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Effects of Dysprosium Oxide Nanoparticles on Escherichia coli

N. M. Anaya
University of Rhode Island

F. Soloman
University of Rhode Island

Vinka Oyanedel-Craver
University of Rhode Island, craver@uri.edu

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Nano Impact Statement

This study offers new insights into dysprosium oxide nanoparticles (nDy_2O_3) exposure on *E. coli* with respect to its metabolic activity and structural integrity. The toxicity of nDy_2O_3 was evaluated for two array-based techniques, Live/Dead and respirometric assays. Our work is novel since new nDy_2O_3 toxicity data was produced covering manufacturer recommendations for toxicity assessments, environmental and industrial nDy_2O_3 effluent concentrations and metabolic activity. This is highly relevant to the evaluation of the toxic effect of nanoparticles since the physicochemical properties of the nanoparticles can differ greatly depending of the physicochemical characteristics of the aqueous solution. With an understanding of the nanoparticles fate in aqueous media, a careful selection of appropriate toxicological methodologies can be made to improve the accuracy of future nanotoxicological studies.

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Effects of Dysprosium Oxide Nanoparticles on *Escherichia coli*

N. M. Anaya [a], F. Solomon [a] and V. Oyanedel-Craver [a]

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There is increasing interest in the study of dysprosium oxide nanoparticles (nDy_2O_3) for biomedical applications due to their fluorescent and paramagnetic properties. However, the fate of nDy_2O_3, and their effects on natural biological systems, are a growing concern.

This study assessed the toxicity of nDy_2O_3 on *Escherichia coli* for concentrations between 0.02 and 2 mg/L, exposed to three concentrations of NaCl (8,500; 850 and 85 mg/L) and three glucose concentrations (35, 70, 140 mg/L). The ranges of these variables were selected to cover manufacturer recommendations of analytical methodologies for toxicity assessment, environmental and industrial nDy_2O_3 effluent concentrations, and metabolic activity. Two array-based toxicity techniques were used to evaluate the 27 combinations of conditions. Fluorescent dyes (Live/Dead) and respirometric assays were used to measure the undisturbed cell membrane (UCM) and remaining respiration percentage (RRP), respectively.

Respirometric tests showed a higher toxic effect than Live/Dead test assays, indicating that metabolic processes are more affected than the physical structure of the cell by exposure to nDy_2O_3. After exposing the bacteria to concentrations of 2.0 mg/L uncoated nDy_2O_3 for 2 hrs at 85 mg/L of NaCl and 140 mg/L of glucose, the RRP and UCM decreased to 43% and 88%, respectively. Dysprosium ions (Dy^{+3}) toxicity measurement suggested that Dy^{+3} was the main contributor to the overall toxicity.

1. Introduction

Gadolinium, holmium and dysprosium belong to the lanthanide oxide-based nanoparticles (LnONps), which have acquired more relevance in recent years in regard to the locating, diagnosing and treating of diseases[1–3]. LnONps have unique paramagnetic properties that allow greater spatial and temporal resolution through a higher signal-to-noise ratio. These properties play a fundamental role in acquiring and enhancing the contrast in T_1 or T_2 magnetic resonance images (MRI) [4,5]. Due to the higher sensitivity provided by the LnONps, the MRI contrast is improved and the T_1 or T_2 relaxation times are discriminatorily shortened in the region of interest[6].

Dysprosium oxide nanoparticles (nDy_2O_3) have recently received increasing attention due to their potential applications in the biomedical field [4,5] including cancer research, new drug screening, and the delivery of drug applications [2,7,8]. However, the fate of nDy_2O_3 and their effects on natural biological systems are growing concerns [9]. nDy_2O_3 will enter into aquatic and land environments through wastewater treatment facility effluent and wastewater sludge due to an inability to retain and or remove these nanoparticles completely [10]. Moreover, the release of nDy_2O_3 into land environments from agricultural applications could transport nanoparticles to surface waters via stormwater runoff and to groundwater via infiltration through the soil [10,11].

Previous studies have provided limited insight into the toxic effects of nDy_2O_3 and Dy ions on natural systems. Kattel et al.[12] investigated the *in vitro* toxicity effect of ultra-small spherical dysprosium oxide and dysprosium hydroxide nanorods [5]. Both nanoparticles were coated with D-glucuronic acid and exposed to DU 145 and NTC 1469 cell lines. These studies showed that the nanoparticles were not toxic to the human cells for concentration values ranging from 0 to 37.3 mg/L.

