HOW DO NANOPARTICLES OF TiO_2, SiO_2 AND ZrO_2 AFFECT MICROORGANISMS IN ACTIVATED SLUDGE?

ROSICKÁ Petra[1, 2], SVOBODOVÁ Lucie[1], COUFALOVÁ Adéla[2], LEDERER Tomáš[1], BAKALOVÁ Totka[1]

[1]*Institute for Nanomaterials, Advanced Technologies and Innovations, Technical University of Liberec, Studentská 2, 461 17 Liberec 1, Czech Republic, EU; e-mail: petra.rosicka@tul.cz*

[2]*Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Studentská 2, 461 17 Liberec 1, Czech Republic, EU*

Abstract

This paper deals with the observation and assessment of the impact of TiO_2, SiO_2 and ZrO_2 nanoparticles on microorganisms in activated sludge. These nanoparticles were tested in three different concentrations – 100 mg/L, 300 mg/L and 500 mg/L. The influence of the nanoparticles was assessed especially through respirometric measurements and fluorescence microscopy using a Live/Dead Cells Kit, which allowed the observation of the activity (O_2 consumption) and viability of the microorganisms in the tested activated sludge. In addition, the morphology of the activated sludge was observed using Gram staining under optical microscopy. The acute toxicity of nanoparticles was evaluated; however, no significant toxic effects for most of the tested nanoparticles were observed. The impact of the tested nanoparticles depends on their type and concentration. The most significant decrease in respiration measurements was observed in a sample containing nanoparticles of zirconium dioxide at a concentration of 500 mg/L, whereby the respiration was reduced by 45.3 % compared to the control sample. In contrast, respiration of samples containing nanoparticles of SiO_2 (300 mg/L and 500 mg/L), TiO_2 (500 mg/L) and ZrO_2 (300 mg/L) was almost the same as the control sample.

Keywords: nanoparticles, toxicity, activated sludge, respiration

1. INTRODUCTION

The increasing use of engineered nanoparticles (NPs) in consumer products and industrial applications leads to the fact that they are released into the natural environment where they may threaten human health and ecosystems [1]. It is already apparent that the release of waste NPs into wastewater treatment plants will sharply increase [2]. Therefore, an understanding of the fate of NPs during wastewater treatment is needed as our knowledge of the environmental risks of nanomaterials is still limited. Wastewater treatment processes may play an important role in determining the environmental pathways and the disposal of the NPs that are incorporated into various different products [1,3].

SiO_2 NPs are some of the most commonly used nanomaterials [4]. They are utilized in many different sectors as construction materials, biomedical applications, filler materials in food packaging and as abrasives [1]. TiO_2 NPs have received increasing interests due to their widespread industrial and medical application. TiO_2 NPs are used in solar cell technologies, self-cleaning surfaces of facades, paints, sunscreens, food additives and in environmental remediation [5,6]. ZrO_2 nanoparticles show photocatalytic activity compared to commercial TiO_2 powders [7]. Precisely because the use of these nanoparticles is still growing, their concentration in wastewater is also increasing [4,8].

The risk of NPs consists in their size, shape, reactivity and other properties. While the size of NPs is critical for maintaining the cell membrane in its original condition, their shape may play a significant role as a contact surface between the NPs and the cell cytoplasmic membrane [9]. Bacteria are a good model organism for studying the toxicity of NPs because it is possible to observe how they affect the cell but also the entire function of the organism [10] and bacteria in activated sludge are not only a model but a good real sample.

This work is focused on an evaluation of the influence of SiO_2, TiO_2 and ZrO_2 NPs on the respiration and viability of microorganisms in activated sludge. TiO_2 and SiO_2 NPs were selected because of their widespread use in commercial products and a high risk of their potential release into the environment [1]. We used a mixed culture of microorganisms of activated sludge from a real wastewater treatment plant in the Czech Republic. SiO_2, TiO_2 and ZrO_2 NPs were tested in three concentrations: 500 mg/L, 300 mg/L and 100 mg/L. The aim of these experiments was to test the effects of different types of NPs and to find out how much their effects depend on their concentration.

