Inhibitory Effects of Titanium Dioxide, Silver and Fullerene Nanoparticles on Activated Sludge from a Municipal Wastewater Treatment Plant

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ABSTRACT

The possibility of negative impacts of three nanomaterials on activated sludge was investigated through respiration inhibition tests following a modified OECD 209 protocol. Titanium dioxide, silver and fullerene nanoparticles were considered. A silver salt was also used as a known toxic and as a basis for comparison with the nanoparticle form. Activated sludge was obtained from a municipal wastewater treatment plant. All materials tested showed respiration inhibition when present at concentrations between 10 mg/l and 100 mg/l, although ionic silver resulted significantly more toxic with an IC_{50} below 5 mg/l.

Keywords: activated sludge, nanomaterials, respiration inhibition.

1 INTRODUCTION

Nanotechnology is reaching a wide variety of fields, from research laboratories, medical purposes and consumer products, leading to an exponential increase in the total mass of materials produced annually. Therefore, it is likely that nanoparticles are detected in natural systems and water treatment facilities[1]. Scientists need to accurately assess the risk involved in its use, also considering interferences with engineering systems. In particular, as they leach out of nano-enabled products and will become part of the normal composition of municipal sewage. Nanoparticles are expected to undergo different transformations under conditions commonly encountered in the environment [2]. In particular, nanomaterials in complex aqueous matrixes such as domestic wastewaters have the potential to undergo a complete and interrelated set of alterations, not to be easily predicted without experimental data.

Titanium dioxide nanoparticles are within the most common nanomaterials, finding applications in cosmetics, paints, catalyst, and many other fields. Production is expected to grow exponentially reaching by 2025 an annual level of 2.5 million metric tons only in the U.S.[3], making the material a potential major contaminant in the environment. Silver nanoparticles is another nanomaterial that has found several applications in everyday products, from textiles to washing machines and refrigerators, all of which will produce leakage of nanoparticles during normal use and wear [3][4]. Fullerenes and its derivatives also show a growing trend. It is estimated that the lower and upper bounds of U.S. production of fullerenes are 2 and 80 tons per year respectively [5]. Although not as widely applied as the previous two, its potential applications in superconductors, sensors, medicine, cosmetics, and advanced materials hint to a rapid increase in the global market for nanoproducts in the next few years [6].

There are few reports on the interactions of nanomaterials in activated sludge wastewater plants. Silver and cerium nanoparticles have been shown to produce respiration inhibition to heterotrophic organisms at concentration levels in the order of 0.1 to 0.3 mg/l [7]. However, different loads and/or stabilizers were not investigated in this study. Size is an important factor, since silver nanoparticles smaller than 5 nm produced more microbial growth inhibition than silver ions, probably due to the ability of the small, uncharged particle to react with the cell membrane and release a large number of silver ions very near or inside the cell [8]. Based on another work, silver nanoparticles had a higher inhibitory effect than both silver chloride colloids and silver ions on nitrifying bacteria, but the reversed was observed on E. Coli bacteria [8]. No significant impact has been reported for microbial communities exposed to titanium dioxide nanoparticles under the experimental conditions tested [7], although some effects have been found on specific organisms commonly found in activated sludge [9]. The fate of fullerene nanoparticles in wastewater treatment plants was investigated, with a focus on the sorption on activated sludge, but the biological interactions were not studied [10] [11].

The objective of this work is to investigate the influence of the presence of three nanomaterials titanium dioxide (TiO_2NP), fullerenes (C_{60}) and silver (AgNP) nanoparticles on the oxygen uptake rate of a mixed microbial community obtained from a municipal wastewater treatment plant.

2 METHODS AND MATERIALS

2.1 Nanoparticles

Titanium dioxide nanoparticles (Aeroxide P25) were obtained from Evonik Degussa Corporation, (Parsippany, NJ), a 99,5% of hydrophilic fumed titanium dioxide with primary size of 21 nm and specific surface area of 50±15 m2/g, as reported by the manufacturer.

Silver nanoparticles were synthesized in the laboratory following Lee and Miesel´s method [12], using analytical grade silver nitrate (Merck) and tri-sodium citrate (AppliChem) as the reducing agent and stabilizer. Briefly, 90 mg of silver nitrate was disolved in 500 ml of distilled water and the solution was heated to boiling. 10 ml of 1% solution of sodium citrate was added with vigorous stirring and kept boiling until a green-gray dispersion indicated the formation of the silver nanoparticles.