Harper et al.[13] tested 11 types of metal oxide nanoparticles, including nDy_2O_3, and found that high mortality of embryonic zebrafish was observed when they were exposed to 250 mg/L of nDy_2O_3 for 5 days of continuous waterborne conditions. In addition, concentrations of 250 mg/L for nDy_2O_3 produced morphological malformations of the zebrafish's jaw and eyes.

Toxicological assessment of nanoparticles can be studied in terms of their impact on metabolic functions and cell structure such as cell viability, membrane permeation, growth and respiration. Live/Dead assay (BacLight viability kit) is a

[a] Department of Civil and Environmental Engineering, Bliss hall 203, University of Rhode Island, Kingston, RI, 02881, USA. E-mail: nelson_anaya@my.uri.edu; fsolomon@my.uri.edu; craver@uri.edu. Phone: (401)-874-2784. Fax: (401)-874-2786

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commonly-used method to measure cell viability [14] and membrane permeation on bacteria through the integrity of cell membranes. The manufacturer of the reagents used for the Live/Dead test recommends that experiments and samples have to be prepared in specific water chemistry conditions (8,500 mg/L of NaCl) to avoid a decrease in staining efficiency [14]. Previously, the metabolic activity of bacteria has been measured using a traditional respirometric bottle test (RT). Water chemistry conditions with monovalent and divalent cations have been successfully used in the range of 10 to 1,000 mg/L; however, high concentrations of glucose (in the order of 300mg/L) and bacteria (in the order of 10^9 CFU/mL) were required to quantify a toxic response[15]. This type of test can be used to measure the interaction and effect of nanoparticles on microorganisms. Nevertheless, each methodology required its own range of optimal conditions, which makes it a complex process to assess toxic effects when identical water chemistry conditions are used. This is highly relevant to the evaluation of the toxic effect of nanoparticles, since the physicochemical properties of the nanoparticles (e.g., charge, aggregation, cell-nanoparticle ratio and dissolution) can differ among each other and also their properties are influenced by the physicochemical characteristics of the aqueous solution.

In this study, we propose to evaluate the use of array-based dyes methods in identical water chemistry conditions and observe the effect of nDy_2O_3 and exposure on *E. coli* metabolic activity and structural integrity of *E. coli* under variable water chemistry conditions.

2. Materials and Methods

2.1. Materials

A non-pathogenic wild strain of *E. coli* (IDEXX laboratory) was selected for this study. *E. coli* is a gram-negative bacterium that has been found to be metabolically active in saline solution without growth [16] and has been extensively studied in nanotoxicological research[15,17,18]. Reagents used to prepare the growth media for the bacteria — sodium chloride (NaCl), yeast extract, and tryptone — were purchased from Sigma Aldrich. Glucose was purchased from Sigma Aldrich and used as received. Tetrazolium dye (Redox Dye Mix A) was purchased from Biolog and used to measure the respiratory responses of *E. coli*. Cell membrane permeation was measured using SYTO 9 and propidium iodide; both reagents were purchased from Invitrogen.

2.2. Methods

Hydrodynamic diameter and zeta potential were measured by Malvern Zetasizer Nano ZS, ZEN 3600, dynamic light scattering (DLS). Data was collected at 0.25 hrs and at 2 hrs after nanoparticles exposure to bacteria to differentiate the effect of aggregation of nanoparticles. Shape characterization of the nanoparticle was obtained by using JEOL JEM-2100 LaB6 transmission electron microscope (TEM) imaging. Ionic release from nDy_2O_3 for each condition was quantified as per Liu and Hurt[19] using centrifugal ultrafilter devices (ultra-4,3K) purchased from Amicon. Inductively-coupled plasma spectroscopy (ICP-OES optima 3100, Perkin Elmer) was used to measure the concentration of nDy_2O_3 and Dy ions before and after the contact times established for each of the water chemistry conditions tested. Samples were digested in nitric acid (2% v/v, HNO_3) before analysis.

Growth media consisted of 10 g/L NaCl, 5 g/L yeast extract, and 10 g/L tryptone. After the solution was prepared, it was autoclaved and then *E. coli* seed was added. *E. coli* was grown for 12 hrs in a culture media at 37°C. Bacteria were harvested during the logarithmic growth phase and centrifuged at 2000 rpm for 0.25 hrs. The supernatant was discarded and the pellet re-suspended in the respective NaCl solution[15]. *E. coli* concentration was fixed to OD670 (optical density at a wavelength of 670nm) of 2.68 using the respective NaCl solution. *E. coli* solutions were refrigerated at least 30 min prior to inoculation into the microplate in order to decrease the metabolism of the bacteria. This allowed for a homogenous metabolic reaction to the glucose.

