

Effect of ZnO nanoparticles in the oxygen uptake during aerobic wastewater treatment

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Abstract The increased use of ZnO nanoparticles (NPs) in everyday products indicates the importance of studying NPs release to the wastewater and its possible effect on biological process for wastewater treatment. Therefore, the aim of this work was to study the effect of the presence of ZnO NPs in aerobic wastewater treatment. The results indicated that the oxygen uptake rate of microorganisms is inhibited for concentrations higher than 473 mg L^{-1} of ZnO NPs. The diversity of microorganisms involved in wastewater treatment was reduced in presence of ZnO NPs. Related to morphological interaction between ZnO NPs and suspended biomass, physical damage in flocs structure were observed in presence of ZnO NPs. However, the internalization of Zn compounds in microorganisms not presented mechanical damage in the membrane cell. These findings suggest that inhibition in oxygen uptake was caused for negative

effect that ZnO NPs induces in aerobic microorganisms involved in wastewater treatment.

Keywords ZnO nanoparticles · Wastewater · Activated sludge · Oxygen uptake · Bacterial diversity · Environmental effects

Introduction

The growth in the use and production of nanomaterials (NMs) due to the incorporation of them in new products has like a consequence the release of them to the environment. It has been reported in 2010 that 15 % of the products that made up the global market were synthesized by nanotechnology in their manufacturing processes (Keller and Lazareva 2013). Some of the common products containing NMs are cosmetics, paints, pigments, and solar coatings. Consequently, NMs can be found in the wastewater streams (Ma et al. 2014). Even though NMs are highly used at industry scale, the impacts of these NMs on the environment and human health are still not clear.

It is logical to think that NMs could arrive to the wastewater treatment plant (WWTP) by following the pathway of the wastewater streams. A recent research has demonstrated the presence of Ag NPs in the influent of nine WWTPs in Germany, suggesting secondary treatment of such influent fraction (Li et al. 2013). Another research (Kiser et al. 2009) also

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reported the occurrence of TiO₂ NPs in the WWTP, which suggested that the presence of NPs with similar behavior could have been through the absorption of the biomass, thereby still remaining in the effluents. Therefore, one of the new fields that have been developed to explore the effect of the NMs is the biological wastewater treatment. Activated sludge represents the main process applied during secondary treatment in probably more than 70,000 WWTPs around the world (Seviour and Nielsen 2010). It is a biological system where even a small change in the process can modify the efficiency of the wastewater treatment; therefore it is important to research the possible effects caused by the input of NPs to the biological wastewater treatment.

Some studies have verified that nanoparticles (NPs) can alter bacterial communities in different environmental matrices such as soil or water. The consequences of these alterations are changes in metabolism and geochemical cycles, reduction of microbial biomass, and changes in the bacterial taxa associated with nitrogen fixation, methane oxidation, and organic compound decomposition (Ge et al. 2012). The majority of ecotoxicological studies have been carried out using carbon nanotubes (Parise et al. 2014); however, ecological studies on the wide range of metallic NPs and oxide metallic NPs is very scarce or even absent. In a recent study, the aggregate stability of ZnO NPs and TiO₂ NPs in wastewater was reported, and therefore study on the effects of the aggregates during the wastewater treatment was performed (Xiao-Hong et al. 2015). In related with the effects on microorganisms, some authors found that there was an impact of Ag NPs and ZnO NPs on the functional bacteria that are commonly present in the activated sludge (Chen et al. 2014). The main mechanisms of NPs antibacterial activity reported are as follows: (1) dissolution of metal ions from NPs and their uptake into cells followed by depletion of intracellular ATP production and disruption of DNA replication (Song et al. 2010), (2) reactive oxygen species (ROS) generation from NP metal oxides and ions with subsequent oxidative damage to cellular structures such as mitochondria (Meruvu et al. 2011), and (3) changes in the membrane permeability which affect the pore size and the dissipation of the proton motive force as a result of accumulation and dissolution of NPs on the membrane (Sirelkhatim et al. 2015). However, it is not clear how these mechanisms of

antibacterial activity affect the aerobic wastewater treatment.

In the present work, the impact of ZnO NPs on aerobic wastewater treatment was studied, through the evaluation of the oxygen consumption by activated sludge exposed to different concentrations of ZnO NPs, the changes in the bacterial populations in contact with ZnO NPs, and finally, the detection of morphologic interactions between flocs and ZnO NPs.

