Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.884528

52 (2014) 3680–3689
May

Taylor & Francis
Taylor & Francis Group

The impacts of aluminum and zirconium nano-oxides on planktonic and biofilm bacteria

Nina Chrzanowska*, Monika Załęska-Radziwiłł

Faculty of Environmental Engineering, Department of Biology, Warsaw University of Technology, Nowowiejska 20, Warsaw 00-653, Poland
Email: nina.chrzanowska@is.pw.edu.pl

Received 18 March 2013; Accepted 8 July 2013

ABSTRACT

Nanoparticles are molecules, whose dimensions are below 100 nm; they are of colloidal particle size and are often smaller than eukaryotic cells and bacteria. Their increasingly common application might cause their release into sewage and the induction of toxic reactions, among others, in micro-organisms participating in wastewater treatment. The aim of this study was to compare the ecotoxicity of commercial nanoparticles of aluminum oxides and zirconium oxides, in relation to two bacterial strains *Pseudomonas putida* and *Aeromonas hydrophila*. These bacteria have an ability to form biofilms, as they are present in planktons and participate in wastewater treatment. The study also includes the assessment of aluminum oxide (Al_2O_3) and zirconium oxides' (ZrO_2) ecotoxicity in order to compare their harmfulness with the nanoparticular form. It has been found that aluminum and zirconium nano-oxides were more harmful to bacteria compared to aluminum and zirconium oxides. Biofilm-forming bacteria were more resistant than planktonic bacteria to the influence of both types of compounds. Aluminum nano-oxide proved to be more toxic than zirconium nano-oxide in relation to both species of bacteria. Nanoparticles appeared to be less toxic towards bacteria with EPS. *A. hydrophila* strain showed lower sensitivity than *P. putida* to the studied nanoparticles.

Keywords: Aluminum nano-oxide; Zirconium nano-oxide; Ecotoxicity; Biofilms; Planktonic bacteria

1. Introduction

Nanoparticles are structures in which at least one of two dimensions does not exceed 100 nm. These particles are characterized by specific properties such as: high surface-to-volume ratio, mechanical strength, chemical reactivity, an ability to create aggregates, and diffusivity. These properties can simultaneously lead to the increase in their bioavailability and toxicity [1–3]. Owing to their chemical and physical properties, nanoparticles have become an attractive material for commercial and technological use [4–7]. It has been

*Corresponding author.

Presented at the 11th Scientific Conference on Microcontaminants in Human Environment. 25–27 September 2013, Wisla, Poland
Organized by Department of Chemistry, Water and Wastewater Technology,
Faculty of Environmental Engineering and Biotechnology, Czestochowa University of Technology

1944-3994/1944-3986 © 2014 Balaban Desalination Publications. All rights reserved.

estimated that investment expenditures in the field of nanotechnology worldwide rose fivefold from 825 million dollars in the year 2000 to 4.1 billion dollars in 2005 [8].

The intensive growth of nanotechnology triggers the increase of nanoparticle content in sewage and waste, which as a consequence makes them enter the surface waters and water intended for human consumption [9].

Nanoparticles can have some negative influence on micro-organisms participating in wastewater treatment and water conditioning. This impact might be clearly observed in appliances in which micro-organisms form biological membranes—biofilms, i.e. in different types of biofilters. A biofilm is a complex of micro-organisms, attached to solid surfaces, capable of producing exopolysaccharides (EPS) which among other things protects them against a disadvantageous influence of environmental conditions [9,10].

In the literary sources there is little data concerning the negative impact of nanoparticles on micro-organisms in wastewater treatment, and if so it mainly concerns free-floating micro-organisms, the so-called bacterioplankton including nitrifying micro-organisms.

Choi's studies demonstrated that during wastewater treatment, the nano-silver with a concentration of 1 mg/L inhibited the growth of autotrophic nitrifying bacteria by about 80% [11]. In turn, the research conducted by Zheng proved that zinc oxide nanoparticles had a disadvantageous influence on biological processes of nitrogen and phosphorus removal as well as on denitrifying bacteria and micro-organisms responsible for phosphorus elimination, the so-called PAO (*polyphosphate accumulating organisms*). Nitrogen removal efficiency decreased from 81.5 to 75.6% and 70.8%, with concentrations of 10 and 50 mg/L nano-ZnO, respectively. These results indicate that zinc oxide nanoparticles lead to the disruption of activated sludge functioning [12].