Three NaCl concentrations were selected: 85 mg/L, 850 mg/L and 8500 mg/L (ionic strength 1.45 mM, 14.5 mM and 145 mM, respectively). The lowest concentration represents the ionic strength commonly found in surface waters,[20] while the highest NaCl concentration was selected based on the manufacturer's recommendations of the Live/Dead test[14]. The middle value was chosen for a three-tier comparison. Glucose concentrations in the range of 35 mg/L to 140 mg/L were used as a carbon source to evaluate nDy_2O_3 toxicity under different aerobic metabolic levels. Preliminary experiments were carried out to determine the glucose concentrations that inhibit the *E. coli* metabolic functions. The glucose concentration of 35 mg/L was the lowest limit of respiratory detection, and 140 mg/L was below the concentration (210 mg/L) that produces an inhibitory effect on *E. coli.*

Three concentrations of non-coated Dy_2O_3 nanoparticles — 0.02 mg/L, 0.2 mg/L and 2.0 mg/L — were used to simulate not only environmental concentrations (0.02 mg/L), but also accidental spill scenarios (2.0 mg/L). Non-coated Dy_2O_3 nanoparticles were prepared based on the method used by Kattel et al. [12].

Table 1 shows the 27 conditions and the blanks tested in this study for the respirometric and Live/Dead tests using the synergy TM MX microplate reader. Blanks without nDy_2O_3, glucose and NaCl were analyzed for each scenario.

Table 1 Condition Matrix tested using respirometric and live/dead tests. Samples in quadruplicate were run. Glucose, nDy_2O_3 and bacteria were prepared at the same NaCl concentration. * represents blanks for each condition.

NaCl [mg/L]	Glucose [mg/L]	nDy_2O_3 [mg/L]
0*	0*	0.02, 0.2, 2
	35	0*, 0.02, 0.2, 2
	70	0*, 0.02, 0.2, 2
	140	0*, 0.02, 0.2, 2
85	0*	0.02, 0.2, 2
	35	0*, 0.02, 0.2, 2

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NaCl [mg/L]	Glucose [mg/L]	nDy_2O_3 [mg/L]
	70	0*, 0.02, 0.2, 2
	140	0*, 0.02, 0.2, 2
850	0*	0.02, 0.2, 2
	35	0*, 0.02, 0.2, 2
	70	0*, 0.02, 0.2, 2
	140	0*, 0.02, 0.2, 2
8500	0*	0.02, 0.2, 2
	35	0*, 0.02, 0.2, 2
	70	0*, 0.02, 0.2, 2
	140	0*, 0.02, 0.2, 2

2.3. Toxicity tests

2.3.1. Respirometric test. Cell respiration in non-growing conditions was quantified through the reduction of tetrazolium dye[21,22]. When a carbon source is consumed, metabolic activity transports electrons from the carbon source to the electron transport chain in the cell membrane where the tetrazolium dye is reduced, which in turn produces a purple colour[23,24]. The purple colour can be quantified in terms of absorbance using a microplate reader at a wavelength of 590nm[25]. The plate was prepared first by adding NaCl, then glucose, nDy_2O_3, tetrazolium dye, and finally a bacteria solution were directly inoculated into a Blank 96 half-area well microplate to achieve the required concentrations. The final volume within each well was 100 µL, mixed thoroughly by pipetting at least 10 times. Glucose, nDy_2O_3, and cell solutions were all prepared in the respective saline solution. Experiments were run for 2.5 hrs and readings were recorded every 0.25 hrs after every horizontal shaking (medium setting) performed by the microplate reader. Data was analyzed at 0.25 and 2 hrs. The two-hour interval was selected in order to distinguish the effect between nanoparticles before and after aggregation. Aggregation increased drastically after 2 hrs, particularly for those conditions with high concentrations of NaCl. Higher exposure time could increase toxicity effect; however, for time periods longer than 2h, the toxicity results will not be because of the dysprosium nanoparticles but because of the dysprosium microparticules. The microplates were incubated at 25°C. A detailed description of the microplate set-up is presented in the Supplemental Information section.

The microplate included nDy_2O_3 blank, and background correction sections to evaluate the interaction between nanoparticles and tetrazolium dye. Lack of coloration in the nDy_2O_3 blank section indicated that no interaction was observed between nDy_2O_3 and the tetrazolium dye. For the background (absorbance of nDy_2O_3) correction, the value obtained in the respective well, was subtracted from the experiment values and also served as a secondary control to confirm that experimental conditions had no reducing effect on the tetrazolium dye without the presence of bacteria.