Materials and methods

ZnO NPs characterization and working solution

The stock of ZnO NPs in powder was purchased from I&D Nanotechnology (México) and was observed by scanning electron microscopy (SEM, Jeol JSM 7401F) and transmission electron microscopy (TEM, Jeol 2200FS HR-FE-TEM). For SEM and TEM characterizations of ZnO NPs, a suspension of the nanoparticles in ethanol (1 mL) was prepared and sonicated for 15 min. 300 µL of this solution was placed onto an aluminum support and then analyzed by SEM operated at 5.0 kV. TEM samples were prepared by placing one drop of the suspension onto nickel grids, and the ethanol was evaporated using plasma for a period of 5 min and then observed (Fig. 1a). The ZnO NPs purity was defined through energy-dispersive spectroscopy (EDS) in the samples exposed to SEM. The size distribution was estimated by measuring NPs in 15 images by the software ImageJ, while the crystalline form was determined by comparing the X-ray diffraction (XRD) spectrum with the patterns in databases (Brayner et al. 2010). Furthermore, the UV–Vis spectrum was used to verify the response of ZnO NPs in this region. After characterization, working solution of ZnO NPs was prepared by adding 6000 mg L⁻¹ in MilliQ water (pH 7), sonicating for 30 min, and then stirring at 200–300 rpm for 24 h before use (Roebben et al. 2011).

Biomass seed and wastewater used in assays

The biomass seed for oxygen consumption assays was taken in a sequential batch reactor (SBR) with an effective volume of 40 L. The SBR pilot reactor was operated for 120 days and was used to treat 20 L of synthetic wastewater per cycle, before supplying