Fullerene nanoparticles (Fullerene C_{60} sublimed 99,9%) were obtained from Sigma Aldrich.

2.2 Synthetic Sewege and Activated Sludge

A synthetic sewage was fabricated in the laboratory as growth media for the microorganisms according to OECD 209 protocol. It was composed by: tryptone (Oxoid) 16 g/l, meat extract (Lab-lemco powder, Oxoid) 11g/l, urea (Anedra) 3 g/l, NaCl (Sigma Aldrich) 0,7 g/l, $CaCl_2 \cdot 2H_2O$ (Anedra) 0.4 g/l, $MgSO_4 \cdot 7H_2O$ (Sigma Aldrich) 0.2 g/l, and K_2HPO_4 (Anedra) 2.8 g/l. All reagents were of analytical grade. The synthetic sewage was prepared fresh, daily, for the tests.

Fresh activated sludge was obtained from the aeration tank of the Champagnat Wastewater Treatment Facility (Pilar, Buenos Aires). In the laboratory, the activated sludge was mixed, kept under aeration, and fed daily with synthetic sewage.

The solids retention time was controlled by daily wasting an appropriate mixed liquor volume and the mixed liquor suspended solids (MLSS) was maintained between 3,600 to 4,000 mg/l as required by the OECD method.

2.3 Characterization

The activated sludge was characterized, before every test, by optic microscopy (DME, Leica) and the Sludge Volume Index (SVI) was measured according to standard methods [13].

The morphology of silver nanoparticles was determined by Scanning Electron Microscopy (SEM), on a Zeiss Supra 40 at 3.00 kV. The SEM samples were prepared by placing a drop of freshly synthesized silver nanoparticles over a gold-coated glass support and immediately dried to minimize concentration effects. The size distribution was evaluated by Dynamic Light Scattering (DLS), using a Zetasizer Nano ZS system (Malvern Instruments). The UV-Vis spectrum was determined with a UV-1650 PC (Shimadzu).

2.4 Respiration Inhibition Test

The activated sludge respiration inhibition test was carried out according to a modified OECD 209 protocol [14]. The test involved the measurement of activated sludge oxygen uptake rate (OUR) from a synthetic substrate to which the test compound had been added at various concentrations. Briefly, 200 ml of activated sludge (AS) and 25 ml of synthetic sewage (SS) were placed in a beaker. The test substance (TS) was added from a stock solution to achieve the desired final concentration. The final volume was adjusted to 500 ml with deionized water. Air was supplied at a rate of 0,5 to 1 liter/hour. After 75 minutes another 25 ml of SS were added to the system. Fifteen minutes later, the air supply was stopped. The oxygen uptake rate was immediately determined by measuring the dissolved oxygen concentration during 10 minutes with an oxygen probe (Sension6, Hach Company). These OUR measurements (OUR_M) were compared to that of a control without the test compound (OUR_C). Another control, consisting in a reference toxicant (3,5-dichlorophenol, Sigma Aldrich) was measured to insure that the test was working properly and that the biomass had the appropriate sensitivity. All tests were carried out in duplicates and the temperature was kept at 20±2°C. The respiration inhibition was calculated as:

$$\%\ Inhibition = \left(1 - \frac{OUR_M}{OUR_C}\right) \times 100$$

3 RESULTS AND DISCUSSION

3.1 Silver Nanoparticles Characterization

The SEM images showed that under the conditions used to synthesize the silver nanoparticles, the particles had roughly spherical shape and diameters could be identified from approximately 10 nm to 80 nm.

The absorption spectra exhibited one surface plasmon absorption band in the visible region with a defined peak at 409 nm. In this case the presence of only one distinctive peak at 409 nm is indicative of the presence of spherical silver particles. [15]

The size distribution of the silver nanoparticles by DLS showed a peak at an average size of 11 nm (mean value weighted according to the number of particles of each size), with a distribution between 7 and 40 nm.

3.2 Activated Sludge Characterization

The MLSS in the activated sludge used during the tests ranged from 3636 to 4016 mg/l, with an average value of 3830 mg/l. The Sludge Volume Index (SVI) was under 100 ml/g in all cases, indicative of the good settling properties of the sludge. This was in concordance with the optic microscopy images that showed a floc forming activated sludge, with abundance of a variety of microorganisms, such as rotifers, vorticellas, and nemathodes besides bacteria.