Each plate has been set up in quadruplicate wells for each condition, and the plates were run in duplicate to quantify the percent of remaining respiration (PRR). The PRR (Eq. 1) is the ratio of slopes between bacteria exposed to nDy_2O_3 and the blank bacteria (samples containing bacteria that were not exposed to nDy_2O_3) at a specific nDy_2O_3 concentration from the absorbance-time graph.

$$\mathbf{PRR} = \frac{P_t}{P_c}.\mathbf{100}\% \quad \text{Eq. 1}$$

Where,

P_t = slope from absorbance-time graph for bacteria exposed to nDy_2O_3

P_c = slope from absorbance-time graph for bacteria control without nDy_2O_3

2.3.2. Live/dead test. The cell membrane permeation of *E. coli* was determined using the Backlight kit (propidium iodide and SYTO 9) with the microplate reader. Propidium iodide becomes intercalated to the DNA within cells, and indicates bacteria that have a damaged membrane. SYTO 9, on the other hand, indicates intact cell membranes[14]. A stain solution composed of SYTO 9 and propidium iodide fluorescent nucleic acid stains was mixed at a 1:1 (v/v) ratio with a subsequent dilution in DI water (12µL of stain mixed solution in 2 mL of DI water). Suspension mixtures of NaCl, glucose, nDy_2O_3, and bacteria were added into separate wells of a 96 well flat-bottom black microplate to achieve the required concentrations. The plate was incubated at 25°C for 2.0 hrs, during which horizontal shaking (medium setting) was performed by the microplate every 0.25 hrs. After incubation, 100 µl of mixed stain solution was added and mixed thoroughly by pipetting at least 10 times for each well. Before reading with the microplate, 0.25 hrs of additional incubation was required in the dark at room temperature. A detailed description of the microplate setup is presented in the Supplemental Information section. Each plate contained quadriplicate wells for each condition, and each plate was run in duplicate to quantify the undisturbed cell membrane (UCM). The UCM (Eq. 2) is the green/red fluorescence ratio between bacteria exposed to nDy_2O_3 and the blank bacteria (bacteria not exposed to nDy_2O_3) at given nDy_2O_3 concentration. Data was analyzed at 0.25 and 2 hrs to differentiate the effect of aggregation of nanoparticles.

$$\mathbf{UCM}=\frac{P_t}{P_c}.\mathbf{100} \quad \text{Eq. 2}$$

Where,

P_t = Green/red fluorescence ratio for bacteria exposed to nDy_2O_3

P_c = Green/red fluorescence ratio for bacteria control without nDy_2O_3

2.3.3. Toxicity tests for nDy_2O_3 ion release. Additional experiments using the respective ions concentration, released at the highest concentration of nDy_2O_3, were performed. These experiments allow us to determine the contribution of Dy ions to the overall toxicity

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on *E. coli.* Similar method was used to prepare the plate, but dysprosium ions were used instead of nDy_2O_3.

2.4. Statistical analysis

The results from each data set were analyzed with SAS statistical software, version 9.1.2. A generalized linear mixer model (GLIMMIX) was used to identify statistical differences among glucose, NaCl, and nDy_2O_3 concentrations because the response was not necessarily normally distributed. A p value of less than 0.05 was considered to indicate significant difference.