2. MATERIALS AND METHODS

2.1. Nanoparticles (NPs)

All three types of the tested NPs were supplied by 'SkySpring Nanomaterials', Inc., Houston, USA. The characteristics of the NPs are as follows: Titanium dioxide NPs (TiO_2, anatase, 99.5%, Product #: 7910DL), size 10 – 25 nm, white nanopowder, specific surface area 50 – 150 m^2/g, morphology: flat texture of the surface with smooth edges; Silicon dioxide NPs (SiO_2, 99.5%, product #: 6807NM), size 15 – 20 nm, porous white nanopowder, specific surface area 640 m^2/g, morphology: porous and nearly spherical; Zirconium dioxide (ZrO_2, 99.9 %, Product #: 8512QI), size 20 – 30 nm, white nanopowder, specific surface area > 35m^2/g, morphology: spherical.

2.2. Sample preparation

Activated sludge from a real wastewater treatment plant in the Czech Republic was used for testing the toxicity of the NPs. The NPs were prepared in a commercial solution for machining. Generally it is assumed that the solution reduces the formation of large agglomerates of NPs. The stock solution was prepared for each type of NP in a concentration of 25 g/L, mixed well and sonicated. The sample for measurement contained 96 mL of activated sludge (dry matter 1 g/L), 2 mL of the concentrated solution with NPs (final concentration 500 mg/L of NPs), and 2 mL of phosphate-buffered saline (PBS) and nutrients – acetate was added at a concentration of 100 mg/L. The stock solution of NPs was subsequently diluted for final concentration of NPs 300 mg/L and 100 mg/L. The amount of solution with NPs added to the sample was the same. A control sample was prepared in the same way but instead of the solution of NPs a clear stabilized solution (no NPs) was added into the sample of activated sludge.

2.3. Respirometry

Respirometry allows the monitoring of a bacterial metabolism by measuring the oxygen consumption and carbon dioxide production. The respiration activity of microorganisms in activated sludge was measured using a Micro-Oxymax respirometer (Columbus Instruments International, USA) according to the standard methodology of EN ISO 9408, using inorganic nanoparticles instead of organic compounds. The acute toxicity of the NPs was observed through respirometry, whereby the samples prepared according to Section 2.2 were measured continuously for 40 hours.

2.4. LIVE/DEAD fluorescence analysis

Fluorescence microscopic assessment of the samples was performed using a ZEISS Axio Imager.M2 fluorescent microscope fitted with an AxioCamICc1 camera with a Colibri.2 fluorescent lamp. The microscope settings matched those of the 62HE B/G/HR filter, i.e. wavelengths of 365 nm, 470 nm and 590 nm. Cell viability was assessed using a LIVE/DEAD® BacLight™ Bacterial Viability Kit, which allows observation and comparison of both living and dead cells (cells with damaged membranes [i.e. dead or dying] turn red while cells with intact membranes turn green). Fluorescence microscopy of the samples was performed on the first day and 40 hours after the end of the respirometric measurements.

2.5. Gram staining

Gram staining (the modified Hücker’s method) allows the structure and morphology of the microorganisms in the activated sludge to be observed as well as the creation the flakes, and it shows the representation of gram positive and gram negative microorganisms.

3. RESULTS AND DISCUSION

The toxic effect of TiO_2, SiO_2 and ZrO_2 NPs was observed using respirometric measurement, which showed the influence of the NPs on the cumulative oxygen consumption of the microorganisms in the activated sludge. The results of the respiration test showed that the toxic effect of NPs depends on their concentration. The most significant difference is shown in the results in Figure 1. The cumulative oxygen consumption of the samples with ZrO_2 NPs at concentrations of 100 mg/L and 300 mg/L is almost the same as the control sample, and no toxic effect of the NPs was observed. On the contrary, the most significant toxic effect of these experiments was observed in the sample with ZrO_2 NPs at a concentration of 500 mg/L. The respiration was reduced by 45.3 % comported to the control sample. The SiO_2 and TiO_2 NPs at concentrations of 500 mg/L showed no decrease in the respiration of the microorganisms. The cumulative oxygen consumption is almost the same as in the control sample, as is shown in Figure 1 and Table 1.

