2SO_4$ and $NaHCO_3$. The

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