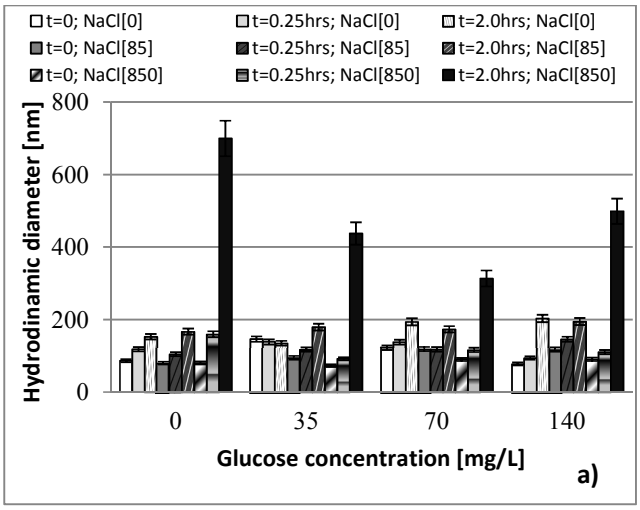
3. Results and Discussion

3.1. Physiochemical characterization of nDy_2O_3

3.1.1. Size and zeta potential without bacteria. Uncoated nDy_2O_3 characterization consisted of size and zeta potential measurements in two of the three water chemistry conditions. Tests with 8500 mg/L NaCl were discontinued due to interference between tetrazolium dye and NaCl. Details will be explained in Section 3.2. Figure 1 shows size (a) and zeta potential (b) measurement at 0, 0.25 and 2.0 hrs for the highest concentration of 2.0 mg/L of nDy_2O_3. nDy_2O_3 sizes from 0 to 0.25 hrs at all water chemistry conditions were in the range of 75 to 150 nm; however, size measurements at 2 hrs showed an increase in nDy_2O_3 particle size, indicating aggregation. It was observed that aggregation increased when the ionic strength was higher (850 mg/L NaCl), and also at all glucose concentrations. This may be attributed to the absence of a coating agent on the nanoparticles surface and lower repelling forces as a result of high ionic strength in the solution. Zeta potential measurements (Figure 1b) showed the instability of nDy_2O_3 with surface charge measurements ranging between 6 and 23 mV in 85 and 850 mg/L of NaCl during 2 hrs of contact time.

3.1.2. Shape and pH. TEM imaging confirmed the shape of the nDy_2O_3 to be spherical (Figure 2). Nanoparticles were found to have an average size of 74.8 $\pm$ 5nm. Changes in H^+ ions before and after nanoparticle exposure were recorded periodically with a pH meter. The pH measurements at t = 0 hrs and t = 2 hrs ranged from 5.5 to 6.2, indicating that pH did not function as an additional stress on bacteria performance. Moreover, there was no statistically significant change.

3.1.3. Ion release. Ion release experiments were conducted for the highest concentration of nDy_2O_3, (2.0 mg/L), which was the most toxic condition for *E. coli.* Dy ions were measured in all water chemistry and glucose conditions to determine the amount of dissolution over time. Table 3-1 shows a sudden increase in Dy ions measured at 0 hrs and 0.25 hrs, with a plateau occurring at 2.0 hrs.



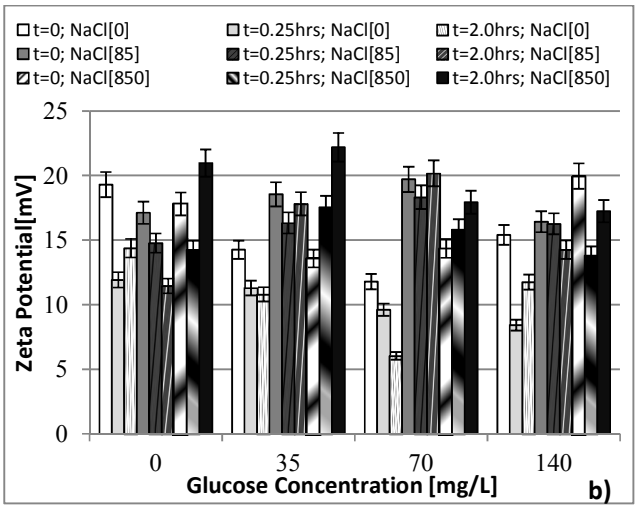


Figure 1 Size (a) and zeta potential (b) measurements in samples containing 35 mg/L, 70 mg/L, and 140 mg/L glucose, and 2.0mg/L nDy_2O_3. Measurements were taken at 0 hrs, 0.25 hrs and 2 hrs.

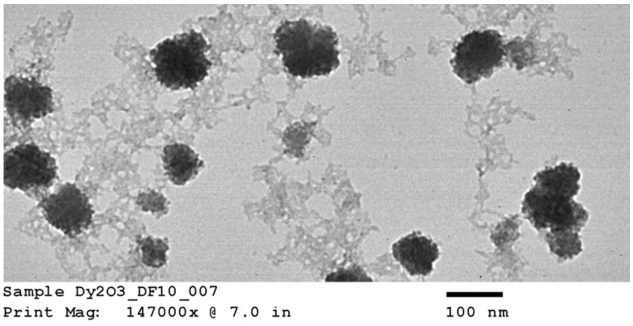


Figure 2 nDy_2O_3 TEM image shows the spherical nanoparticles with average diameter of 74.8 nm.

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Table 2 Ion release concentration for 2.0mg/L nDy_2O_3 in samples containing 85 mg/L and 850 mg/L NaCl, and 35 mg/L, 70 mg/L and 140 mg/L glucose. Measurements were taken at 0 hrs, 0.25 hrs and 2.0 hrs.