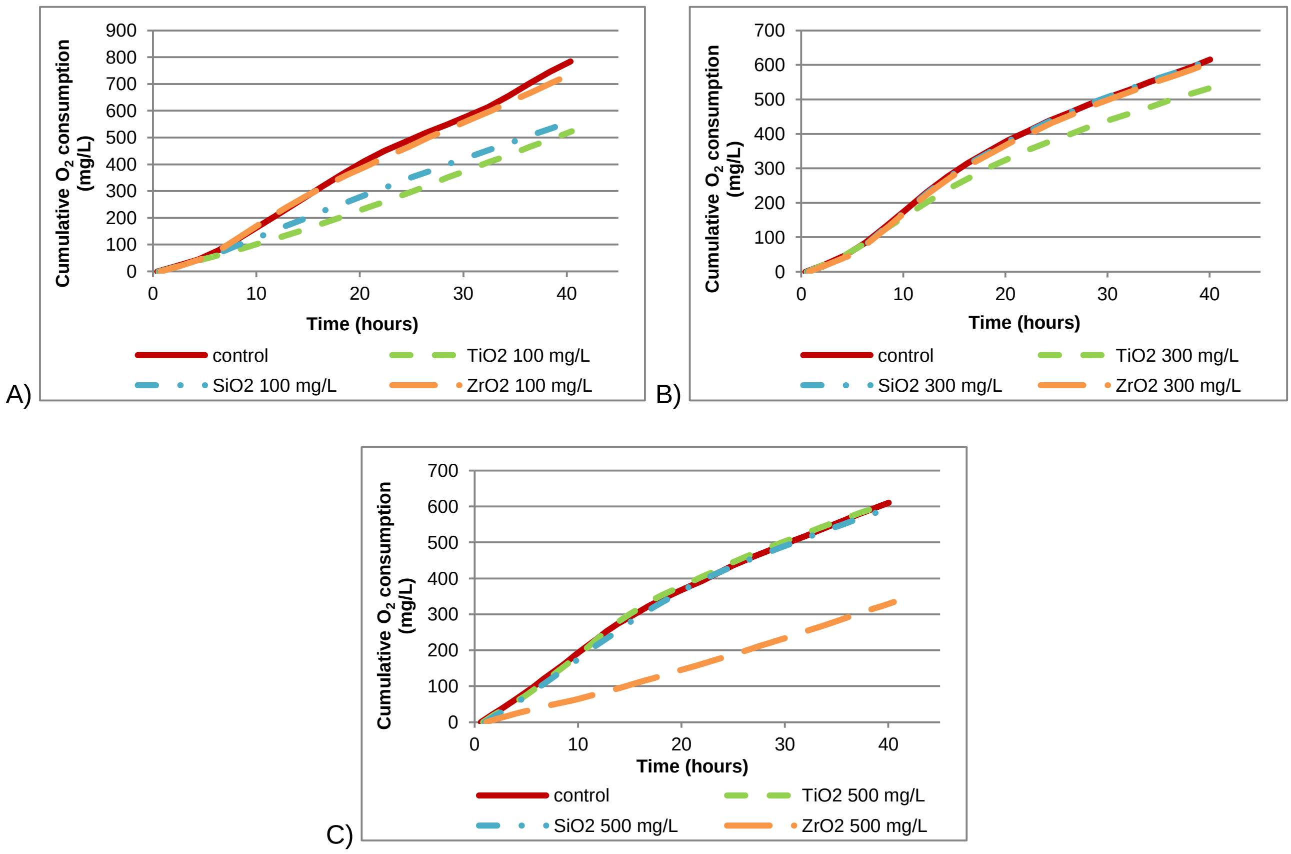

Fig. 1 Cumulative oxygen consumption of microorganisms in activated sludge with 3 different concentrations of NPs (A) 100 mg/L NPs, (B) 300 mg/L NPs and (C) 500 mg/L NPs

It was found that the lower concentration of TiO_2 NPs has a greater toxic effect on the microorganisms. The maximum cumulative oxygen consumption is 100.4 % in the sample with a concentration of 500 mg/L of TiO_2 NPs (Table 1). When the lower concentration of 300 mg/L was used then the maximum respiration was reduced by 13.2 %. In the sample with the lowest tested concentration of 100 mg/L of TiO_2 NPs respiration was reduced by 33.3 %. SiO_2 NPs had an effect on the microorganisms only at a concentration of 100 mg/L, and the cumulative oxygen consumption was approximately 28.1 % lower than in the control sample.

Table 1 Comparison of maximum respiration of activated sludge with NPs at three different concentrations

	Maximum respiration (%) for each concentration		
Sample/Concentration of NPs	**100 mg/L**	**300 mg/L**	**500 mg/L**
Control (no NPs)	**100**	**100**	**100**
TiO_2	66.7	86.8	100.4
SiO_2	71.9	100.4	98.1
ZrO_2	94.5	99.1	54.7

Figure 2 shows the viability of the microorganisms from the fluorescence analysis of live/dead cells from the first day at time zero and then after 40 hours of exposure to the NPs. The results obtained from the fluorescence microscopy tend to correspond with the respirometry data. According to the fluorescence analysis the ZrO_2 NPs are the most toxic at a concentration of 500 mg/L and the most significant effect from all of the tested NPs was observed in the samples with ZrO_2.