This study presents the research on the influence of aluminum oxide nanoparticles (nano-Al_2O_3, <50 nm) and zirconium oxide nanoparticles (nano-ZrO_2, <100 nm) on the survival of two bacterial strains which are capable of biofilm formation. Little information is available on the fate, transport, and effects of nanomaterials, including metal-based particles such as nano-sized Al_2O_3 and ZrO_2, in the environment. Nano-sized aluminum is currently being used by the military and in commercial industries in many applications including coatings, thermites, and propellants. The use of nano-aluminum in various applications may cause a release of the oxidized form,

Table 1
Effect of aluminum nano-oxide and aluminum oxide on *A. Hydrophila* biofilm

Sample type	Range of concentration tested [mg/L]	Biofilm-forming bacteria											
		A. hydrophila with EPS						*A. hydrophila* without EPS					
		EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h	EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h
Nano-Al_2O_3	1,000 – 0.48	>1,000	<0.48	>1,000	<0.48	709.68 (±35.48)	<0.48	>1,000	<0.48	>1,000	<0.48	102.62 (±5.13)	<0.48
Al_2O_3		>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48

nano-Al_2O_3, into the environment. As utilization of nanomaterials is on the rise, it is increasingly important to determine their potential environmental fate and the effects [13]. Recently, such issues as preconcentration and separation of trace elements and organic compounds in the sample solutions by means of nanoparticles ZrO_2 have been discussed in various literary sources. These nanoparticles have unique properties, so they are promising solid-phase extractants and have contaminant scavenging mechanisms [14,15]. Therefore, the explosion of nanotechnology applications makes it inevitable that nano-Al_2O_3 and nano-ZrO_2 will be released into domestic and industrial waste streams. These nanoparticles exert toxic effects on micro-organisms, so their release into wastewater systems may adversely affect the microbial communities found in biological treatment processes [9].

The ecotoxicity of these compounds has been determined in relation to the bacteria in biofilm and to those remaining in mid-water (planktonic). Also, the influence of nanoparticular forms of aluminum oxide and zirconium oxide on micro-organisms has been compared to the effect of their "macro" forms.

2. Materials and methods

2.1. Chemicals

Aluminum nano-oxide, Al_2O_3, nanopowder <50 nm with a specific surface area >40 m^2/g, and zirconium nano-oxide ZrO_2, nanopowder <100 nm with a specific surface area ≥25 m^2/g, were obtained from Sigma-Aldrich. The stock solutions of nano-oxides and oxides (Sigma-Aldrich) with a concentration of 1,000 mg/L were prepared in deionized water. Because nanoparticles are able to form aggregates, the stock dispersion was sonicated (0.4 kW, 20 kHz) for 30 min to break aggregates before being diluted to the exposure concentrations. The stock solutions were diluted (using the medium with respect to the procedures of tests) in descending order with a geometric series of quotient $q = 2$ to obtain final 1,000–0.48 mg/L.

2.2. Bacterial strains

Heterotrophic gram-negative rods of *Pseudomonas putida* and *Aeromonas hydrophila* were isolated from the activated sludge working in laboratory conditions in the Department of Biology, Faculty of Environmental Engineering at Warsaw University of Technology. Bacteria were isolated from the activated sludge working in laboratory conditions. Aseptic technique was used throughout the testing process. Constant bacterial cultures were maintained throughout the experimentation and incubations for all tests were conducted for 48 h at 37°C.