NaCl [mg/L]	Glucose [mg/L]	Dy ions [mg/L]		
		t = 0hrs	t = 0.25 hrs	t = 2 hrs
85	35	0.40	0.65	0.70
	70	0.42	0.68	0.72
	140	0.43	0.69	0.75
850	35	0.47	0.64	0.73
	70	0.48	0.68	0.75
	140	0.49	0.68	0.79

3.2. Toxicity tests

Respirometric tests at 8,500 mg/L of NaCl detected interference between tetrazolium dye and NaCl. Bacteria at 85 and 850 mg/L of NaCl without stress conditions maintained an absorbance value after the dye was reduced. On the other hand, bacteria at 8,500 mg/L showed the opposite behaviour (see SI). Interference with glucose was discarded because Live/Dead tests showed no problems under similar condition. Although, absorbance measurements could not be obtained at high NaCl concentration, it is known that *E. coli* may survive under high ionic strength conditions similar to those presented in this study [26–28].

3.2.1. Respirometric test. The PRR compares the slope values representing respiration of the remaining bacteria after nDy_2O_3 exposure with the slope values of the control wells. In the control wells, bacteria were not exposed to nDy_2O_3. Figure 3 shows the PRR for all water chemistry conditions tested at 0.25 and 2 hrs. For 0.25 hrs, no toxicity effect was observed for most glucose concentrations of 35 and 70mg/L, except for 35 mg/L of glucose and 0.02mg/L of nDy_2O_3. This could be due to the fact that the nDy_2O_3 contact time was not enough to produce a significant toxicity effect in the bacteria. After 2.0 hrs, there was an increase in toxicity for some water chemistry conditions. For 2.0 mg/L nDy_2O_3, the toxicity effect was more pronounced at all glucose and NaCl concentrations. The greatest toxicity effect (PRR=43%) was observed at 85 mg/L NaCl and 140 mg/L glucose. The high PRR values may be associated with the metabolic activity at high carbon concentration levels and more stable nanoparticles at low concentrations of NaCl. *E. coli* increases the carbon mineralization at glucose concentration of 140 mg/L, where Dy ions can be transported inside the bacteria that are coupled to an essential metal uptake by the cells[29].

3.2.2. Live/Dead test. The undisturbed cell membrane (UCM) results are shown in Figure 4. Live/Dead experiments detected a very low toxicity effect of nDy_2O_3 on *E. coli* under the same conditions used for the respirometric microarray tests. The results showed no significant membrane permeation, which means minimal (UCM= 94.9) to no physical damage to *E. coli* occurred at nDy_2O_3 concentrations ranging from 0.02 to 0.2 mg/L. Only at high concentrations of nDy_2O_3 (2 mg/L) and at ionic strength (850 mg/L NaCl), a slight toxicity effect (UCM= 88.5) was observed on bacteria. However, it was less pronounced compared to the toxicity effect results obtained for the respirometric test. The increasing concentrations of glucose seem to show no constant trend. Thus, glucose concentration could not have influence the toxicity effect results.

3.2.3.Effect of Dy ions on *E. coli* Toxicity. Additional experiments were performed to determine the contribution of Dy ions to the overall toxicity on *E. coli*. The tests were performed using the respective ions concentration released at the highest concentration of nDy_2O_3 (table 2), as that exhibited the highest toxicity effect on bacteria. The results showed that cell viability was predominantly lost due to interactions of Dy^{+3} ions with *E. coli* rather than nDy_2O_3 (Figure 5). This suggests that nDy_2O_3 could have caused damage to the cell membrane, and Dy^{+3} could have entered into the cell and disturbed intracellular activities, as previously presented.

3.3. Comparison between toxicology methodologies

This study performed a comparison between two toxicological tests using nDy_2O_3 on *E. coli*. It was found that for both methodologies, and at the same conditions, nDy_2O_3 is most toxic at high concentrations [2.0 mg/L]. The respirometric test showed a more prominent response (PRR=43%) compared to Live/Dead (UCM=88%) test. This indicates that metabolic responses are more sensitive to toxicity than cell physiology when bacteria are exposed to nDy_2O_3.

