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Ecotoxic Effects of Cerium Oxide Nanoparticles on Bacteria

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Abstract. In this study, the ecotoxic effects of cerium oxide nanoparticles (CeO_2 NPs) on both gram positive *Bacillus subtilis* and gram negative *Escherichia coli* bacteria were investigated. CeO_2 NPs were prepared in synthetic water solutions having different water contents (low, median and high ionic strength and conductivity, pH 5.5 and 6.5). Bacteria were exposed to CeO_2 NP solutions in absence and presence of light conditions for 1 h. Different NP concentrations (10, 100, 500 and 1000 mg/L) were used, and environmental scanning electron microscopy imaging was performed for morphological examination of the bacteria. Results showed an aggregation of NPs relating to both high NP concentrations and high ionic strength of the water solutions. Regardless of the test condition, CeO_2 NPs highly inhibited the bacterial growth.

Keywords*:Bacillus subtilis*, *Escherichia coli*, CeO_2 nanoparticle, inhibition, synthetic water solutions.

Seryum Oksit Nanopartiküllerinin Bakteriler Üzerindeki Ekotoksik Etkileri

Özet. Bu çalışmada, seryumoksit nanopartiküllerinin (CeO_2 NP) gram pozitif *Bacillus subtilis* ve gram negatif *Escherichia coli* bakterileri üzerindeki ekotoksik etkileri incelenmiştir. CeO_2 NPleri farklı içeriğe sahip (düşük, orta ve yüksek iyonik güç ve iletkenlik, pH 5,5 ve 6,5) sentetik su çözeltileri içinde hazırlanmıştır. Bakteriler ışıklı ve ışıksız ortamlarda CeO_2 NPlerine 1 saat süreyle maruz bırakılmıştır. Farklı NP konsantrasyonları (10, 100, 500 and 1000 mg/L) kullanılmış ve çevresel taramalı electron mikroskopi görüntüleme ile bakterilerin morfolojik incelemesi yapılmıştır. Sonuçlar yüksek NP konsantrasyonu ve yüksek iyonik güce bağlı olarak NPlerin agregasyona uğradığını göstermiştir. Test koşullarından bağımsız olarak CeO_2 NPleri bakteriyel büyümeyi yüksek oranda inhibe etmiştir.

***AnahtarKelimeler*:** *Bacillus subtilis*, *Escherichia coli*, CeO_2 nanopartikülü, inhibisyon, sentetik su çözeltisi.

1. INTRODUCTION

Engineered nanoparticles (NPs) are used in almost every field from imaging technology to food, agriculture and cosmetics to the pharmaceutical industry. Due to this intensive use, titanium dioxide (TiO_2), zinc oxide (ZnO), cerium oxide (CeO_2) and silver NPs have the highest production volumes of 100-1000 tons/year [1]. In addition to these metal oxide NPs, cerium (Ce) with 0.0046% of rare elements, is as abundant as copper (Cu) in the earth's crust [2]. In Europe, the median Ce concentration detected as 48.2 mg/kg, 66.6 mg/kg, and 55 ng/L in soil, sediment and water,

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respectively [3]. European Union (EU) Commission estimated the global production amount of nano-CeO_2 around 10000 tons in 2012 [4]. A study by the Future Markets Company predicted that the production of nano-CeO_2 in 2011 was between 7500 and 10000 tons [5]. According to Statista, the price of CeO_2 was 98217 US-$/ton in 2011, 5516 US-$/ton in 2018 and the predicted price will be 308 US-$/ton in 2025 [6]. The American Geological Survey Unit (USGS) reported that 80% of the global CeO_2 production potential was reported in China [7] and other important nano-CeO_2 producer countries were located in Asia, Australia and Europe [8].

CeO_2 NPs are employed in electronics and optics [4, 9], polishing products [4, 10], exterior facade paints [4], metallurgy [4], as fuel additives due to its catalytic properties, wood coating material [11], petroleum refinery, and in fluid catalytic cracking [3]. Moreover, in medicine [12, 13], environmental chemistry [2] and in removal of pollutants from industrial wastewaters [14] CeO_2 NPs have been employed. Dai et al. [14] used SiO_2/CeO_2 catalyst (Fe_3O_4 magnetic core covered with SiO_2 in middle layer and CeO_2 in outer layer) in catalytic ozonation for acetylsalicylic acid degradation in the aqueous solution and obtained 81% removal efficiency within 1 hour.

As a result of high production volumes, high amounts of NPs tend to release into receiving environments. There are studies showing TiO_2, ZnO, Ag and CeO_2 NPs in surface waters, wastewater treatment plant effluent and sludge, sediments and landfill areas [15]. CeO_2 NP concentrations estimated in treated wastewater was between 0.003 and 1.17 µg/L, and in sludge was between 0.53 and 9.10 mg/kg [16]. It is also predicted that the increasing concentrations of NPs in receiving environments may result in ecotoxicity to organisms [15, 17, 18]. When CeO_2 NPs released into the receiving environments, it is inevitable to emerge in aquatic environments [16], and rapidly undergo an aggregation/agglomeration and interact with aquatic organisms [19, 20]. With this interaction, microorganisms, algae and macroinvertebrates, which are the basic building blocks of ecological food web, are the first and most exposed organisms to the ecotoxic effects of NPs [21].