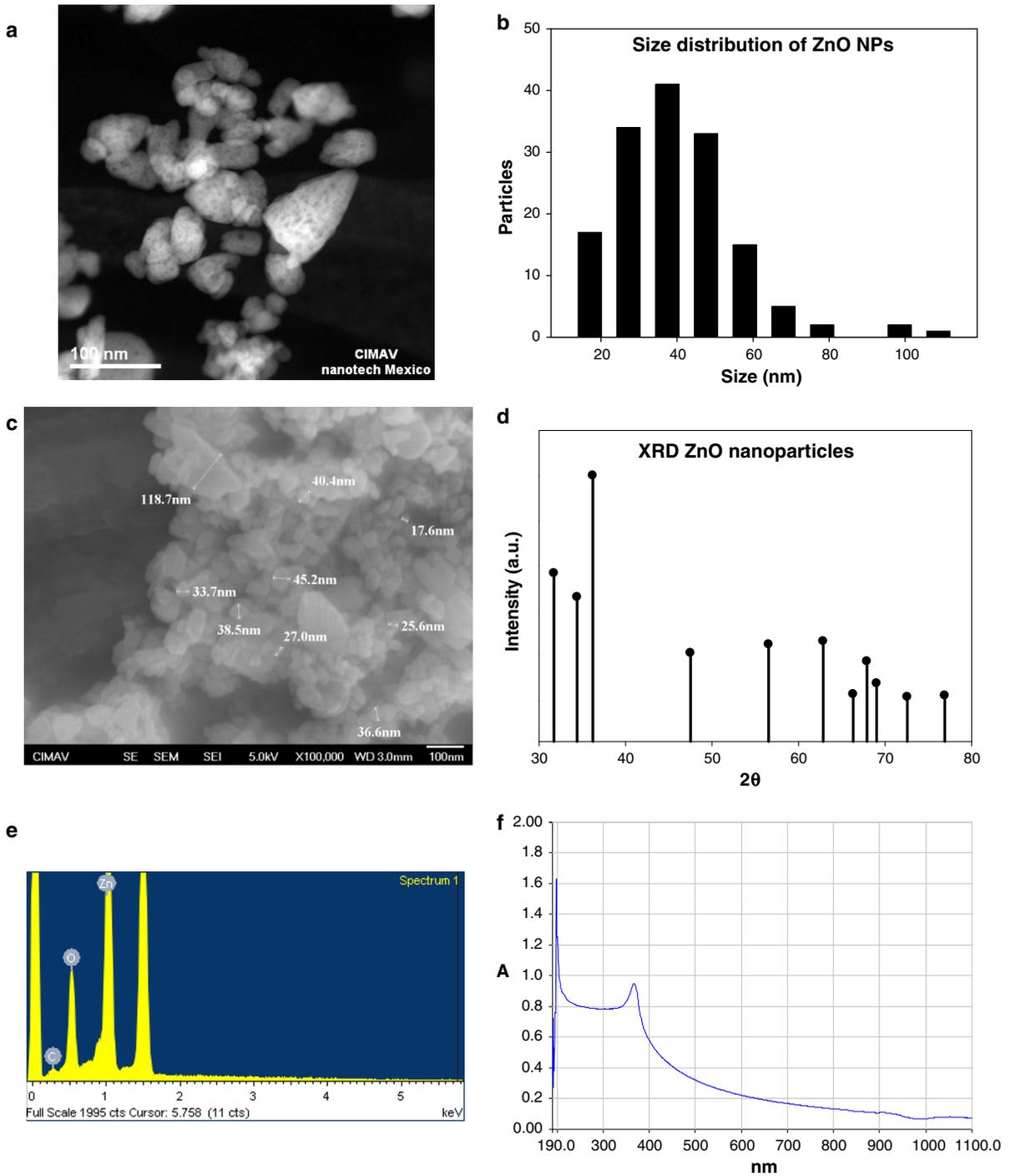


Fig. 1 Characterization of ZnO NPs. **a** TEM image **b** size distribution, **c** SEM image, **d** XRD pattern, **e** EDS and **f** UV-Vis spectra

biomass for the seeding oxygen consumption tests. Each cycle consisted of 15 min of filling, 4.5 h of anoxic reaction, 18.5 h aerobic reaction, 30 min of settling and 15 min for decanting.

The synthetic wastewater used in SBR had a C:N:P ratio of 100:5:1 and contained dextrose (1902 mg L^{-1}), NH_4Cl (344 mg L^{-1}), and K_2HPO_4 (72 mg L^{-1}) as main nutrients, while $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (44 mg L^{-1}),

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (19 mg L^{-1}), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.18 mg L^{-1}), H_3BO_3 (0.29 mg L^{-1}), $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$ (0.28 mg L^{-1}), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.28 mg L^{-1}), and NaCl (71 mg L^{-1}) as trace nutrients (Cuevas-Rodríguez et al. 2015).

Respirometry assays

The oxygen consumption was evaluated through respirometry assays. The respirometry used gas-static-static (GSS) principle, in which the oxygen transferred from the gas phase to the liquid phase was measured through the change in pressure at $20 \text{ }^\circ\text{C}$ in a closed bottle (Hach BOD Track II, USA). The respirometry tests consisted in measuring oxygen uptake every 20 min for 8 h, which is the typical hydraulic retention time (HRT), in activated sludge. The liquid phase inside the bottle contained a total volume of 95 mL: 40 mL of biomass seed, 40 mL of synthetic wastewater, and 15 mL of ZnO NPs working solution. The concentrations of ZnO NPs tested in respirometry tests through GSS principle were 283, 300, 473, 568, 750, 900, 1200, 1500, 1800, and 2000 mg L^{-1} . The controls of respirometry tests consisted of 40 mL of biomass seed, 40 mL of synthetic wastewater, and 15 mL of MilliQ water (pH 7) without ZnO NPs. The biomass seed contained volatile suspended solids (VSS) at a concentration of $2540 \pm 530 \text{ mg L}^{-1}$ in respirometry assays. The assay was conducted in triplicate, and the concentrations of ZnO NPs and controls were evaluated each time. Data analysis was performed using one-way ANOVA, followed by Dunnett test to determine the significant difference by comparing with the control. Results with $p < 0.05$ were considered significant.

Bacterial diversity

After respirometry assays, control sample and samples exposed to ZnO NPs at different concentrations of 473, 900, 1500, and 2000 mg L^{-1} were used to determine the bacterial diversity through the detection of terminal restriction fragment length polymorphism (T-RFLP). 0.2 g of suspended biomass was used to extract the total DNA with the help of Soil DNA Isolation Kit (Mo BioTM Laboratories, USA) and was quantified using Qubit[®] fluorometer (InvitrogenTM, USA), according to the instructions of the manufacturer. DNA solutions were preserved at $-20 \text{ }^\circ\text{C}$ until use.