Bacterial cultures were then subjected to biochemical reaction. A gram stain was implemented to determine whether the bacteria were positive or negative. Oxidase test was performed by adding bacterial smear to filter paper containing oxidase reagent (a mixture of dimethyl-4-phenylenediamine hydrochloride and α-naphthol), and color development was observed within 1 min. Catalase test was performed by adding one drop of 30% hydrogen peroxide (Aflofarm, Pabianice, PL) to a slide that contained bacterial smear. Bubbling reaction was observed within 1 min. API 20 NE bacterial identification was performed according to manufacturer's instruction (bioMérieux, Durham, NC).

The selection of strains was based upon the ability of these bacteria to be used as xenobiotics, as the source of carbon and energy, and their adhesive capacity and biofilm formation capacity in compliance with quorum sensing [16–20]. The strains were multiplied in nutrient broth at a temperature of 26°C for 18 h until the commencement of the logarithmic growth phase.

2.3. Removal of the loosely bound EPS surface layer

The EPS components contained in biofilm that can be readily removed are defined as "loosely bound EPS." Those EPS that need special removal processes are defined as "tightly bound EPS." Sheng's procedure

Table 2
Effect of aluminum nano-oxide and aluminum oxide on planktonic *A. Hydrophila* cells

		Planktonic bacteria					
		P. putida					
Sample type	Range of concentration tested [mg/L]	EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h
Nano-Al_2O_3	1,000 – 0.48	>1,000	<0.48	228.57 (±13.71)	<0.48	16.33 (±0.81)	<0.48
Al_2O_3		>1,000	<0.48	>1,000	<0.48	111.21 (±5.56)	<0.48

was applied in the experiments, allowing the extraction of the loosely bound EPS layer and maintaining cell viability. Biofilms were smoothly scraped off the polystyrene plates and suspended in 5 mL 1% phosphate buffered saline (PBS, 8 g NaCl, 0.2 g KH_2PO_4, 1.11 g Na_2HPO_4 0.2 g KCl). A 30-s vortex was performed to mix biofilm fragments with PBS. The biofilm suspension was vortexed at the maximum speed for 1 min, then centrifuged at 4˚C, 4,000 g, for 20 min. The pellets were resuspended in 5 mL of 1% PBS, vortexed, and centrifuged again. Pellet resuspension, vortexing, and centrifugation were repeated two times [9].

2.4. Studying the effects of nanoparticles on biofilms

Cultures *P. putida* and *A. hydrophila* in the logarithmic growth phase were diluted with nutrient broth to optical density OD_{600} in the range of 0.1–0.15, inserted into the wells in polystyrene plates (96 well) 25 μL in each, and incubated at a temperature of 26˚C for 48 h. Biological membranes started to form in the wells. After 24 h the wells were rinsed three times with 0.9% NaCl in order to remove the unattached cells. Aluminum and zirconium nano-oxides with concentrations of 1,000–0.48 mg/L were inserted in the rinsed plates. Wells with medium were considered a negative control. Prepared plates were protected by a cover.

After a given period of incubation, the plates were rinsed with distilled water, and the each well was filled with ethanol 250 μL for cell preservation and incubated at room temperature for 15 min. After alcohol was removed and cells dried at 37˚C, 250 μL of 0.1% crystal violet solution was added to each well in order to stain the bacterial cells [21–23]. After 15 min, the plates were rinsed with water and each well was filled with 250 μL of 33% acetic acid solution. The next step was to measure the absorbance at a wavelength of $\lambda = 570$ nm in the wells. The absorbance value equalled the density of cells after they were released from the foundation. The measurement was performed with a Bio-Rad microplate reader. The above-mentioned procedure was also applied to evaluate the impact of the aluminum and zirconium oxides in their "macro" on the bacteria in both biofilm types.