There are limited number of studies on the ecotoxic effects of nano-CeO_2 particles to microorganisms [3, 22-27]. Thill et al. [22] showed the effects of 7 nm particle sized CeO_2 NPs on gram (-) *Escherichia coli* with an EC50 value of 5 mg/L. Moreover, they depicted that CeO_2 – *E. coli* interaction was directly by electrostatic attraction to the negatively charged bacteria wall due to the positively charged physiological pH value. Pelletier et al. [23] presented inhibitions of *E. coli* and *Bacillus subtilis*, and no effect on *Shewanella oneidensis* under CeO_2 NP treatment. In the study, different NP concentrations (50–150 µg/mL), NP sizes (6–40 nm), pH and medium were used to determine the effects of CeO_2 NPs. Dar et al. [24] treated *E. coli* HB101 K12 strains with four different sizes (3,5–6,5 nm) of CeO_2 NPs and showed the inhibition of bacteria. The studies showed the effect mechanisms of CeO_2 NPs on bacteria mostly related to a direct contact with bacterial cell wall [22, 25, 27], membrane deformation [26], cell disintegration [27] and release of free Ce(III) [25]. The evaluation of those studies revealed that the data on the ecotoxic effects of CeO_2 NPs are still inadequate, and especially the systems that represent real environmental conditions need to be carried out.

In this study, the effects of CeO_2 NPs on *E. coli* and *B. subtilis* were evaluated in terms of bacterial inhibition and cell membrane deformation. The real environmental conditions were partly fulfilled and in order to simulate them, CeO_2 NPs were prepared in synthetic water solutions (SWSs) with different water contents (low, median and high ionic strength and conductivity, pH 5.5 and 6.5). Bacteria were treated in CeO_2 NP solutions under dark and illuminated conditions. Different NP concentrations (0, 1, 10, 50 and 100 mg/L) and morphological examination were used to determine the bacterial inhibition.

2. MATERIALS AND METHODS

2.1. Preparation of Bacterial Cultures

E. coli bacteria were kindly gifted by Biology Department of Akdeniz University. Frozen bacterial culture of 1 mL was inoculated in 100 mL LB Broth and incubated at 37°C for 18 h (OD_{600}= 1.05). Lyophilized *B. subtilis* (ATCC6633) bacteria were purchased from American Type Culture Collection (ATCC, Wesel, Germany). A pellet of lyophilized bacteria was inoculated in 100 mL of LB Broth at 37°C for 18 h (OD_{600}= 1.03). After the incubation period, the cultures were transferred into 2 mL eppendorf tubes and centrifuged at 10,000 rpm for 5 min. Supernatants were removed and the pellets were dissolved in 10% glycerol + 90% LB Broth media. The cultures were stored at -20°C until upon use.

2.2. Nanoparticle Solutions and Characterization

The CeO_2 NPs used in the experiments were purchased (Alfa aesar, Karlsruhe, Germany). The NPs were in 15- 30 nm size range and have 32- 40 m^2/g of surface areas. A stock NP solution of 1000 mg/L was freshly prepared in SWSs with different ionic strength (10, 50 and 100 mM), pH (5.5 and 6.5) and conductivity (0.6, 3 and 6 mS/cm). The stock solution was then diluted to 10, 100 and 500 mg/L before the experiments. All test units were continuously shaken at 100 rpm.

In order to characterize the NPs, size distribution was calculated using dynamic light scattering (DLS) technique in Dynapro Nano star particle sizer (Wyatt Tech. Corp., CA, USA), particle size analysis was performed by scanning electron microscopy (SEM, Quanta FEG 250, FEI, Hillsborough, OR, USA), zeta potential was tested using NanoZS zetasizer (Malvern Panalytical Inc., Westborough, MA, ABD), and the structure of the NPs was detected by energy dispersive X-ray (EDX) spectroscopy (Apollo X AMATEK).

2.3. Bacterial Inhibition Analysis

Overnight grown bacteria of 1 mL was added into 49 mL of NP solutions and exposed to environmental conditions (different NP concentrations, pH, ionic strength, conductivity, and dark and light) for 1 h at 150 rpm. The used light intensity was 2.1 mW/cm^2. At the end of test duration, serial dilutions were made and bacteria+NP solutions of 100 µL was inoculated into petri dishes. The petri dishes were incubated at 37°C for 24 h. The number of the colonies on petri dishes were counted using a colony counter and reported as CFU/mL. Inhibition tests were triplicated and values were calculated as percentages (%).

2.4. Morphological Examination

Environmental scanning electron microscopy (E-SEM) imaging analysis was performed with FEI Quanta250 FEG model E-SEM in Material Research Laboratory, Izmir Institute of Technology. In fixation procedure: 2 mL of samples were centrifuged at 6000 rpm for 10 min, were washed in phosphate buffer solution (PBS, pH 7.4). Washed sample was centrifuged again and pellet was stored in PBS+cold ethanol (1:1 v/v) at -4°C for 12 h. In E-SEM imaging, 100 µL of sample was placed onto specimen stub and dried at 30°C for 15 min. Sample was then analyzed at 5 kV high vacuum and 50000× magnification.