For T-RFLP analysis, the 16S rDNA gene was amplified by PCR using primers 8F and 1492R (5'-AGAGTTTGATCCTGGCTAG-3' and 5'-GGTTACCTTGTTACGACTT-3'; Lane 1991). The primer 8F was labeled with carboxyfluorescein (6-FAN). The reaction mixture for PCR contains 1X PCR buffer, 1.5 mM MgCl_2 , 0.2 mM of each dNTPs, 1.25 U Taq Polymerase (EurobioTM, France), and 1 μL of template DNA. The reactions were cycled in a PTC 200 Thermal cycler (MJ Research Inc., USA) with an initial denaturation step at $94 \text{ }^\circ\text{C}$ for 10 min, followed by 35 cycles of denaturation at $94 \text{ }^\circ\text{C}$ for 1 min, annealing at $52 \text{ }^\circ\text{C}$ for 1.5 min, extension at $72 \text{ }^\circ\text{C}$ for 1 min, and a final extension step at $72 \text{ }^\circ\text{C}$ for 15 min. PCR products were purified with the GFX PCR DNA purification kit (GE Healthcare Inc.) and digested for separation with 10U of restriction enzymes HaeIII, HinfI, or AluI at $37 \text{ }^\circ\text{C}$ for 3 h. Approximately 100 ng of digested DNA was mixed with 0.25 μL of ROX ladder (Applied Biosystem[®], USA) and 8.75 μL of formamide. The lengths of the terminal restriction fragments (TRFs) were determined by capillary electrophoresis on an ABI Prism[®] 3130XL Genetic Analyzer (Applied Biosystems[®], USA). After an injection step of 10 s, electrophoresis was carried out for 30 min at 15 kV. The T-RFLP profiles were analyzed using the Gene Scan Software (Applied Biosystems[®], USA). Datasets lower than 0.05 % were eliminated as instrumental background noise. After the data have been normalized, the TRFs representing $<1 \%$ of the total fluorescence were removed (Hewson and Fuhrman 2006). Statistical analysis was carried out with MVSP Software (Multi-variance Statistical Package 3.1, Kovach Computing Services, UK).

TEM imaging

Following respirometry assays, control sample and samples exposed to ZnO NPs at different concentrations of 473, 900, 1500 and 2000 mg L^{-1} of ZnO NPs were fixed with 3 % glutaraldehyde for 3 h at room temperature, washed 4 times with sodium cacodylate buffer (0.1 M, pH 7.4), and 1 % osmium tetroxide was added to the sodium cacodylate buffer for 1 h. After fixation, samples were washed again 4 times with the same sodium cacodylate buffer. Subsequently, the samples were dehydrated with ethanol, and washed twice with 10, 30, 50, 70, 90, 96, and 100 % ethanol

after each step. Finally, the samples were embedded in EPON812 resin. The resin blocks were cut in sections of 60 nm using an ultramicrotome. Observations were made with a transmission electron microscope at 80 kV (Jeol JM-1010). Additionally, images in Z-contrast were captured to develop chemical mapping to determine zinc inside the cells. This technique was developed using a high-angle annular dark field (HAADF) detector in scanning transmission electron microscopy (STEM) mode, operated at 300 kV (FEI Titan 80-300).

Results and discussion

ZnO nanoparticles characterization

SEM and TEM characterizations of ZnO NPs revealed nanorod morphology (Fig. 1a, c), and were between 20 and 60 nm in size (Fig. 1b). The crystalline phase detected by XRD spectrum was wurtzite (Fig. 1d) and was compared with the database (Brayner et al. 2010). The presence of other metals related to ZnO NPs was not detected on EDS (Fig. 1e). The UV–Vis spectrum of the ZnO NPs was also analyzed, and a typical peak at 365 nm in the UV region was found (Sinhamahapatra et al. 2012).

Preparation of biomass seed

The main operating conditions of SBR for seeding supply are given in Table 1. These operating conditions applied in the SBR are essential in an activated sludge system to achieve a steady state of the biomass (Tchobanoglous et al. 2008). The macronutrients monitored in the influent and effluent of the SBR were organic matter measured as chemical oxygen demand (COD), ammonium ($\text{NH}_4\text{-N}$), and orthophosphate ($\text{PO}_4\text{-P}$). The concentrations in the effluent correspond to values reported in literature where has been successfully used this technology for domestic wastewater treatment, so this behavior suggests the stability of the system (Tchobanoglous et al. 2008). The steady biomass present in SBR was used as an inoculum for respirometry assays.