Statistical analysis (for details, see Supplemental Information section) confirmed a correlation — first with the nDy_2O_3 concentrations ($p<0.0001$), then with NaCl concentrations ($p=0.0040$) and finally with glucose concentrations ($p=0.0074$) on the exposure response to nDy_2O_3 for the metabolic activity of the cell. For both methods, nDy_2O_3 concentration was the most influential variable and determined the magnitude of the exposure response. In addition, a strong correlation was observed when the combined effect between NaCl and nDy_2O_3 ($p=0.0148$) was analyzed. This is consistent with the results obtained, which showed higher toxicity effect in conditions with low NaCl and high nDy_2O_3 concentrations. Also, NaCl and glucose were analysed simultaneously showed a significant correlation with a p value of 0.0566. Similar trend was observed in the high toxicity effect of nDy_2O_3 on *E. coli* when bacteria were more active at high glucose concentrations and nDy_2O_3 more stable at low concentrations of NaCl.

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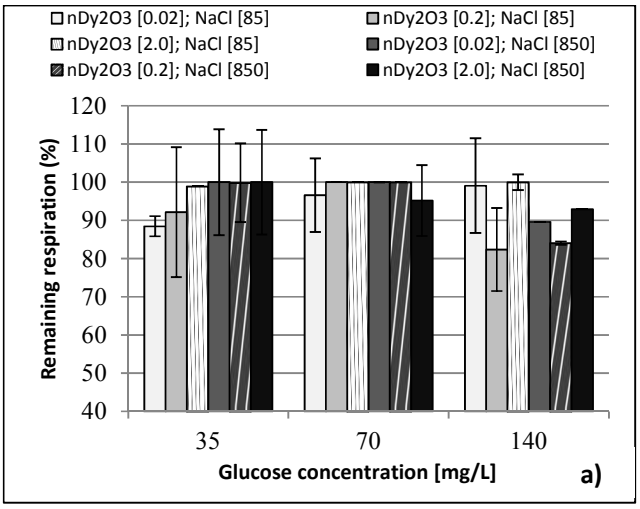

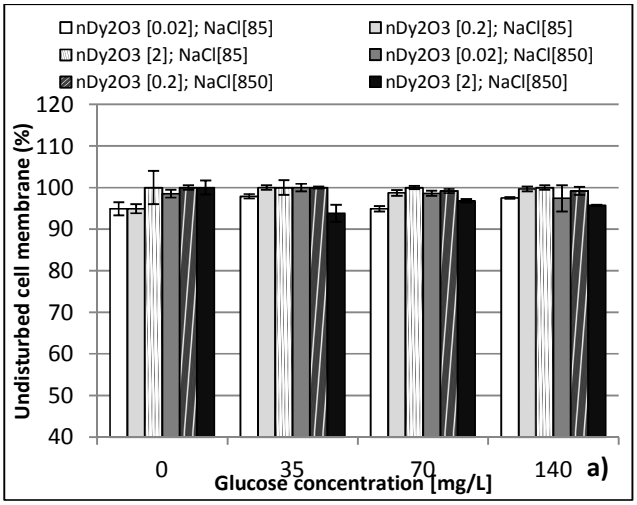

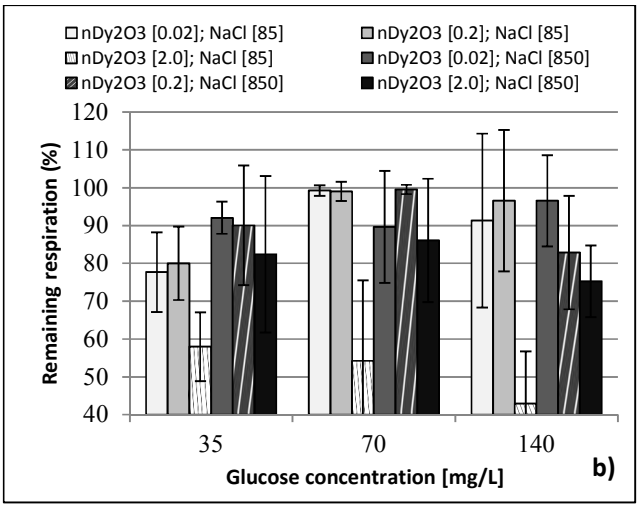

Figure 3 Percent remaining respiration (PPR) of *E. coli* under different water chemistry conditions, (NaCl 85 mg/L and 850 mg/L), and glucose concentration (35 mg/L, 70 mg/L and 140 mg/L). PPR values were calculated for three concentrations of nDy_2O_3, 0.02 mg/L, 0.2 mg/L and 2.0 mg/L at (a) 0.25 hrs and (b) 2.0 hrs. Values are mean from 4 wells and duplicate plates.