3. RESULTS AND DISCUSSION

3.1. Nanoparticle Characterization

The size of the CeO_2 NPs was measured by SEM-EDX and DLS, and the zeta potential was measured by zeta sizer. Figure 1a shows the SEM image of the CeO_2 NPs (100 mg/L in ultrapure water) with agglomerated particle shapes. The average particle size was calculated as 45±1.2 nm, which is almost 1.5 to 3 times larger than those of the retailer's info. EDX analysis was applied on three different areas (yellow areas, Figure 1a), and the purity of NP sample was shown in Figure 1b that the sample was only composed of CeO_2 particles (Ce: 40.3±8.7% and O: 55.9±10.6%).

The particle size results obtained from DLS method is given in Table 1. DLS method has been effectively used in particle size measurements and usually an optimum concentration of 50 mg/L has been chosen [28, 29]. A 50 mL of NP concentration prepared in 10 and 50 mM ionic strength, 0.6 and 3

mS/cm conductivity, and pH 5.5 and 6.5 were used. The measurements were obtained at time 24 h. Our results show that the aggregated particle size was strongly ionic strength and pH dependent. At lower ionic strength (10 mM) and pH (5.5) values, the particle size was 343±11 nm. However, when the ionic strength (50 mM) and pH (6.5) increased, micrometer sized (1497±242 nm) particles were observed. Similar finding was also reported in Kosyan et al. [30] that the CeO_2 NP size in the test media was ranged between 192±62 nm and 1.5 µm. Leung et al. [31] showed that size of the particle was highly affected by the medium content.

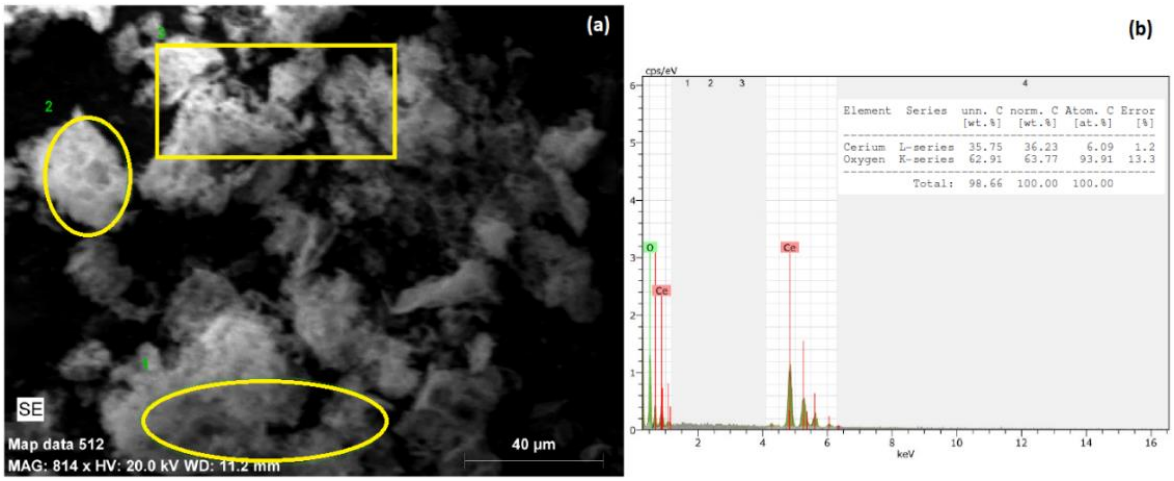

Figure 1. Scanning electron microscopy (SEM) (a) and energy dispersive X-ray (EDX) images (b) of CeO_2 nanoparticles.

Table 1. The effect of ionic strength (IS), conductivity (Cond.) and pH on CeO_2 nanoparticle size (Nanoparticle concentration: 50 mg/L)

Measurement Conditions	Particle Size (nm)
IS: 10 mM, Cond.: 0.6 mS/cm, pH: 5.5	343 ± 11
IS: 50 mM, Cond.: 3 mS/cm, pH: 5.5	1259 ± 224
IS: 10 mM, Cond.: 0.6 mS/cm, pH: 6.5	463 ± 54
IS: 50 mM, Cond.: 3 mS/cm, pH: 6.5	1497 ± 242

The zeta potential values of CeO_2 NPs used in this study were measured as 8.9 mV and 2.1 mV at pH 5.5 and 6.5, respectively. The pH, ionic strength, natural organic matter and etc. of the aquatic environment can highly effect the surface charge of nano-CeO_2 particles. Especially the surface charge of CeO_2 NPs can be negative or positive due to the pH of the solution [3, 4]. In our study, CeO_2 NPs were positively charged. According to studies, at low pH values CeO_2 NPs were positively charged and at high pH values they were negatively charged, and their isoelectric point is pH 8 [32, 33]. Buettner et al. [34] reported that CeO_2 NPs' isoelectric point is between pH 6.5 and 8.1, Berg et al. [35] noted that value as pH 6.71.

3.2. Bacterial Inhibition

The antibacterial effects of CeO_2 NPs on *B. subtilis* is depicted in Figure 1. High ionic strength and conductivity showed high negative effect on *B. subtilis*. Especially at 1000 mg/L NP concentration, 100 mM ionic strength, and 6 mS/cm conductivity under dark condition, maximum inhibition results were calculated as 64% and 68% at pH 5.5 and 6.5, respectively (Fig 2A and 2C). When 2.1 mW/cm^2 of light intensity was applied, the highest inhibitions were resulted from highest NP concentration and ionic strength conditions. The most antibacterial results were calculated as 92% and 95% at pH 5.5 and 6.5 in presence of light, respectively (Fig 2B and 2D).