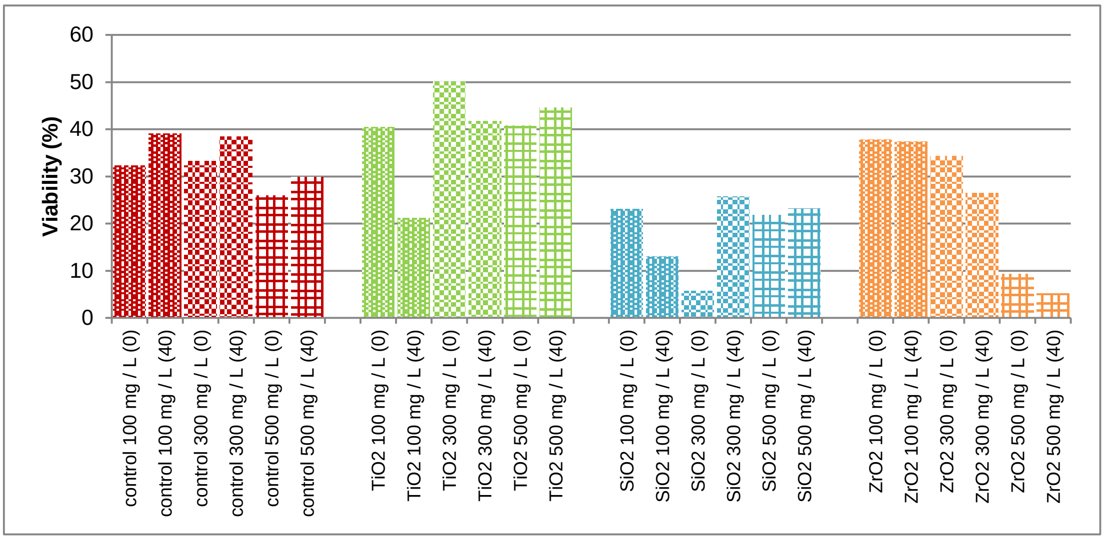

Fig. 2 Viability (%) of microorganisms from fluorescence analysis

The evaluation of the structure of the activated sludge through Gram staining showed no significant effect of TiO_2 and SiO_2 NPs on the structure of activated sludge flakes. In samples with ZrO_2 a slight effect of the NPs was observed, whereby the sludge flakes were not as compact as in the control sample.

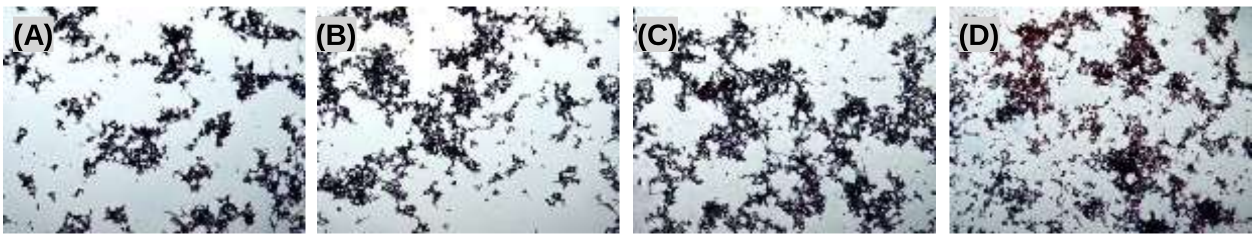

Fig. 3 Gram staining of activated sludge (A) control sample, (B) TiO_2 500 mg/L, (C) SiO_2 500 mg/L, (D) ZrO_2 500 mg/L

4. CONCLUSION

The toxicity of NPs highly depends on their concentration. The results indicate that nanoparticles of TiO_2 (500 mg/L), SiO_2 (300 mg/L; 500 mg/L) and ZrO_2 (100 mg/L; 300 mg/L) have almost no toxic effect on microorganisms in activated sludge. The most significant toxic effect was observed in a sample with the highest concentration of ZrO_2 (500 mg/L), whereby respiration was reduced by 45.3 %. A reverse trend was observed in the samples with TiO_2 NPs. The lower the concentration of TiO_2 NPs (100 mg/L) the more pronounced the decrease in respiration of microorganisms in the activated sludge. Both methods (respirometry and fluorescence microscopy) confirmed that ZrO_2 NPs at a concentration of 500 mg/L were the most toxic.