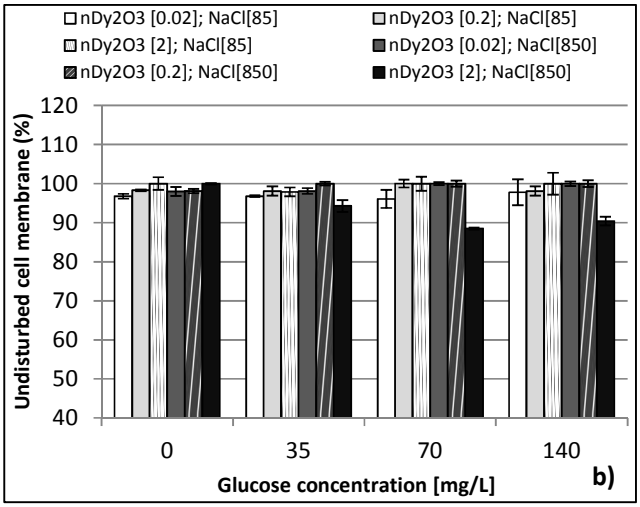

Figure 4 Undisturbed cell membrane (UCM) of *E. coli* under different water chemistry conditions, (NaCl 85 mg/L and 850 mg/L), and glucose concentration (35 mg/L, 70 mg/L and 140 mg/L). UCM values were calculated for three concentrations of nDy_2O_3, 0.02 mg/L, 0.2 mg/L and 2.0 mg/L at (a) 0.25 hrs and (b) 2.0 hrs. Values are mean from 4 wells and duplicate plates.

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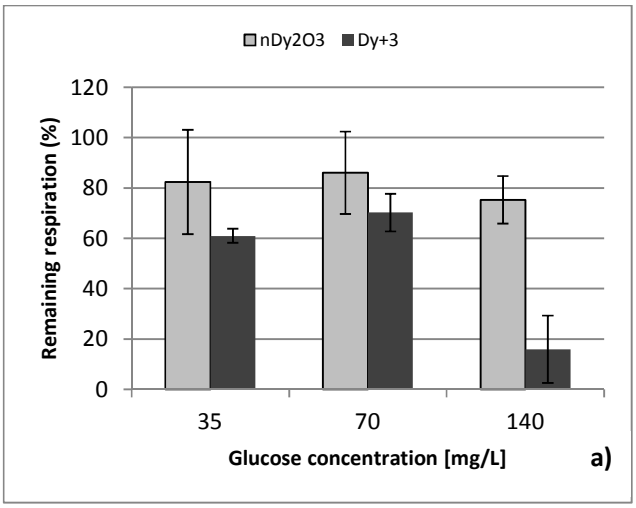

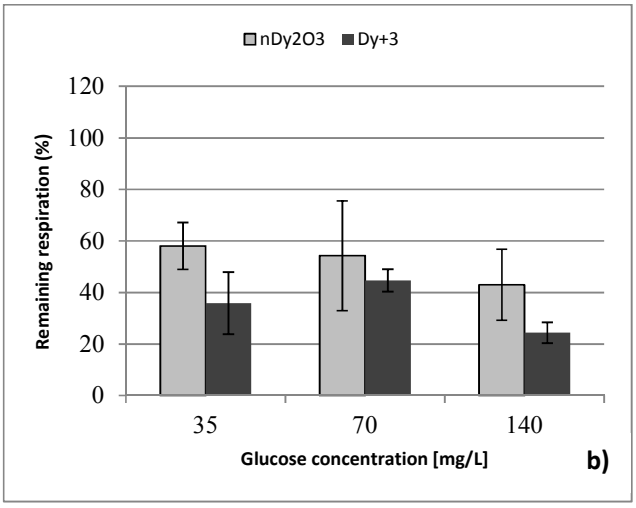

Figure 5 Toxicity effect of dysprosium ions (Dy^{+3}) and nDy_2O_3 under different water chemistry conditions: (a)850 mg/L and b)85 mg/L) and glucose concentration (35 mg/L, 70 mg/L, and 140mg/L) at the highest concentration of ions released after 2 hrs for 2mg/L of nDy_2O_3.

Conclusion

The results showed that respirometric and permeation membrane tests can be used to provide a comprehensive assessment of nanoparticle toxicity on microorganisms. This study evaluated the performance of two toxicity methodologies: the Live/Dead assay to evaluate the membrane permeation, and the respirometric assay to evaluate the metabolic activity of bacteria. The respirometric microarray test proved to be more sensitive than the Live/Dead test in measuring nanoparticle toxicity.

With an understanding of the fate of nanoparticles in aqueous media, a careful selection of appropriate toxicological methodologies can be made to improve the accuracy of future nanotoxicological studies.

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