3.3 Respiration Inhibition Test

The experimental details of the different tests conducted are presented in Table 1, indicating the volume of activated sludge and synthetic sewage used, milligrams of test substance added and final volume, as described in the previous section. The final concentration of each test substance was calculated as the mass of the TS divided by the final volume at the beginning of the test (500 ml), neglecting the 25 ml of synthetic sewage added at 75 minutes. All tests were conducted in the presence of natural light except for TiO_2NP that was evaluated in the dark.

	AS (ml)	SS (ml)	TS (mg)	Final volume (ml)	Conc. (mg/l)
Control	200	25(0´) 25(75´)	0	500	0
Ag +	200	25(0´) 25(75´)	0,5	500	1
			2,5		5
			5		10
AgNP	200	25(0´) 25(75´)	2,5	500	5
			5		10
			10		20
			25		50
TiO_2NP	200	25(0´) 25(75´)	5	500	10
			10		20
			25		50
			50		100
C_{60}	200	25(0´) 25(75´)	5	500	10
			10		20
			25		50
			50		100

Table 1: Experimental details for the respiration inhibition tests.

Figure 1 shows the degree of respiration inhibition in the activated sludge as a function of concentration for the three selected nanoparticles: AgNP and C_{60} nanoparticles in presence of light and TiO_2NP in the absence of light. At all concentration levels investigated, the highest respiration inhibition was observed for TiO_2NP, while AgNP and C_{60} showed lower values. Additionally, there was no significant effect for these two nanomaterials at 10 mg/l, while inhibition started for the 20 mg/l level. Inhibition for all the studied nanoparticles increased linearly with concentration, within the range of expressed effects. Within the experimental error, there was no significant difference in the inhibition presented by AgNP and C_{60}.

The inhibitory effect of AgNP was also investigated in comparison with silver ion (Ag+), as Ag+ toxicity towards microbes is well established, and related to its interaction with thiol groups of vital enzymes and proteins, affecting cellular respiration and transport of ions across the membrane[16]. The results are shown in Figure 2. The respiration inhibition of silver ions was almost 30 % for the lowest concentration tested (1 mg/l), and increased rapidly with increasing levels of Ag+, reaching total inhibition (100%) for concentrations of 10 mg/l or higher. In contrast, the effect of AgNP was not observed for concentration up to 20 mg/l, with a modest inhibition for both the 20 and 50 mg/l levels. Other studies [7] have reported an inhibitory effect of silver nanoparticles in ordinary heterotrophic organisms at concentrations as low as 0.13 mg/l for a 4 hour exposure, although for 1 hour exposure there was no noticeable effect. Silver ions release kinetics from nanoparticles depends on pH and oxygen concentration in the medium [17], leading to an enhance liberation of silver ions in an activated sludge process that may induce inhibition. However, for shorter tests as the one presented in this study, a major contribution to inhibition through dissolved silver from the nanoparticles is not expected, and therefore results presented here may be attributed almost exclusively to microorganism-particle interactions. The results of this work suggest that silver nanoparticle toxicity is significantly lower than that of silver ions, although increased toxicity may be expected if dissolution occurs.

Due to the well-known photocatalytic properties of TiO_2NP, the effect of light on the inhibitory behavior of this material was further investigated. Evaluation of tests conducted (data not presented) without exposure to light showed a significant increase in the consumption of oxygen compared to the blank (no nanoparticles). This effect seems to be associated with the presence of live organisms; since it was not detected in tests performed without activated sludge or sterilized microorganisms. A similar observation was reported by Brunet et al. [18], where growth of E. Coli was enhanced by the presence of TiO_2NP at low concentrations.

4 CONCLUSIONS

All three nanomaterials tested did not show inhibitory behavior towards the mixed community for concentrations below 10 mg/l, but presented negative impacts for higher concentrations.

The inhibitory effect of silver nanoparticles in contrast with silver ions is indicative of silver ions higher toxicity. The inhibition mechanism of the silver nanoparticles is still unknown, but the results of this study suggests that it may be related, in part, to the dissolution of silver ions.

TiO_2NP expressed a differentiated behavior in the presence and absence of light, with a significant increase in the consumption of O_2 when exposed to natural light and respiratory inhibition in the dark. The effect is not expressed in the absence of live organisms. Future research is needed in order to elucidate the mechanisms involved in this observation.