2.5. Studying the effects of nanoparticles on biofilm bacteria without loosely bound EPS

Following the isolation of loosely bound EPS (look section 2.4.) the obtained residue of *P. putida*

Table 3
Effect of aluminum nano-oxide and aluminum oxide on *P. putida* biofilm

Sample type	Range of concentration tested [mg/L]	Biofilm-forming bacteria						*P. putida* without EPS					
		P. putida with EPS											
		EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h	EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h
Nano-Al_2O_3	1,000 – 0.48	1,000	<0.48	>1,000	<0.48	480.63 (±24.03)	<0.48	>1,000	<0.48	463.68 (±23.18)	<0.48	10.75 (±0.53)	<0.48
Al_2O_3		>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	791,15 (±47.46)	<0.48

and *A. hydrophila* sustained in 1% PBS was inserted in the wells in polystyrene plates (96 well) containing specified concentrations of aluminum nano-oxide and zirconium nano-oxide. The samples were incubated at 26˚C for 24–72 h. The measurements of the optical density of the samples with a wavelength of $\lambda = 600$ nm were performed to evaluate the biofilm bacteria growth without EPS. The above procedure was also used to evaluate the influence of aluminum and zirconium oxides on bacterial growth.

2.6. Studying the effects of nanoparticles on planktonic bacteria

The plate wells with a broth base with specified concentrations of aluminum nano-oxide and zirconium nano-oxide were filled with bacterial suspension of optical density OD_{600} amounting to 0.1–0.15. The cultures were incubated at 26˚C for 24–72 h. The measurements of the optical density of the samples were performed with a wavelength of $\lambda = 600$ nm in order to evaluate bacterial growth. The above procedure was also used to evaluate the influence aluminum and zirconium oxides on bacterial growth.

The experiment was repeated 10 times and the results constitute the arithmetic mean of the measurement values. Additionally, represented in the results was the initial turbidity of the samples following the addition of Al and Zr oxides and nanoparticles and the absorbance measurement with a wavelength of $\lambda = 600$ nm.

2.7. Calculation procedures

2.7.1. Bacterial growth inhibition

Bacterial growth inhibition was determined on the basis of the following formula:

$$I = \frac{B_c - B_n}{B_c - B_0} \times 100$$

where I—percentage of inhibition; B_c—optical density of suspension in 1 mL of control sample after time t; B_n—optical density of suspension in 1 mL of the sample examined after time t; and B_0—optical density of suspension in 1 mL of control sample after time t_0.

2.7.2. Calculation of concentrations EC_{50} and no observed effect of concentration (NOEC)

Effect of concentrations (EC50-t) were calculated using probit analysis [24]:

$$EC50 = N\lg\frac{5 - \bar{y} + b\bar{x}}{b}$$

where Nlg—antilog; $\bar{y}$—the average probit corresponding to the percent inhibition of growth or other effect for each concentration, as:

$$\frac{1}{k}\sum_{i-1}^{k} y_i$$

k—number of levels included in the calculations, y_i—probit corresponding to the percentage effect for i concentration, 5—probit constant value corresponding to 50% of the effect, and $\bar{x}$—the mean value of logarithms of particular concentrations, calculated according to the formula:

$$\frac{1}{k}\sum_{i-1}^{k} x_i$$

x_i—logarithm of i concentration and b—regression coefficient, calculated according to the formula:

$$b = \frac{\sum_{i=1}^{k} x_i y_i - \bar{x}\sum_{i=1}^{k} y_i}{\sum_{i=1}^{k} x_i^2 - \bar{x}\sum_{i=1}^{k} x_i}$$

Table 4
Effect of aluminum nano-oxide and aluminum oxide on planktonic *P. putida* cells

		Planktonic bacteria					
		P. putida					
Sample type	Range of concentration tested [mg/L]	EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h
Nano-Al_2O_3	1,000 – 0.48	>1,000	<0.48	59.08 (±3.54)	<0.48	3.34 (±0.20)	<0.48
Al_2O_3		>1,000	<0.48	>1,000	<0.48	61.39 (±3.06)	<0.48

NOEC was determined with single-factor analysis of variance (ANOVA, $p < 0.05$) and Tukey's test [25].

3. Results and discussion

The results of the examination of the influence of aluminum and zirconium nanoparticles on *P. putida* and *A. hydrophila* biofilms and planktonic forms are presented in Tables 1–8. All the compounds analyzed showed a negative effect on micro-organisms and the obtained effective concentrations revealed their diversified susceptibility to the nanocompounds studied.