Respirometry assays

The respirometry method GSS was very effective at detecting the oxygen uptake by microorganisms

because this technique detected only the oxygen transferred from the gas to liquid phase (Spanjers et al. 1998). Comparing all respirometry assays results, the aerobic microorganisms exposed to 283, 300, and 473 mg L^{-1} of ZnO NPs statistically presented the same variations in oxygen uptake compared to the controls. Thus, the oxygen uptake of activated sludge presented tolerance to concentrations lower than 473 mg L^{-1} of ZnO NPs. In concentrations from 568 to 900 mg L^{-1} of ZnO NPs, similar inhibitory effect in oxygen uptake were detected (Fig. 2). For concentrations higher than 900 mg L^{-1} of ZnO NPs was observed a remarkably linear decrease in the oxygen uptake.

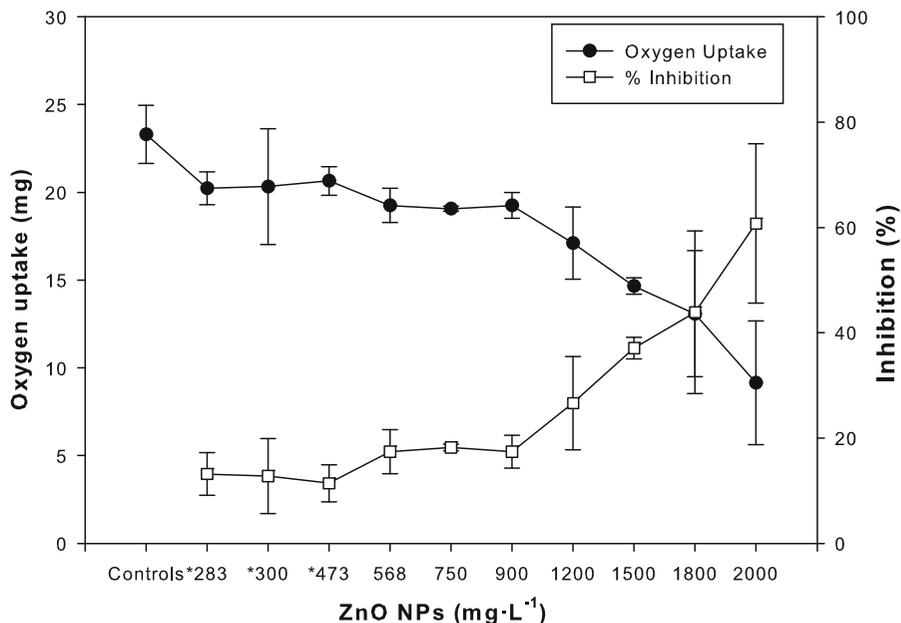
The inhibition percentage of oxygen uptake was estimated by comparing the average data of the treatment system with controls (Fig. 2). One of the comparative estimation is the 50 % of inhibition in the oxygen uptake of the microorganisms (IC50), which in this study was found at 1800 mg L^{-1} . This value is higher than reported in the literature, where using similar system of activated sludge exposed to ZnO NPs, values for IC50 were found around 300 mg L^{-1} of ZnO NPs after 4 h of experimentation (Liu et al. 2011). In another research, the resulting time course of microelectrode measurements demonstrated that ZnO NPs inhibited respiration within 2 h in the presence of 50 mg L^{-1} (Hou et al. 2014). Thereby, the inhibition in oxygen uptake by microorganism is related to time of exposition to ZnO NPs.

Figure 3 shows the plot of biochemical oxygen demand (BOD) at the end of experiments versus different concentrations of ZnO NPs. The decrease in the BOD with the increasing concentration of ZnO NPs can be attributed to the exhaustion of organic matter which is readily biodegradable due to possible transformation of the organic compounds into complexes—in other words addition of Zn to organic molecules (Dubey et al. 2015). Another possible cause may be the reduction in the diversity of the microorganisms in the culture, affecting the number of heterotrophic bacteria due to the presence of ZnO NPs (Chen et al. 2014). Releasing Zn ions which can penetrate the membrane cell or even the internalization of ZnO NPs is the main mechanism reported by which ZnO NPs cause damage to microorganisms (Sirelkhatim et al. 2015). The excess of metallic ions inside the cell can alter some metabolic activities such as the oxidation of metabolites, production of ATP and