Although nanoparticle levels in this study were higher than those expected to occur in municipal wastewater

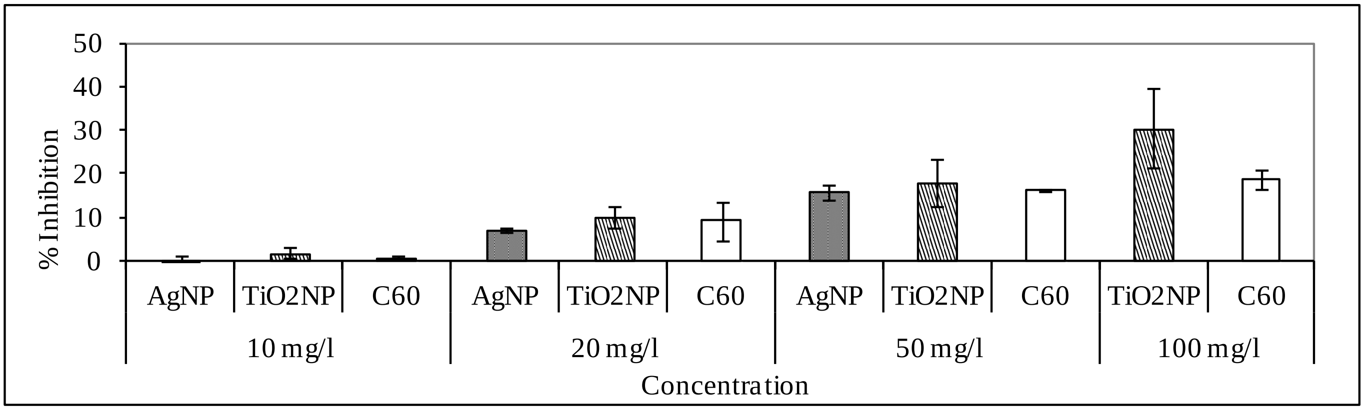

Figure 1: Respiration inhibition of AgNP, TiO_2NP, C60.

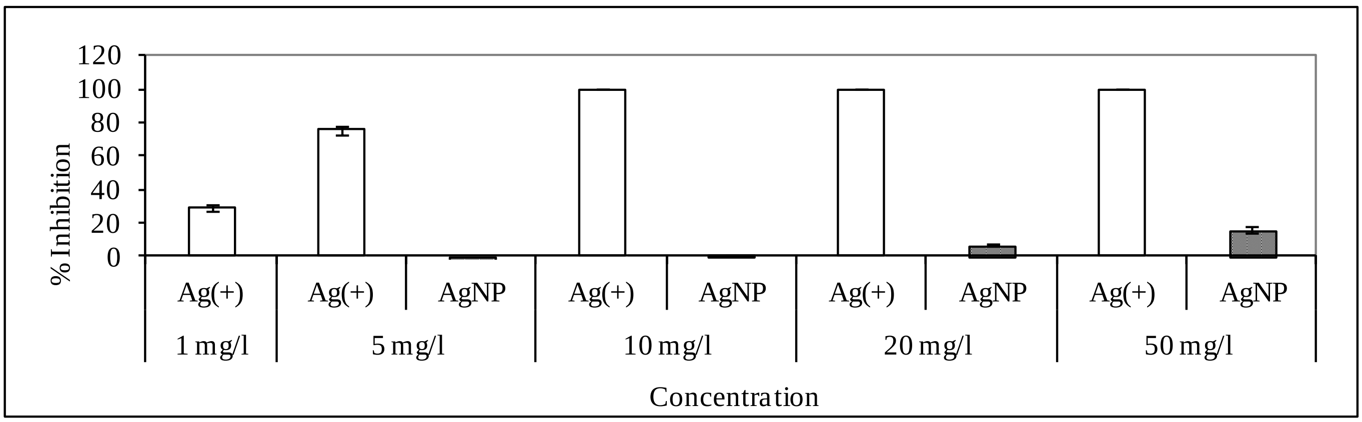

Figure 2: Respiration inhibition of silver ions and AgNP

plants, the evidence of acute toxicity presented here hints to possible negative impacts at lower concentrations and much larger exposure times, as may happen if nanomaterials become an everyday constituent of domestic effluent.

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