On the basis of a colorimetric test with crystal violet, which is used for assessing the influence of the studied compounds on the biofilm from bacterial strains, it was demonstrated that in the presence of aluminum nano-oxide, the concentration values of EC_{50} after 72 h were within the range of 709.68–480.63 mg/L for *A. hydrophila* (Table 1) and *P. putida* (Table 3), respectively.

The analyzed strains proved to be less susceptible to the influence of zirconium nano-oxide. The determined effective concentration values after 72 h amounted to 651.67 mg/L for *P. putida* (Table 7) and 1,000 mg/L for *A. hydrophila* (Table 5). The NOEC value after 72 h for *A. hydrophila* amounted to <0.48 mg/L as is the case with *P. putida* (Tables 5 and 7).

The nanoparticles proved to be more toxic towards the biofilm bacteria without extracellular envelope EPS. *P. putida* without the loosely bound EPS proved to be sensitive to nano-Al_2O_3—$EC_{50–72\ h}$ was 10.75 mg/L (Table 3). This compound was less toxic to *A. hydrophila*—$EC_{50–72\ h}$ amounting to 102.62 mg/L. The NOEC was <0.48 mg/L for both bacterial strains. Zirconium nano-oxide was less toxic than nano-Al_2O_3 in comparison to the biofilm bacteria without EPS. The effective concentration after 72 h for *P. putida* was 28.32 mg/L (NOEC < 0.48 mg/L) (Table 7) and 530.75 mg/L (NOEC < 0.48 mg/L) for *A. hydrophila* (Table 5).

The planktonic bacteria proved to be more susceptible to the influence of nanoparticles than the biofilm bacteria. EC_{50} value of aluminum nano-oxide for *P. putida* after three days was 3.34 mg/L (Table 4). A slightly lower susceptibility towards these bacteria was demonstrated by nano-ZrO_2. The effective concentration value which was obtained in the study was 14.53 mg/L (Table 8). *A. hydrophila* strain showed lower susceptibility to the nanoparticles studied. The specified EC_{50} values after 72 h were 16.33 mg/L for aluminum nano-oxide (Table 2) and 172.06 mg/L for zirconium nano-oxide (Table 6). Values of the highest concentrations not causing harmful effects (NOEC) for

Table 5
Effect of zirconium nano-oxide and zirconium oxide on *A. Hydrophila* biofilm

Sample type	Range of concentration tested [mg/L]	Biofilm-forming bacteria											
		A. hydrophila with EPS						*A. hydrophila* without EPS					
		EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h	EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h
Nano-ZrO_2	1,000 – 0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	530.75 (±26.53)	<0.48
ZrO_2		>1,000	0.48 (±0.02)	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48

the two nanocompounds were below 0.48 mg/L for both *P. putida* and *A. hydrophila* (Tables 2, 4, 6, and 8).

Data from the literary sources also indicate that the biofilms can be up to 1.000 times more resistant to toxicants than the planktonic cells [25,26]. Choi pointed out that the concentration of nano-silver inhibiting the growth of *Escherichia coli* in the biofilm form was fourfold higher (38 mg/L) than the concentration inhibiting bacterial growth in the planktonic form [26]. Furthermore, it was indicated that silver nanoparticles at the concentration of 228 μg/L caused bacterial growth in the biofilm form, but they turned out to be toxic to the planktonic bacteria, causing the reduction of the number of bacteria by over 80% [27].

Bacterial resistance to the influence of harmful substances might result from the specific biofilm structure and can be regulated by a number of specific mechanisms. It appears that this results from the biofilms' cells surrounded by extracellular polysaccharide EPS [25,26]. Exopolysaccharide reduces diffusion of antimicrobial substances into the biofilm. As the biofilm grows, the participation of polysaccharide components increases in its envelope, which also increases the number of free functional groups and contributes to the protection of microorganisms. Following the removal of the loosely bound EPS, cells of the micro-organisms in the biofilm became more susceptible to the influence of nano-Al_2A_3 and nano-ZrO_2 in comparison to the bacterial cells in the biofilm with EPS.

