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Comparison of the influence of nanoparticles on *Escherichia coli* and *Pseudomonas aeruginosa* bacterial populations

Petra Škodová, Lucie Křiklavová, Tomáš Lederer, Totka Bakalova, Lukáš Voleský

Institute for Nanomaterials, Advanced Technologies and Innovation | Bendlova 1409/7 | 461 17 Liberec | Czech Republic

E-mail: petra.skodova@tul.cz

Abstract

As use of nanoparticles continues to increase in various fields of human activity, it becomes increasingly important to understand all interactions that occur between nanoparticles and cells. In this experiment, we explore the influence of several types of nanoparticle on populations of facultative anaerobic bacteria (*Escherichia coli*) and aerobic bacteria (*Pseudomonas aeruginosa*). Nanoparticle toxicity (nanoparticle concentration 1 g/l) was evaluated through respirometry and microscopic fluorescence analysis, which allows for observation and comparison of both living and dead cells in a sample. Nanodiamond proved to be the least toxic of the nanoparticles tested, while nanoparticles of praseodymium had the most toxic effect on bacterial populations. A scanning electron microscope (SEM) was used to assess both the appearance and the approximate sizes of the nanoparticles and bacteria. SEM confirmed colonization of nanoparticle aggregate surfaces by bacteria in almost all samples.

Introduction

The use of nanoparticles in commercial products and industrial applications has increased dramatically, despite a general lack of understanding of molecular-level reaction mechanisms between nanoparticles and biological systems. The first interaction with nanoparticles occurs at the cell membrane, the actual interaction being dependent on the physicochemical property of the nanoparticle concerned. Small change in size, shape, charge, or chemical composition can result in radically different interactions with living systems.

As a first step in obtaining comprehensive information on the interaction of nanoparticles with biological agents, we assessed the influence of selected nanoparticles on bacterial populations of *E. coli* and *P. aeruginosa*. In both cases, the experiment was performed in an aqueous culture medium in order to obtain information on the preparation of appropriate future testing methodologies.

Methods

- Tested nanoparticles: diamonds, TiO_2, SiO_2, Bi, Ce, Pb and Pr
- Culture media: soya broth and BSM medium
- Effect of nanoparticles was evaluated using:
  - respirometry
  - microscopic fluorescence analysis (live/dead cells)
  - determination of colony forming units (CFU/ml)
  - SEM microscopy
- Experimental design:
  - 18 ml media + 2 ml saline solution containing either *E. coli* or *P. aeruginosa* – initial absorbance ~ 0,9 at 600 nm + 1 g/l nanoparticles
- Respiration was measured for 5 days, microscopic fluorescence analysis and determination of CFU/ml were performed 1st and 5th day of the experiment.

Results

- Nanoparticles of Bi Ce, Pb and Pr decreased the respiratory activity of bacteria in all samples when compared to the control. Greatest toxicity was observed in samples containing nano-Pr (Fig. 1).
- The effect of nanoparticles on cumulative oxygen consumption in samples containing *E. coli:* nanodiamond – generally higher respiratory activity than the control; nano-SiO_2, TiO_2 – results close to or just above the control (Fig. 3)
- Cumulative oxygen consumption in samples containing *P. aeruginosa* was almost the same for all nanoparticles tested and did not differ from the control (Fig. 5).

