COMPARISON OF THE INFLUENCE OF NANOPARTICLES ON *ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA* BACTERIAL POPULATIONS

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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. These two methods were supplemented by determination of colony forming units. Basal salt medium (BSM) with differing concentration of glucose and a soya broth medium were used for both bacterial populations as a culture medium. Nanodiamond proved to be the least toxic of the nanoparticles tested (though these contained impurities that may have affected the results), 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. The bacteria clearly differed in size, with *E. coli* averaging 3-4 μm and *P. aeruginosa* averaging 1.5 μm. The various nanoparticles ranged between 50 and 200 nm. SEM confirmed colonization of nanoparticle aggregate surfaces by bacteria in almost all samples.

Keywords: Nanoparticles, toxicity, scanning electron microscopy (SEM), *Escherichia coli*, *Pseudomonas aeruginosa*

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 [1]. As nanoparticles are now being released into the air, water and soil, it is possible that they may affect human health. Though the effects of many common metals and materials on human health and the environment are reasonably well known, there is a clear need for further studies on their effects when these same materials are produced at nanometer size. In particular, it is the size and shape of nanoparticles that poses a potential threat [2]. In biological materials, the first interaction with nanoparticles occurs at the cell membrane, the actual interaction being dependent on the physicochemical property of the nanoparticle concerned. Small changes in size, shape, charge, or chemical composition can result in radically different interactions with living systems [3, 4].

Nanomaterial toxicity can be caused by a range of factors. It has been shown, for example, that nanostructures have electronic, optical and magnetic properties that are related to their physical dimensions and disintegration of these agglomerated nanostructures could lead to unique toxic effects that are difficult to predict [5, 6]. Furthermore, nanostructure surfaces are involved in many catalytic and oxidative reactions. Should such reactions induce cytotoxicity, the resulting toxicity may be greater than that from microparticles of the same material due to the greater surface-to-volume ratio at nanoscales [6].

At present, there are no standardized methods for testing the potential toxicity of nanoparticles released into different components of the environment. 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 Escherichia coli and Pseudomonas 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.

1. METHODS

Two basic methods were selected to evaluate the effect of nanoparticles on *E. coli* and *P. aeruginosa*: respirometry and microscopic fluorescence analysis.

Experimental design for respirometry - Bacterial respiration activity was measured using a Micro-Oxymax respirometer (Columbus Instruments International, USA) according to standard methodology EN ISO 9408 of the Czech Office for Standards, Metrology and Testing, using inorganic nanoparticles instead of organic compounds. Respiration was measured for 5 days on a sample comprising 18 ml media and 2 ml of saline solution containing either *E. coli* or *P. aeruginosa*. Two culture media were used during the experiment, a basal salt medium (BSM) with differing concentrations of glucose, and a soya broth medium. The BSM comprised 0.043 g KH_2PO_4, 0.033 g K_2HPO_4, 0.178 g $(NH_4)_2SO_4$, 0.085 g $MgCl_2.6H_2O$ and 0.25 ml of a trace element solution (1 g/l $MnCl_2.4H_2O$, 0.196 g/l $CaCl_2$, 0.6 g/l $FeSO_4.7H_2O$, and 2 g/l $Na_2MoO_4.2H_2O$) dissolved in 250 ml of distilled water. Initial absorbance of the test solution (with bacterial population) was ~0.9 at 600 nm measured with a DR 6000 UV-VIS spectrophotometer (Hach-Lange, Germany). Respiration growth curves were produced for samples containing nanoparticles at 1 g/l and for control media without nanoparticles.

Experimental design for microscopic fluorescence analysis - Microscopic evaluation 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 the 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). Microscopic analysis was performed on the first and fifth day of the experiment.

These methods were complemented by determination of colony forming units (measured as CFU/ml) according to standard methodology EN ISO 6222 of the Czech Office for Standards, Metrology and Testing. Samples for CFU determination were taken during deployment and termination of the experiment. **Nanoparticles used for toxicity analysis -** Nanoparticles of diamond, TiO_2, SiO_2, Pr, Pb, Ce and Bi were selected for evaluation of toxicity to *E. coli* and *P. aeruginosa*.

