EFFECTS OF TiO_2 AND SiO_2 NANOPARTICLES ON ACTIVATED SLUDGE

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Abstract:
This paper deals with the observation and evaluation of the effects of TiO2 and SiO2 nanoparticles on microorganisms in activated sludge. The selected nanoparticles were tested at three different concentrations - 0.1 g/l, 0.3 g/l, and 0.5 g/l. Respirometry measurements were mainly used to evaluate the effects of TiO2 and SiO2 nanoparticles on microorganisms, as they allowed us to observe their activity, i.e. consumption of O2. Fluorescence microscopy, for which a Live/Dead Cells Kit was used, enabled us to observe and compare the viability of microorganisms in the activated sludge. The acute toxicity of these nanoparticles was primarily tested during these experiments; however, no significant toxic effects were observed. It was found that the concentration of nanoparticles has an effect on their toxicity to microorganisms. In the case of TiO2 nanoparticles the greatest toxic effect on the microorganisms was observed at a concentration of 0.1 g/l. The highest tested concentration did not show any effect.

Key words:
Nanoparticles, toxicity, activated sludge, respirometry

Introduction

The use of nanoparticles in commercial products and industrial applications leads to the release of nanoparticles into the environment, where they can threaten human health and ecosystems (Park et al., 2013). It is also clear that this will increase the concentrations of nanoparticles in sewage treatment (Eduok et al., 2013). Therefore, it is important to determine the fate of the nanoparticles during wastewater treatment; however, our knowledge about the environmental risks of nanomaterials remains limited (Park et al., 2013; Brar et al., 2010).

SiO_2 nanoparticles are among the most commonly used nanomaterials in construction materials, in biomedical applications, as filling material in food packaging, and abrasives (Otero-González et al., 2015; Park et al., 2013). In addition, there is an increasing interest in TiO_2 nanoparticles due to their wide use in industrial and medical applications. TiO_2 nanoparticles are also used in solar cell technologies, for self-cleaning surfaces of facades and in environmental remediation (Mu et al., 2011; Farkas et al., 2015).

This work was focused on evaluating the effects of SiO_2 and TiO_2 nanoparticles on the respiration and viability of microorganisms in activated sludge. Nanoparticles of TiO_2 and SiO_2 were selected for their widespread use in commercial products and the associated high risk of potential release to the environment (Park et al., 2013). We used a mixed culture of microorganisms in activated sludge taken from a real sewage treatment plant. The nanoparticles were tested at concentrations of 0.1 g/l, 0.3 g/l and 0.5 g/l. The objective of this experiment was to determine the effects of the nanoparticles on the microorganisms and the roles played by the different concentrations used.

Material and methods

Nanoparticles (NP) - both types of nanoparticles were supplied by SkySpring Nanomaterials Inc. Houston, USA. **TiO_2:** anatase, 99.5% purity, size 10 to 25 nm, a white powder with a specific surface area of 50 -150 m^2/g. **SiO_2**: 99.5% purity, size 15 to 20 nm, a porous white powder with a specific surface of 640 m^2/g.

Sample preparation
The nanoparticles were mixed into a solution, which contained a mixture of amines and detergents in order to prevent the formation of agglomerates. A stock solution was prepared for both types of nanoparticles with a concentration of 25 g/l NPs. The test samples contained 96 ml of the activated sludge, 2 ml of the NPs stock solution (with a final concentration of 0.5 g/l), 2 ml of a phosphate buffer and nutrients - sodium acetate in a concentration of 100 mg/l. For the lower concentrations tested, the NPs stock solution was first diluted in order for the same volume to be added. A control sample was prepared in a same manner; however, a clear solution was added instead of the solution of nanoparticles.

Respirometry
Respirometry allows us to observe bacterial metabolism measured by the consumption of O_2 and production of CO_2. The respiratory activity of the microorganisms in the activated sludge was measured using a Micro-Oxymax respirometer (Columbus Instruments International, USA), pursuant to ČSN EN ISO 9408, inorganic nanoparticles were used instead of organic compounds. Respiration was measured after 40 hours.

Live/Dead fluorescence analysis
An evaluation of the samples was performed in terms of florescence using a Zeiss Axio Imager.M2 fluorescence microscope with an AxioCamICc1 camera and a Colibri 2 fluorescent lamp. The configuration corresponds to a 62HE B/G/HR filter i.e. wavelengths of 365 nm, 470 nm and 590 nm. Cell viability was evaluated using a LIVE/DEAD BacLight Bacterial Viability Kit, which allows us to compare the representation of live and dead cells. Fluorescence microscopy was carried out at time 0 and after respirometry i.e. 40 hours.

Results and discussion
The toxic effects of nanoparticles of TiO_2 and SiO_2 observed using respirometric measurements showed an effect of the nanoparticles on the cumulative oxygen consumption of the microorganisms in the activated sludge. The results showed that the toxic effect depends on the concentration of the nanoparticles (Table. 1). While in both cases the highest tested concentration of nanoparticles had almost no effect, the lowest concentration conversely decreased respiration in the samples containing both the SiO_2 and TiO_2 nanoparticles. The largest decrease in the maximum cumulative oxygen consumption was observed in the sample containing TiO_2 nanoparticles at a concentration of 0.1 g/l, where the respiration was reduced by 33.3%. A slight decrease in respiration was observed at a concentration of 0.3 g/l. The SiO_2 nanoparticles had a negative effect on the microorganisms only at a concentration of 0.1 g/l (respiration was reduced by 28.1%). Fluorescence analysis of living and dead cells after 40 hours of exposure to the nanoparticles showed a similar trend to the respiration of the samples (Tab. 2). Nanoparticles of TiO_2 at a concentration of 0.1 g/l resulted in a decrease in viability of 47.5% after 40 hours compared to time 0, whereas a slight increase (9.4%) was observed for the highest tested concentration. A huge increase in the viability of the sample with nanoparticles of SiO_2 at a concentration of 0.3 g/l was probably due to poor sample collection at time 0.

Tab. 1: Maximum respiration of microorganisms for each individual concentration

	Maximum respiration (%)		
Sample/ NP concentration	**0.1 g/l**	**0.3 g/l**	**0.5 g/l**
Control	100	100	100
TiO_2	66.7	86.8	100.4
SiO_2	71.9	100.4	98.1

Tab. 2: Viability of microorganisms after 40 hours

	Viability (%)		
Sample / NP concentration	**0.1 g/l**	**0.3 g/l**	**0.5 g/l**
Control	121.2	115.8	115.2
TiO_2	52.5	83.3	109.4
SiO_2	56.5	452.0	106.2

Conclusion
The toxicity of nanoparticles depends greatly on their concentration. The results show that nanoparticles of TiO_2 at a concentration of 0.5 g/l and SiO_2 at a concentration of 0.3 and 0.5 g/l do not display any toxic effects on the microorganisms in the activated sludge. The most important toxic

effect was observed in the sample with the lowest concentration of TiO_2 nanoparticles, where respiration was reduced by 33.3%. A trend was observed in the case of TiO_2 nanoparticles whereby their toxicity lowered with increasing concentrations (in the range of 0.1 - 0.5 g/l). The results of the fluorescence analysis showed the same trend for TiO_2 nanoparticles as the results of the respiratory tests.

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