ACKNOWLEDGEMENTS

This paper was supported through the OP VaVpI project "Innovative products and environmental technologies", registration number CZ.1.05/3.1.00/14.0306. The results of project LO1201 were obtained through financial support of the Ministry of Education, Youth and Sports under the framework of targeted support within the "National Programme for Sustainability I" and the OPR&DI project Centre for Nanomaterials, Advanced Technologies and Innovation (CZ.1.05/2.1.00/01.0005). The work of P. Rosická was supported by the Ministry of Education of the Czech Republic within the SGS project No. 21066/115 at the Technical University of Liberec.

REFERENCES

[1] H.-J. Park, H. Y. Kim, S. Cha, C. H. Ahn, J. Roh, S. Park, S. Kim, K. Choi, J. Yi, Y. Kim, and J. Yoon, "Removal characteristics of engineered nanoparticles by activated sludge," *Chemosphere*, vol. 92, no. 5, pp. 524–528, Jul. 2013.

[2] S. Eduok, B. Martin, R. Villa, A. Nocker, B. Jefferson, and F. Coulon, "Evaluation of engineered nanoparticle toxic effect on wastewater microorganisms: Current status and challenges," *Ecotoxicol. Environ. Saf.*, vol. 95, pp. 1–9, Sep. 2013.

[3] S. K. Brar, M. Verma, R. D. Tyagi, and R. Y. Surampalli, "Engineered nanoparticles in wastewater and wastewater sludge – Evidence and impacts," *Waste Manag.*, vol. 30, no. 3, pp. 504–520, Mar. 2010.

[4] L. Otero-González, J. A. Field, I. A. C. Calderon, C. A. Aspinwall, F. Shadman, C. Zeng, and R. Sierra-Alvarez, "Fate of fluorescent core-shell silica nanoparticles during simulated secondary wastewater treatment," *Water Res.*, vol. 77, pp. 170–178, Jun. 2015.

[5] H. Mu, Y. Chen, and N. Xiao, "Effects of metal oxide nanoparticles (TiO2, Al2O3, SiO2 and ZnO) on waste activated sludge anaerobic digestion," *Bioresour. Technol.*, vol. 102, no. 22, pp. 10305–10311, Nov. 2011.

[6] J. Farkas, H. Peter, T. M. Ciesielski, K. V. Thomas, R. Sommaruga, W. Salvenmoser, G. A. Weyhenmeyer, L. J. Tranvik, and B. M. Jenssen, "Impact of TiO2 nanoparticles on freshwater bacteria from three Swedish lakes," *Sci. Total Environ.*, vol. 535, pp. 85–93, Dec. 2015.

[7] T. Sreethawong, S. Ngamsinlapasathian, and S. Yoshikawa, "Synthesis of crystalline mesoporous-assembled ZrO2 nanoparticles via a facile surfactant-aided sol–gel process and their photocatalytic dye degradation activity," *Chem. Eng. J.*, vol. 228, pp. 256–262, Jul. 2013.

[8] P. A. Holden, F. Klaessig, R. F. Turco, J. H. Priester, C. M. Rico, H. Avila-Arias, M. Mortimer, K. Pacpaco, and J. L. Gardea-Torresdey, "Evaluation of Exposure Concentrations Used in Assessing Manufactured Nanomaterial Environmental Hazards: Are They Relevant?," *Environ. Sci. Technol.*, vol. 48, no. 18, pp. 10541–10551, Sep. 2014.

[9] M. Mahmoudi, J. Meng, X. Xue, X. J. Liang, M. Rahman, C. Pfeiffer, R. Hartmann, P. R. Gil, B. Pelaz, W. J. Parak, P. del Pino, S. Carregal-Romero, A. G. Kanaras, and S. Tamil Selvan, "Interaction of stable colloidal nanoparticles with cellular membranes," *Biotechnol. Adv.*, vol. 32, no. 4, pp. 679–692, Jul. 2014.

[10] W. Jiang, H. Mashayekhi, and B. Xing, "Bacterial toxicity comparison between nano- and micro-scaled oxide particles," *Environ. Pollut.*, vol. 157, no. 5, pp. 1619–1625, May 2009.