The appearance and size of the nanoparticles were evaluated using a Carl Zeiss Ultra Plus scanning electron microscope (SEM) with SE2, InLens, AsB and EsB detectors. In this experiment Au was used as a conductive coating on the sample. Imaging took place at an acceleration voltage of 2.5 kW and at a working distance of between 1 and 15 mm.

2. RESULTS

The effect of nanoparticles on cumulative oxygen consumption in samples containing *E. coli* varied, with nanodiamond generally showing higher respiratory activity than the control (soya broth pH7 without nanoparticles) and other nanoparticles providing results close to or just above the control (Fig. 1). Cumulative oxygen consumption in samples containing *P. aeruginosa* was almost the same for all nanoparticles tested and did not differ significantly from the control (Fig. 2).

Presence of nano-Bi, Ce, Pb and Pr had a clear effect on the respiratory activity of *E. coli* in BSM. These nanoparticles decreased the respiratory activity of bacteria in all samples when compared to the control with no added nanoparticles. Greatest toxicity was observed in samples containing nano-Pr (Fig. 3).

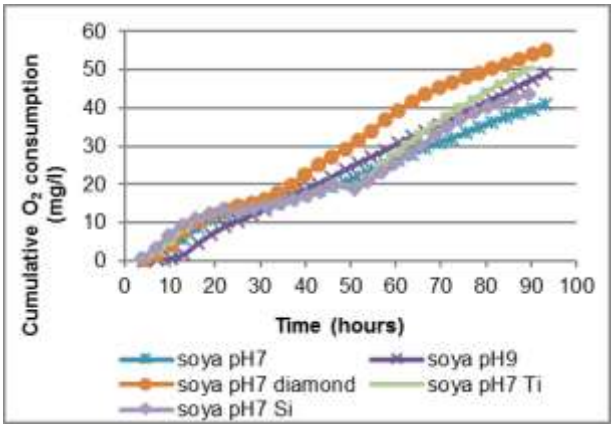

Fig. 1 Cumulative oxygen consumption of *E. coli* in soya broth media containing different nanoparticles

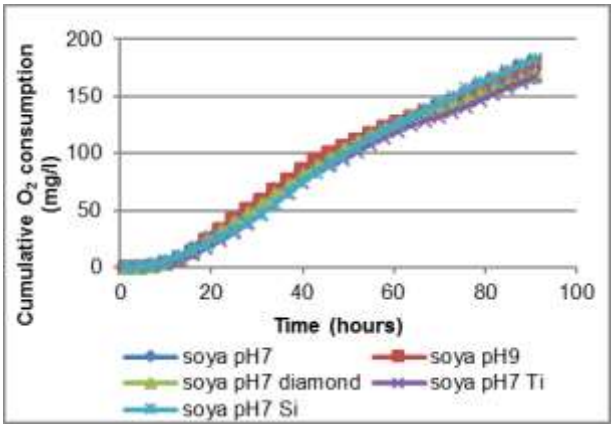

Fig. 2 Cumulative oxygen consumption of *P. aeruginosa* in soya broth media containing different nanoparticles

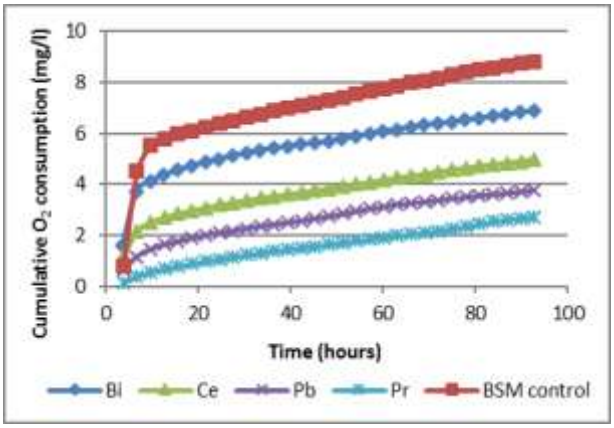

Fig. 3 Cumulative oxygen consumption of *E. coli* in BSM media containing different nanoparticles