Al-Shawafi et al. [36] studied the antimicrobial activity of CeO_2 NPs synthesized with different molar ratios of $Ce(NO_3)_3.6H_2O$ and $C_6H_{12}N_4$ (1:20, 5:20, 7:20, 12:20 and 20:20) on *E. coli* and *B. subtilis* (bacteria), and *Saccharomyces cerevisiae* (yeast). CeO_2 NPs with 12:20 ratio showed an inhibitory effect of 67% on *B. subtilis*, where NPs with 5:20 ratio displayed >70% inhibition on *E. coli*. The least antimicrobial result was obtained from NPs with 20:20 ratio on *S. cerevisiae* (45%). Krishnamoorthy et al. [36] showed that the antibacterial effect of CeO_2 NPs on *B. subtilis, E. coli, Salmonella typhimurium and Enterococcus faecalis* can be attributed to membrane stress.

In this study, the optimization of the variables that could affect the extraction efficiency was carried out by monitoring the recovery. Recovery for each variable was calculated according to the following formula.

$$\text{Recovery, \%R} = \frac{C_{int}.V_{int} - C_{final}.V_{final}}{C_{int}.V_{int}}\ 100$$

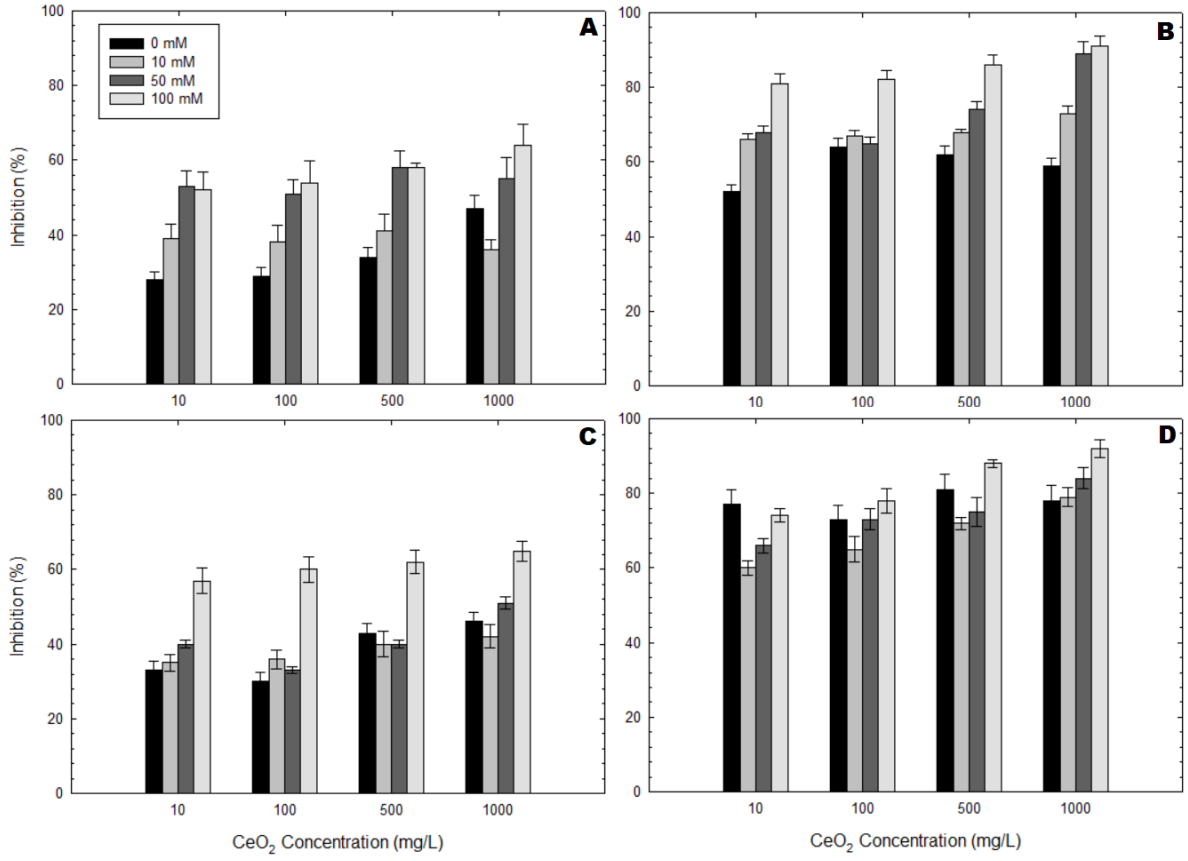

Figure 2. The inhibition effect of CeO_2 NPs on *B. subtilis* bacteria (A: Dark, pH 5.5; B: Light, pH 5.5; C: Dark, pH 6.5; D: Light, pH 6.5).

In Figure 3, the antibacterial effects of CeO_2 NPs on *E. coli* are given. The results from dark and light conditions clearly showed that illumination has a negative impact on bacteria. It is a well-known phenomenon that when CeO_2 NPs are illuminated with a light source (especially UV light), reactive oxygen species (ROS) can be formed. ROS then may disrupt cell membrane and cause stress on bacteria [38]. The most antibacterial result in absence of light was calculated as 52% when test conditions of 10 mM ionic strength, 500 mg/L NP concentration, pH 5.5 were applied. (Fig 3A). However, when light was used, the inhibition increased to 98-99% at test conditions of 50 mM ionic strength and 100–1000 mg/L NP concentration (Fig 3B). At pH 6.5, higher antibacterial effect was observed under dark that the inhibition of bacteria increased to 78% (50 mM, 1000 mg/L) (Fig 3C). On the other hand, lower inhibition values were calculated from illuminated samples that highest result was 84% (50 mM, 100 mg/L) (Fig 3D).