In the experiments with titanium dioxide nanoparticles conducted by Liu et al. it was pointed out that the biofilm-forming bacteria with EPS were more resistant to the influence of nanoparticles rather than the biofilm's micro-organism without EPS. The number of heterotrophic bacteria of the biofilm with EPS in the presence of TiO_2 nanoparticles was about 10^2 cfu/mL, and without EPS was only <10 cfu/mL [28].

The research conducted in this paper demonstrated the protective action of EPS. Furthermore, high resistance of the bacteria in biofilm to the influence of chemical substances results, among other reasons, from the reduction of the cell dimensions, the inhibition of physiological processes, the production of enzymes catalyzing the decomposition of harmful substances, and the increased production of antioxidants (e.g. glutathione). It is also assumed that some of them might modify the structure of antibacterial substances, thus changing their properties [9,29,30].

Table 6
Effect of zirconium nano-oxide and zirconium oxide on planktonic *A. Hydrophila* cells

		Planktonic bacteria					
		A. hydrophila					
Sample type	Range of concentration tested [mg/L]	EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h
Nano-ZrO_2	1,000 – 0.48	>1,000	<0.48	250.07 (±12.50)	<0.48	172.06 (±10.32)	<0.48
ZrO_2		>1,000	<0.48	>1,000	<0.48	>1,000	<0.48

Table 7
Effect of zirconium nano-oxide and zirconium oxide on *P. putida* biofilm

Sample type	Range of concentration tested [mg/L]	Biofilm-forming bacteria											
		P. putida with EPS						*P. putida* without EPS					
		EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h	EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOEC 72 h
Nano-ZrO_2	1,000 – 0.48	>1,000	<0.48	>1,000	<0.48	651.67 (±39.10)	<0.48	>1,000	<0.48	>1,000	<0.48	28.32 (±1.69)	<0.48
ZrO_2		>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48	>1,000	<0.48

Table 8
Effect of zirconium nano-oxide and zirconium oxide on planktonic *P. putida* cells

Sample type	Range of concentration tested [mg/L]	Planktonic bacteria					
		A. hydrophila					
		EC_{50} 24 h [mg/L]	NOEC 24 h	EC_{50} 48 h	NOEC 48 h	EC_{50} 72 h	NOE 72 h
Nano-ZrO_2	1,000 – 0.48	>1,000	<0.48	245.91 (±12.29)	<0.48	14.53 (±0.72)	<0.48
ZrO_2		>1,000	<0.48	>1,000	<0.48	>1,000	<0.48

The obtained results also indicate that the studied nanocompounds are more toxic than the same compounds in their "macro" forms (Tables 2–8). It has been found that nano-ZrO_2 is more toxic over the test time (24, 48, and 72 h) on bacteria than the ZrO_2 oxide. It might have resulted due to different properties of nanoparticles such as: high surface-to-volume ratio, a high chemical reactivity, an ability to form aggregates, diffusivity, and mechanical strength. Therefore, nanocompounds can show a different mechanism of action and can pose greater danger to the environment than the same substance in its "macro" form [31].

4. Conclusions and summary

The conducted studies concerning the ecotoxicity of the two types of zirconium (nano-ZrO_2) and aluminum (nano-Al_2O_3) nano-oxides for *P. putida* and *A. hydrophila* in biofilms and planktonic forms allowed formulating the following conclusions:

- Aluminum nano-oxides and zirconium nano-oxides had harmful influences on *P. putida* and *A. hydrophila* in planktonic and biofilm forms; nano-Al_2O_3 proved to be more toxic.
- The planktonic bacteria were more susceptible to the influence of both the compound types than the biofilm forming bacteria; biofilms are tolerant to nano-Al_2O_3 and ZrO_2 treatment. After the removal of loosely bound extracellular polymeric substances (EPS), the viability of wastewater biofilms was reduced.
- Toxicity of aluminum oxide (Al_2O_3) and zirconium oxide (ZrO_2) in molecular forms was definitely lower than in the case of the nano forms.