Maximum oxygen consumption differed between samples containing *E. coli* and *P. aeruginosa* (Figs. 1, 2; Tables 1, 2), with maximum respiration activity for *E. coli* at 55.1 mg/l (corresponds to 135 %) with nanodiamond after 90 hours in soya broth media, while the results for *P. aeruginosa* indicate a maximum value of 182.2 mg/l (corresponds to 109 %) with nano-Si in soya broth media. The results obtained from fluorescence microscopy tend to correspond with the respirometry data.

Table 1: Maximal respiration of *E. coli*

Sample	Max. O_2 consumption *E. coli* (%)
soya pH7 (control)	100
soya pH9	120
soya pH7 diamond	135
soya pH7 Ti	124
soya pH7 Si	107

Table 2: Maximal respiration of *P. aeruginosa*

Sample	Max. O_2 consumption *P. aeruginosa* (%)
soya pH7 (control)	100
soya pH9	107
soya pH7 diamond	101
soya pH7 Ti	100
soya pH7 Si	109

SEM confirmed colonization of aggregated nano-diamond, nano-SiO_2 and nano-TiO_2 by *E. coli* (Fig. 4). In almost all images, the cell's shape appears to adapt to the surface of the aggregated nanoparticles and the nanoparticles appear to enter the cell walls. The size of *E. coli* in the samples was around 3.7 µm while cells of *P. aeruginosa* were usually around 1.5 µm. The nanoparticles of diamond, SiO_2 and TiO_2 differed slightly in shape (Fig. 4) and ranged in size from 50 to 200 nm.

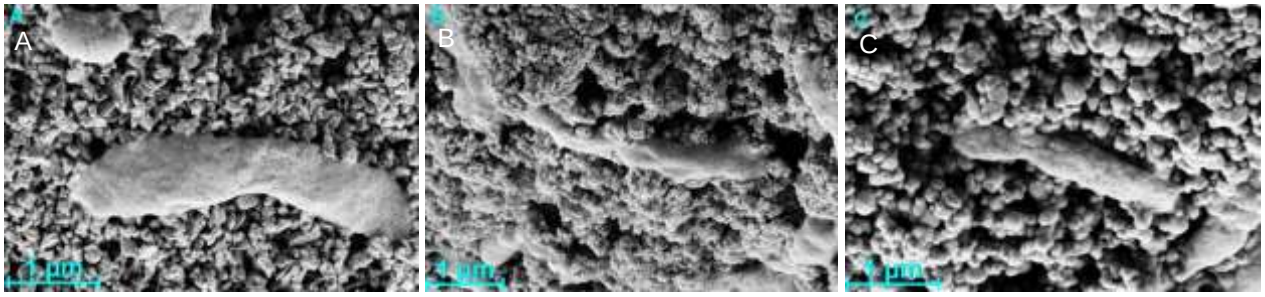

Fig. 4 Scanning electron microscope images showing (A) *Escherichia coli* colonizing the surface of aggregated diamond nanoparticles; (B) *E. coli* colonizing SiO_2 nanoparticles, and (C) *E. coli* colonizing TiO_2 nanoparticles.

CONCLUSION

The results indicate that nanoparticles of Bi, Ce, Pb and Pr do have a toxic effect on populations 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 TiO2, SiO2 or diamond; rather, E. coli tended to grow faster in the presence of these nanoparticles. In samples containing nanodiamond at a concentration of 1 g/l in a culture medium, E. coli cells proliferated around 35 % faster than the control sample. It is likely that the nanodiamond contained contaminants in the form carbon that could be used by the bacteria as a C-source.

SEM indicated that both E. coli and P. aeruginosa colonized the nanoparticle aggregate surfaces in all samples. In almost all images the bacteria appear to adapt their shape to the aggregated nanoparticle surface and the nanoparticles appear to penetrate the cell walls.

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