Agarwal et al. [39] showed a 94% inhibition in the growth of *E. coli* after 9 h of visible light exposure. Thill et al. [22] reported the antibacterial effect of CeO_2 NPs (size: 7 nm) dispersed in water on *E. coli* and showed an electrostatic affinity between positively charged NPs and the negatively-charged outer membrane of bacteria. They conclude that cytotoxic effect of CeO_2 NPs on *E. coli* was due to the close contact of NPs and bacteria, and oxidative response. Thill et al [22] and He et al. [40] have also suggested that changing the exposure media may reverse the cytotoxic effect of CeO_2 NPs on *E. coli*, since surface charge density is largely responsible for antibacterial effect.

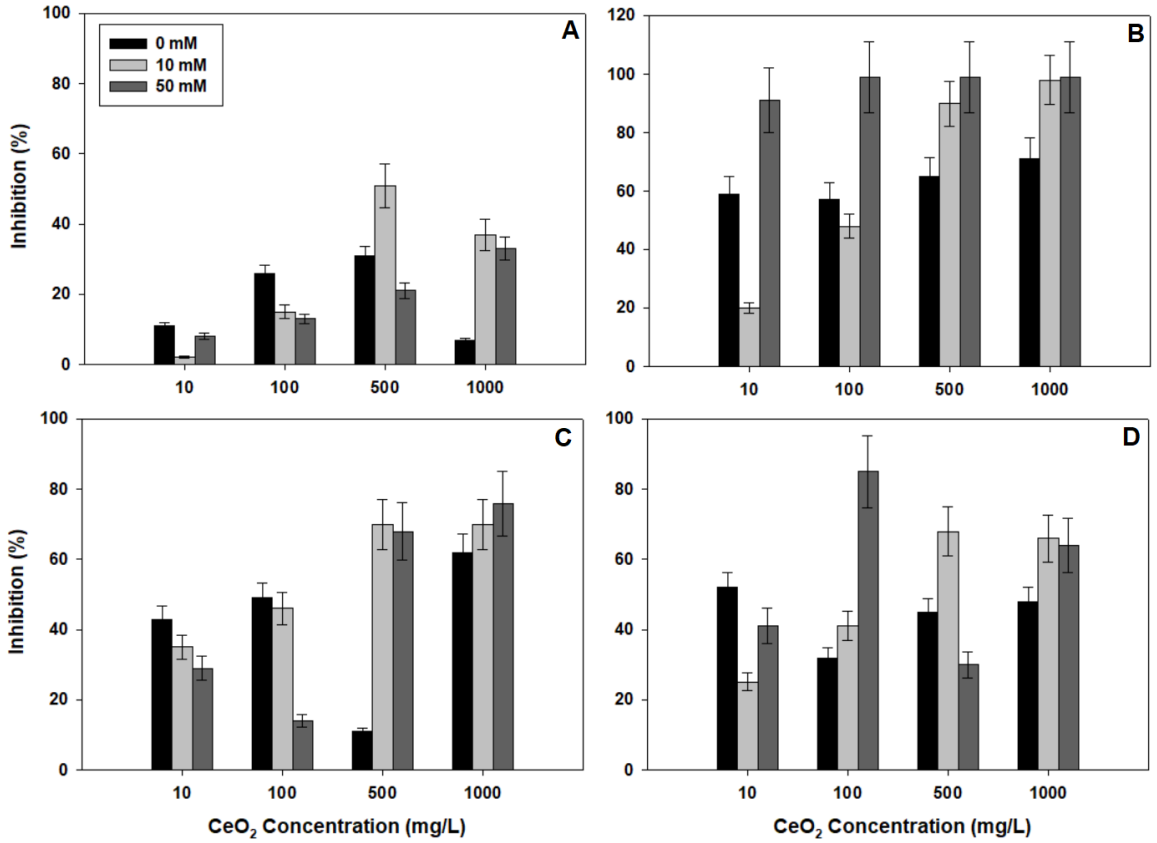

Figure 3. The inhibition effect of CeO_2 NPs on *E. coli* bacteria (A: Dark, pH 5.5; B: Light, pH 5.5; C: Dark, pH 6.5; D: Light, pH 6.5).

In Figure 4, E-SEM images of CeO_2 NPs and *B. subtilis*+CeO_2 NPs are given. Both samples were treated in 100 mg/L of NP concentration, pH 5.5, 50 mM of ionic strength, and 3 mS/cm of conductivity at 23±1.2°C under light (light intensity: 2.1 mW/cm^2). The E-SEM image of CeO_2 NPs clearly shows that high NP concentration and high ionic strength lead to an aggregation/agglomeration of NPs. The NP size distribution was varied between 35 nm and 3.5 µm (Fig 4A). The bacteria + CeO_2 NP image was analyzed, a direct contact, more specifically an adsorption of CeO_2 NPs onto bacteria, was observed. The deformed bacteria were hidden under CeO_2 NPs and their different sizes were shown with red crosses in Figure 4B. The aggregated bacteria + NP size was varied between 65 nm and 6 µm. Similar results were also reported in the literature that CeO_2 NPs had a direct contact with bacterial cell wall [22, 25, 27]. This direct contact mostly resulted in membrane deformation [26] and cell disintegration [27].