This research confirmed the data found in literary sources and showed that the presence of nanoparticles might negatively influence the communities of micro-organisms participating in biological processes of wastewater treatment by lowering the effectiveness of the pollutants' removal. It is important to use biofilm-forming bacteria to estimate the influence of nanoparticles on wastewater treatment systems, as they show different susceptibility to nanoparticles than planktonic bacteria.

It was found that nano forms of the tested compounds were posing greater risks to environment than the same compounds in the "macro" form. Therefore, available ecotoxicity data about compounds in their "macro" forms cannot be used to assess the harmfulness of their nano form counterparts.

This study increased the ecotoxicological knowledge and database in relation to the effect of aluminum nano-oxide and zirconium nano-oxide on micro-organisms. Test results can be used in the safety data sheets.

It is also important to study the interaction mechanism of nanoparticles with microbial cells on the molecular level because of the small dimensions of these compounds.

References

[1] A. Nel, T. Xia, L. Madler, N. Li, Toxic potential of materials at the nanolevel, Science 311 (2006) 622–627.
[2] M. Heinlaan, A. Ivask, I. Blinova, H.C. Dubourguier, A. Kahru, Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria Vibrio fischeri and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*, Chemosphere 71 (2008) 1308–1316.
[3] N. Chrzanowska, M. Załęska-Radziwiłł, Interdyscyplinarne Zagadnienia w Inżynierii Środowiska i Ochronie Środowiska [Interdisciplinary Issues in Environmental Engineering and Environmental Protection], Oficyna Wydawnicza Politechniki Wrocławskiej [The Publishing House of Wroclaw University of Technology], Wrocław, 2012, pp. 111–122.
[4] J. Fabrega, S.N. Luoma, Ch.R. Tyler, T.S. Galloway, J.R. Lead, Silver nanoparticles: Behaviour and effects in the aquatic environment, Environ. Int. 37 (2011) 517–531.
[5] S.K. Brar, V. Mausam, R.D. Tyagi, R.Y. Surampalli, Engineered nanoparticles in wastewater and wastewater sludge—Evidence and impacts, Waste Manage. 30 (2008) 504–520.
[6] G. Bystrzejewska-Piotrowska, J. Golimowski, P.L. Urban, Nanoparticles: Their potential toxicity, waste and environmental management, Waste Manage. 29 (2009) 2587–2595.
[7] M. Łebkowska, M. Załęska-Radziwiłł, Ekotoksyczność nanocząstek [Ecotoxicity of nanoparticles], Och. Śr. 33 (2011) 23–26.
[8] M.C. Roco, International perspective on government nanotechnology funding in 2005, J. Nanopart. Res. 7 (2005) 707–712.
[9] Z. Sheng, Y. Liu, Effects of silver nanoparticles on wastewater biofilms, Water Res. 45 (2011) 6039–6050.
[10] J.W. Costerton, P.S. Stewart, E.P. Greenberg, Bacterial biofilms: A common cause of persistent infections, Science 284 (1999) 1318–1322.
[11] O. Choi, K.K. Deng, Jr. Kim, L. Ross, R.Y. Surampalli, Z. Hu, The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth, Water Res. 42 (2008) 3066–3074.
[12] X. Zheng, R. Wu, Y. Chen, Effects of ZnO nanoparticles on wastewater biological nitrogen and phosphorus removal, Environ. Sci. Technol. 45 (2011) 2826–2832.
[13] J.G. Coleman, D.R. Johnson, J.K. Stanley, A.J. Bednar, C.A. Weiss Jr, R.E. Boyd, J.A. Steevens, Assessing the fate and effects of nano aluminum oxide in the terrestrial earthworm, Eisenia fetida, Environ. Toxicol. Chem. 29 (2010) 1575–1580.