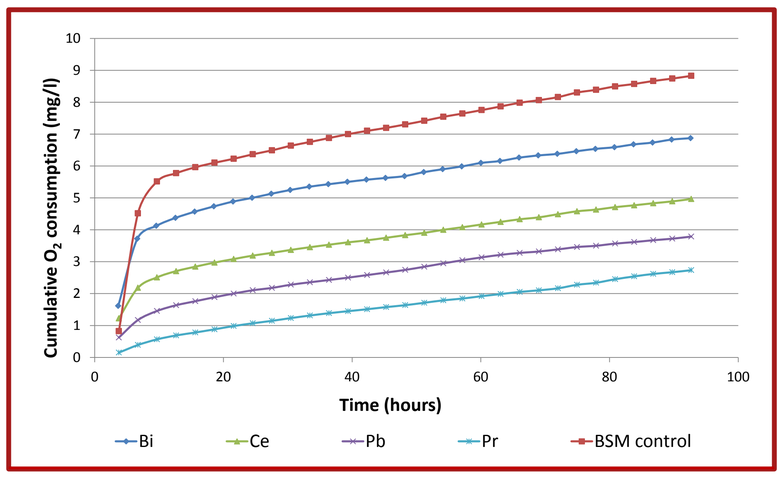

Fig. 1: Cumulative oxygen consumption of *E. coli* in BSM media containing different nanoparticles

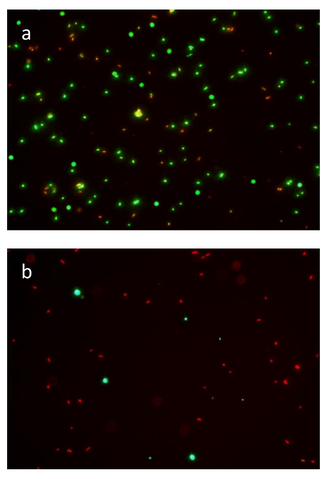

Fig. 2: Fluorescence analysis of *E. coli* with Bi
a) 1st day; b) 5th day

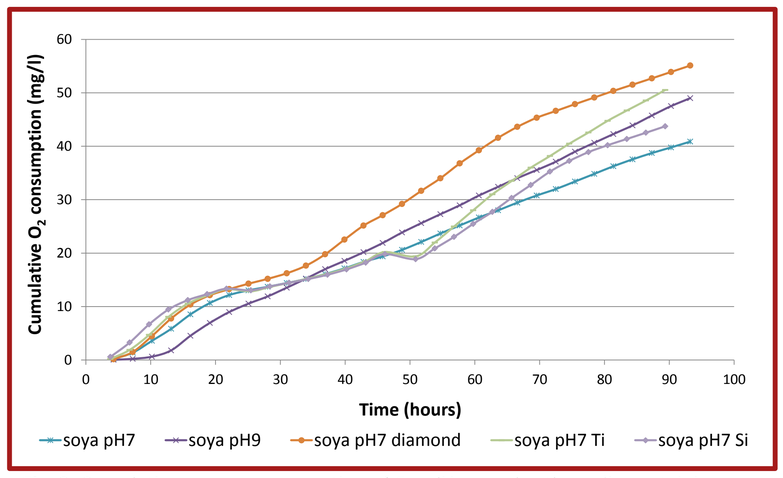

Fig. 3: Cumulative oxygen consumption of *E. coli* in soya broth media containing different nanoparticles

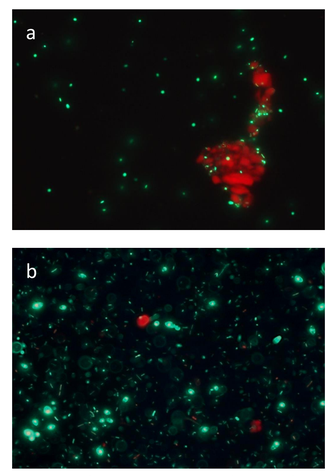

Fig. 4: Fluorescence analysis of *E. coli* with nano-SiO_2
a) 1st day; b) 5th day