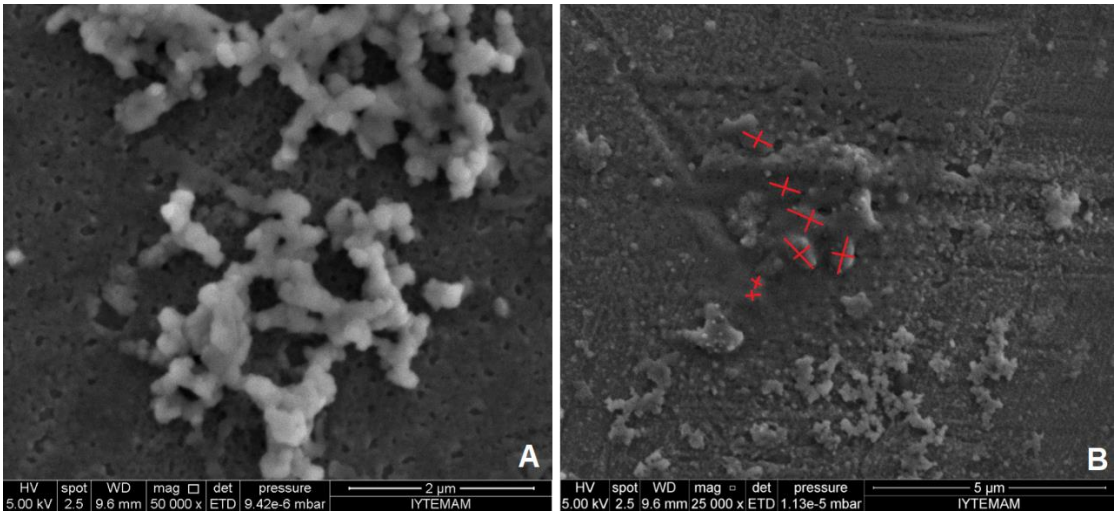

Figure 4. E-SEM images of CeO_2 NPs (A) and *B. subtilis*+ CeO_2 NPs (B) (Condition: NP concentration: 100 mg/L, pH: 5.5, ionic strength: 50 mM, conductivity: 3 mS/cm, temperature:23±1,2ºC and light intensity: *2.1 mW/cm^2)*

4. CONCLUSION

The present study examined the potential ecotoxicity of CeO_2 NPs on *B. subtilis* and *E. coli* bacteria. Regardless of the test conditions, CeO_2 NPs exhibited growth inhibition on both two bacteria. Higher growth inhibitions of bacteria in absence of light were observed compared to the those from literature. This may be attributed to cellular adsorption due to different ionic strength and high NP concentrations. In presence of light, similar results were obtained in this study that high bacterial inhibitions were resulted from high ionic strength and high NP concentration test conditions. The effects of pH varied on bacterial inhibition. It was shown that *B. subtilis* was more susceptible at pH 5.5 and *E. coli* was more sensitive at pH 6.5. The coverage of CeO_2 NPs on bacteria was clearly seen from E-SEM images, and it is suspected that cell damage was mainly caused by the membrane deformation.

As a conclusion of this study, the results suggest that bacteria-NP interaction is the most important factor in explaining the ecotoxic effect of CeO_2 NPs on *B. subtilis* and *E. coli*. However, it is still not clear whether this inhibition effect of CeO_2 NPs can be directly attributed to cellular adsorption. Therefore, further investigations need to be conducted to understand the interaction of NPs with bacteria, the main mechanism of growth inhibition of bacteria, and the fate of NPs in the receiving environments.

In general, CeO_2 NPs have been used in several sectors due to their excellent properties. Based on the results from the literature, many studies have focused on the synthesis of CeO_2 NPs and few of them have defined the factors leading the ecotoxic effects. The synthesis technique of the CeO_2 NPs should be highly efficient, economic, and practical without creating any ecotoxic effects. In overall, more researches need to be focused on environmental- friendly synthesis approaches and a life cycle assessment should be applied on all newly synthesized NPs.

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REFERENCES

[1] Piccinno, F., Gottschalk, F., Seeger, S., Nowack, B., Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J Nanopart. Res., 14 (2012) 1-11.

[2] Sun, C., Li, H., Chen, L., Nanostructured ceria-based materials: synthesis, properties, and applications. Energy Environ. Sci. 5 (2012)

8475-8505.

[3] Collin, B., Auffan, M., Johnson, A.C., Kaur, I., Keller, A.A., Lazareva, A., Lead, J.R., Ma, X., Merrifield, R.C., Svendsen, C., White, J.C., Unrine, J.M., Environmental release, fate and ecotoxicological effects of manufactured ceria nanomaterials. Environ. Sci.: Nano, 1-6 (2014) 533-548.

[4] EU, Commission Staff Working Paper: Types and Uses of Nanomaterials, Including Safety Aspects, Adres: https://ec.europa.eu/health//sites/health/files/nanotechnology/docs/swd_2012_288_en.pdf, Brussels, 2012.