[14] A. Kaur, U. Gupta, A review on applications of nanoparticles for the preconcentration of environmental pollutants, J. Mater. Chem. 19 (2009) 8279–8289.
[15] M. Hossein Habibi, E. Askari, M. Habibi, M. Zendehdel, Novel nanostructure zinc zirconate, zinc oxide or zirconium oxide pastes coated on fluorine doped tin oxide thin film as photoelectrochemical working electrodes for dye-sensitized solar cell, Spectrochim. Acta, Part A 104 (2013) 197–202.
[16] N.C.M. Gomes, I.A. Kosheleva, W.-R. Abraham, K. Smalla, Effects of the inoculant strain *Pseudomonas putida* KT2442 (pNF142) and of naphthalene contamination on the soil bacterial community, FEMS Microbiol. Ecol. 54 (2005) 21–33.
[17] P.G. Ward, M. Goff, M. Donner, W. Kaminsky, K.E. O'Connor, A two-step chemo-biotechnological conversion of polystyrene to a biodegradable thermoplastic, Environ. Sci. Technol. 40 (2006) 2433–2437.
[18] A. Ruiz-Manzano, L. Yuste, F. Rojo, Levels and activity of the *Pseudomonas putida* global regulatory protein Crc vary according to growth conditions, J. Bacteriol. 187 (2005) 3678–3686.
[19] E.V. Kompanets, N.M. Isaeva, I.A. Balakhnin, Bacteria of the genus Aeromonasand their role in aquaculture, Mikrobiol Zh. 54 (1992) 89–99.
[20] M.J. Lynch, S. Swift, D.F. Kirke, C.W. Keevil, C.E. Dodd, P. Williams, The regulation of biofilm development by quorum sensing in Aeromonas hydrophila, Environ. Microbiol. 4 (2002) 18–28.
[21] S. Stepanovic, I. Cirkovic, L. Ranin, M. Svabic-Vlahovic, Biofilm formation by Salmonella spp. and Listeria monocytogenes on plastic surface, Lett. Appl. Microbiol. 38 (2004) 428–432.
[22] Y. Kim, X. Wang, Q. Ma, X.-S. Zhang, T.K. Wood, Toxin-antitoxin systems in *Escherichia coli* influence biofilm formation through YjgK (TabA) and Fimbriae, J. Bacteriol. 191 (2009) 1258–1267.
[23] K. Flemming, C. Klingenberg, J.P. Cavanagh, M. Sletteng, W. Stensen, J.S. Svendsen, T. Flaegstad, High *in vitro* antimicrobial activity of synthetic antimicrobial peptidomimetics against staphylococcal biofilms, J. Antimicrob. Chemother. 63 (2009) 136–145.
[24] E. Weber, Grundniss der biologischen Statistok für Naturwissen schaftler, Landwirte und Medizimer, Veb. Fischer Verlag, Jena, 1972.
[25] P.M. Berthouex, L.C. Brown, Statistic for environmental engineers, Lewis Publishers, C.R.C. Press, Boca Raton, 1994.
[26] O. Choi, Effect of silver nanoparticles on planktonic and biofilm cell growth, A Dissertation presented to the Faculty of the Graduate School at the University of Missouri-Columbia, Columbia, 2009.
[27] J. Fabrega, The impacts of silver nanoparticles on planktonic and biofilm bacteria, A Dissertation presented to the University of Birmingham, Birmingham, 2009.
[28] M. Łebkowska, Występowanie bakterii antybiotykoodpornych w wodzie przeznaczonej do spożycia przez ludzi [Antibiotic resistant bacteria in drinking water], Ochr. Śr. 31 (2008) 11–15.
[29] B. Kołwzan, Analiza zjawiska biofilmu –warunki jego powstawania i funkcjonowania [Analysis of biofilms – their formation and functioning], Ochr. Śr. 4 (2011) 3–14.
[30] Y. Liu, J. Li, X. Qiu, C. Burda, Bactericidal activity of nitrogen-doped metal oxide nanocatalysts and the influence of bacterial extracellular polymeric substances (EPS), J. Photochem. Photobiol., A 190 (2007) 94–100.
[31] T. Snopczyński, K. Góralczyk, K. Czaja, P. Struciński, A. Hernik, W. Korcz, J.K. Ludwicki, Nanotechnologia —możliwości i zagrożenia [Nanotechnology – possibilities and hazards], Roczn. Pzh 2 (2009) 101–111.