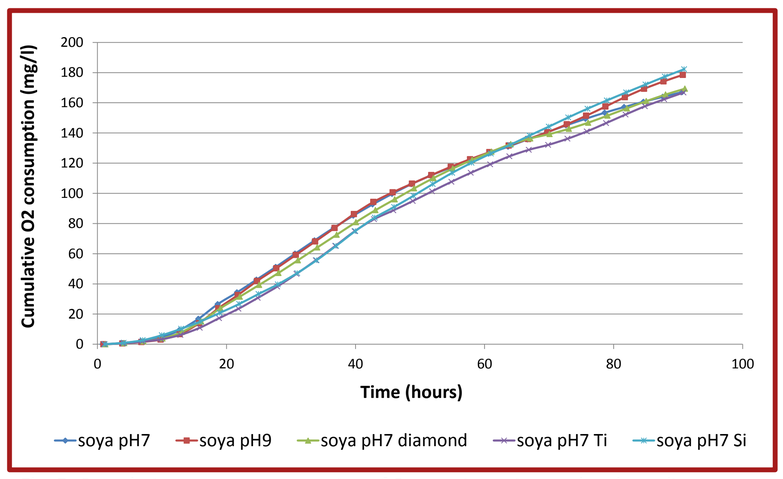

Fig. 5: Cumulative oxygen consumption of *P. aeruginosa i*n soya broth media containing different nanoparticles

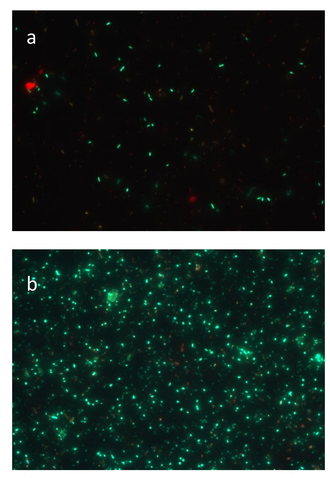

Fig. 6: Fluorescence analysis of *P. aeruginosa* with nano-SiO_2
a) 1st day; b) 5th day

- SEM confirmed colonization of aggregated nanodiamond, nano-SiO_2 and nano-TiO_2 by *E. coli* (Fig. 7).

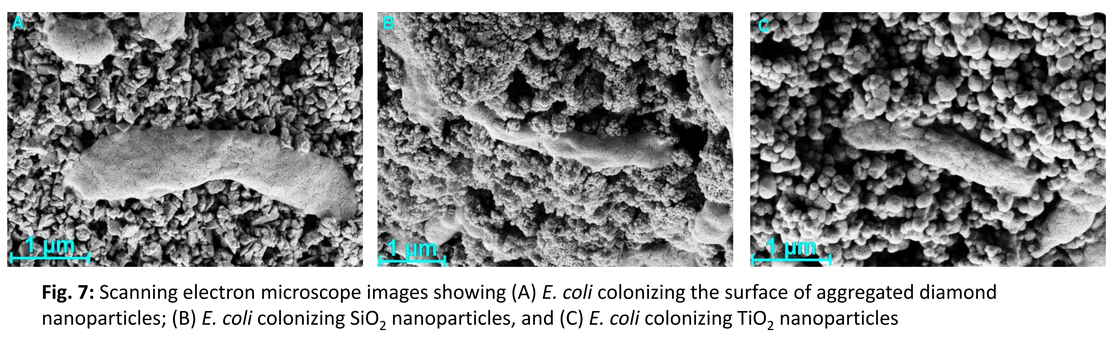

Fig. 7: Scanning electron microscope images showing (A) *E. coli* colonizing the surface of aggregated diamond nanoparticles; (B) *E. coli* colonizing SiO_2 nanoparticles, and (C) *E. coli* colonizing TiO_2 nanoparticles

Conclusion

The results indicate that nanoparticles of Bi, Ce, Pb and Pr do have a toxic effect on population of *E. coli*. Respirometry indicated that nanoparticles of Pr were the most toxic to *E. coli*, which was also confirmed by microscopic fluorescence analysis. No toxic effect was observed for nanoparticles of TiO_2, SiO_2 or diamond. *E. coli* tended to grow faster in the presence of these nanoparticles. In samples containing nanodiamond at concentration of 1 g/l in culture medium, *E. coli* cells proliferated around 35 % faster then the control sample. Respirometry indicated that nanoparticles of TiO_2, SiO_2 or diamond do have no effect on population of *P. aeruginosa*. The results of the sample with nanoparticles were almost the same as control sample. SEM indicated that both *E. coli* and *P. aeruginosa* colonized the nanoparticle aggregate surfaces in all samples.

Acknowledgement

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