[5] Future Markets Inc, The Global Market for Nanomaterials 2002-2016: Production volumes, revenues and end user markets. http://www.futuremarketsinc.com/, 2012.

[6] Statista, The Statistics Portal, Cerium oxide price worldwide from 2009 to 2025 (in U.S. dollars per metric ton). Adres: https://www.statista.com/statistics/450146/global-reo-cerium-oxide-price-forecast/, 2019.

[7] Bleiwas, D.I., Potential for recovery of cerium contained in automotive catalytic converters, in Open-File Report 2013-1037. U.S. Geological Survey. Adres: https://pubs.usgs.gov/of/2013/1037/OFR2013-1037.pdf, 2013.

[8] Hendren, C.O., Mesnard, X., Droge, J., Wiesner, M.R., Estimating production data for five engineered nanomaterials as a basis for exposure assessment. Environ. Sci. Technol., 45 (2011) 2562-2569.

[9] Jasinski, P., Suzuki, T., Anderson, H.U., Nanocrystalline undoped ceria oxygen sensor. Sensors and Actuators B: Chemical, 95 (2003) 73-77.

[10] Armini, S., De Messemaeker, J., Whelan, C., Moinpour, M., Maex, K., Composite polymer core-ceria shell abrasive particles during oxide cmp: A defectivity study. J Electrochem. Society, 155 (2008) H653-H660.

[11] Auffan, M., Masion, A., Labille, J., Diot, M.A., Liu, W., Olivi, L., Proux, O., Ziarelli, F., Chaurand, P., Geantet, C., Bottero, J.Y., Rose, J., Long-term aging of a CeO_2 based nanocomposite used for wood protection. Environ. Pollut. 188 (2014) 1-7.

[12] Mandoli, C., Pagliari, F., Pagliari, S., Forte, G., Di Nardo, P., Licoccia, S., Traversa, E., Stem cell aligned growth induced by CeO_2 nanoparticles in PLGA scaffolds with improved bioactivity for regenerative medicine. Adv. Functional Mat., 20 (2010) 1617-1624.

[13] Colon, J., Herrera, L., Smith, J., Patil, S., Komanski, C., Kupelian, P., Seal, S., Jenkins, D.W., Baker, C.H., Protection from radiation-induced pneumonitis using cerium oxide nanoparticles. Nanomed.: Nanotechnol., Biol. Med., 5 (2009) 225-231.

[14] Dai, Q., Wang, J., Yu, J., Chen, J., Chen, J., Catalytic ozonation for the degradation of acetylsalicylic acid in aqueous solution by magnetic CeO_2 nanometer catalyst particles. Chem. Appl. Cat. B: Environ., 144 (2014) 686-693.

[15] Gottschalk, F., Sun, T.Y., Nowack, B., Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. Environ. Pollut., 181 (2013) 287-300.

[16] Lazareva, A., Keller, A.A., Estimating potential life cycle releases of engineered nanomaterials from wastewater treatment plants. ACS Sustainable Chem. Eng., 2 (2014) 1656-1665.

[17] Mueller, N.C. and Nowack, B., Exposure modeling of engineered nanoparticles in the environment. Environ. Sci. Technol., 42 (2008) 4447-4453.

[18] Gottschalk, F. and Nowack, B., The release of engineered nanomaterials to the environment. J. Environ. Monit., 13 (2011) 1145-1155.

[19] Quik, J.T.K., Lynch, I., Van Hoecke, K., Miermans, C.J.H., De Schamphelaere, K.A.C., Janssen, C.R., Dawson, K.A., Stuart, M.A.C., Van de Meent, D., Effect of natural organic matter on cerium dioxide nanoparticles settling in model fresh water. Chemosphere, 81 (2010) 711-715.

[20] Zhang, P., He, X., Ma, Y., Lu, K., Zhao, Y., Zhang, Z., Distribution and bioavailability of ceria nanoparticles in an aquatic ecosystem model. Chemosphere, 89:5 (2012) 530-535.

[21] Jabiol, J., McKie, B.G., Bruder, A., Bernadet, C., Gessner, M.O., Chauvet, E., Trophic

complexity enhances ecosystem functioning in an aquatic detritus-based model system. J. Anim. Ecol., 82 (2013) 1042-1051.

[22] Thill, A., Zeyons, O., Spalla, O., Chauvat, F., Rose, J., Auffan, M., Flank, A.M. , Cytotoxicity of CeO_2 nanoparticles for *Escherichia coli*: Physico-chemical insight of the cytotoxicity mechanism. Environ. Sci. Technol., 40 (2006) 6151-6156.

[23] Pelletier, D.A., Suresh, A.K., Holton, G.A., McKeown, C.K., Wang, W., Gu, B.H., Mortensen, N.P., Allison, D.P., Joy, D.C., Allison, M.R., Brown, S.D., Phelps, T.J., Doktycz, M.J., Effects of engineered cerium oxide nanoparticles on bacterial growth and viability. Appl. Environ. Microbiol., 76 (2010) 7981-7989.

[24] Dar, M.A., Gul, R., Alfadda, A.A., Karim, M.R., Kim, D.W., Cheung, C.L., Almajid, A.A., Alharthi, N.H., Pulakat, L., Size-dependent effect of nanoceria on their antibacterial activity towards *Escherichia coli*. Sci. Adv. Mat., 9:7 (2017), 1248-1253.

[25] Zeyons, O., Thill, A., Chauvat, F., Menguy, N., Cassier-Chauvat, C., Orear, C., Daraspe, J., Auffan, M., Rose, J., Spalla, O., Direct and indirect CeO_2 nanoparticles toxicity for *Escherichia coli* and *Synechocystis*. Nanotoxicology, 3 (2009) 284-295.

[26] Fang, X.H., Yu, R., Li, B.Q., Somasundaran, P., Chandran, K., Stresses exerted by ZnO, CeO_2 and anatase TiO_2 nanoparticles on the Nitrosomonas europaea. J. Colloid Interface Sci., 348 (2010) 329-334.

[27] Rodea-Palomares, I., Boltes, K., Fernández-Piñas, F., Leganés, F., García-Calvo, E., Santiago, J., Rosal, R., Physicochemical characterization and ecotoxicological assessment of CeO_2 nanoparticles using two aquatic microorganisms. Toxicol. Sci., 119 (2011) 135-145.

[28] Kato, M., Suzuki, M, Fujita, K, Horie, M, Endoh, S, Yoshida, Y, Iwahashi, H, Takahashi, K, Nakamura, A, Kinugasa, S,, Reliable size determination of nanoparticles using dynamic light scattering method for in vitro toxicology assessment. Toxicol. In Vitro, 23 (2009) 927-934.

[29] Kato, H., Fujita, K, Horie, M, Suzuki, M, Nakamura, A, Endoh, S, Yoshida, Y, Iwahashi, H, Takahashi, K, Kinugasa, S., Dispersion characteristics of various metal oxide secondary nanoparticles in culture medium for in vitro toxicology assessment. Toxicol. In Vitro, 24 (2010) 1009-1018.

[30] Kosyan D.B., Y.E.V., Vasilchenko A.S., Vasilchenko A.V., Miroshnikov S.A., Toxicity of SiO_2, TiO_2 and CeO_2 nanoparticles evaluated using the bioluminescence assay. Int. J. Geomate, 13-40 (2017), 66-73.

[31] Leung, Y.H., Yung, M.M.N., Ng, A.M.C., Mab, A.P.Y., Wong, S.W.Y., Chan, C.M.N., Ng, Y.H., Djurisic, A.B., Guo, M., Wong, M.T., Leung, F.C.C., Chan, W.K., Leung, K.M.Y., Lee, H.K., Toxicity of CeO_2 nanoparticles – The effect of nanoparticle properties. J. Photochem. Photobiol. B: Biology, 145 (2015) 48-59.

[32] Baalousha, M., Ju-Nam, Y., Cole, P.A., Gaiser, B., Fernandes, T.F., Hriljac, J.A., Jepson, M.A., Stone, V., Tyler, C.R., Lead, J.R., Characterization of cerium oxide nanoparticles-part 1: size measurements. Environ. Toxicol. Chem., 31-5 (2012), 983-993.

[33] Baalousha, M., Ju-Nam, Y., Cole, P.A., Hriljac, J.A., Jones, I.P., Tyler, C.R., Stone, V., Fernandes, T.F., Jepson, M.A., Lead, J.R., Characterization of cerium oxide nanoparticles. Part 2: Nonsize measurements. Environ. Toxicol. Chem., 31-5 (2012) 994-1003.

[34] Buettner, K.M., Rinciog, C.I., Mylon, S.E., Aggregation kinetics of cerium oxide nanoparticles in monovalent and divalent electrolytes. Colloid. Surface. A, 366 (2010) 74-79.

[35] Berg, J.M., Romoser, A., Banerjee, N., Zebda, R., Sayes, C.M., The relationship between pH and zeta potential of ~ 30 nm metal oxide nanoparticle suspensions relevant to in vitro toxicological evaluations. Nanotoxicology, 3-4 (2009), 276-283.

[36] Al-Shawafi, W.M., Salah, N., Alshahrie, A., Ahmed, Y.M., Moselhy, S.S., Hammad, A.H., Hussain, M.A., Memic, A., Size controlled ultrafine CeO_2 nanoparticles produced by the microwave assisted route and their

antimicrobial activity. J Mater Sci: Mater Med, 28-177 (2017), 1-10.

[37] Krishnamoorthy, K., Veerapandian, M., Zhang, L.H., Yun, K., Kim, S.J., Surface chemistry of cerium oxide nanocubes: Toxicity against pathogenic bacteria and their mechanistic study. J Ind Eng Chem, 20-5 (2014), 3513-3517.

[38] Collin, B., Oostveen, E., Tsyusko, O.V., Unrine, J.M., Influence of natural organic matter and surface charge on the toxicity and bioaccumulation of functionalized ceria nanoparticles in *Caenorhabditis elegans*. Environ Sci Technol, 48-2 (2014), 1280.

[39] Agarwal, C., Aggrawal, S., Dutt, D., Mohanty, P., Cerium oxide immobilized paper matrices for bactericidal application. Materials Science and Engineering: B, 232-235 (2018) 1-7.

[40] He, X., Kuang, Y., Li, Y., Zhang, H., Ma, Y., Bai, W., Zhang, Z., Wu, Z., Zhao, Y., Chai, Z., Changing exposure media can reverse the cytotoxicity of ceria nanoparticles for *Escherichia coli*. Nanotoxicology, 6 (2012) 233-240.