Table 1 Operating conditions of activated sludge SBR pilot reactor for supplying biomass seeding to respirometry tests. V_r volume of replacement, V_t total volume of the reactor

Parameter	Units	Average	SD
Organic load applied	(Kg COD Kg VSS ⁻¹ d ⁻¹)	0.48	0.06
V_r/V_t	–	0.5	–
VSS	(mg L ⁻¹)	2.9	410
Hydraulic retention time	(Hours)	24	–
Sludge retention time	(Days)	8	–
pH	–	6.87	0.7
Dissolved oxygen	(mg L ⁻¹)	3.55	0.9
COD			
Influent	(mg L ⁻¹)	1916.55	250.02
Effluent	(mg L ⁻¹)	131.56	42.88
NH ₄ -N			
Influent	(mg L ⁻¹)	71.82	15.52
Effluent	(mg L ⁻¹)	22.76	6.15
PO ₄ -P			
Influent	(mg L ⁻¹)	14.1	6.45
Effluent	(mg L ⁻¹)	1.96	1.9

Fig. 2 Mass of oxygen uptake during respirometry tests and inhibition percentage in the presence of ZnO NPs compared with control group. Mark (*asterisk*) represents no significant difference with controls



electron transport chain. Besides, the presence of excessive Zn ions can lead to the overproduction of ROS and induce apoptosis in some microorganisms. If bacterial diversity is altered, then the wastewater treatment process could be affected. For this purpose, the diversity of the population of microorganisms in the system was studied, which is described in “[Bacterial diversity](#)”.

In Fig. 4 is shown the OUR performance for 8 h of ZnO NPs at different concentrations. The control OUR values were within the range reported for aerobic biomass in the presence of readily biodegradable substrate (HagMan and La Cour 2007). The behavior of OUR control was similar to the OUR in presence of 473 and 900 mg L⁻¹ of ZnO NPs, which indicate that aerobic microorganisms can tolerate the presence of ZnO NPs.

Fig. 3 BOD at the end of the experiment in the presence of ZnO NPs compared with control group. Mark (*asterisk*) represents no significant difference when compared with control group

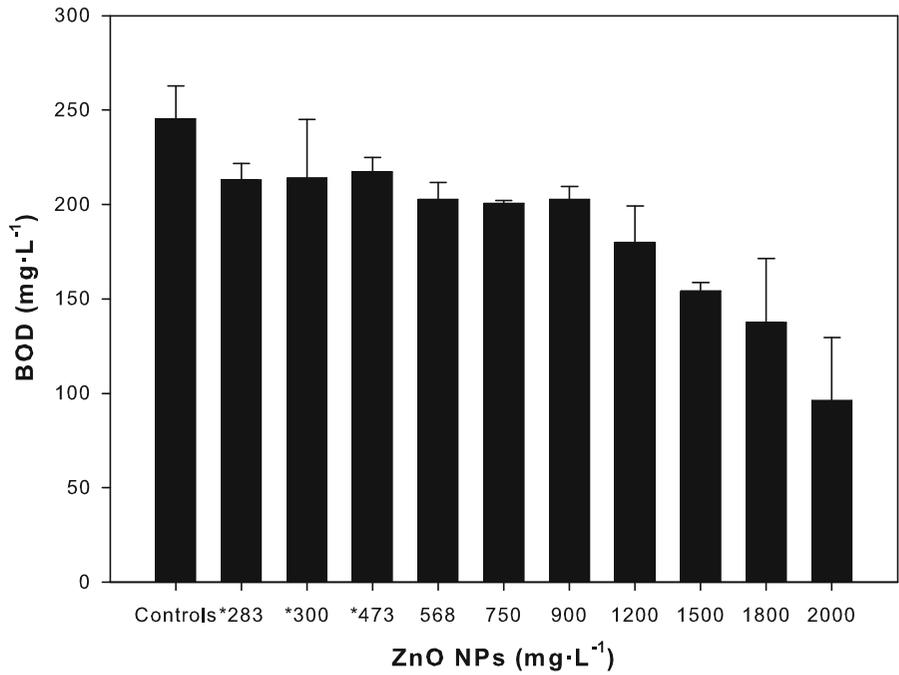
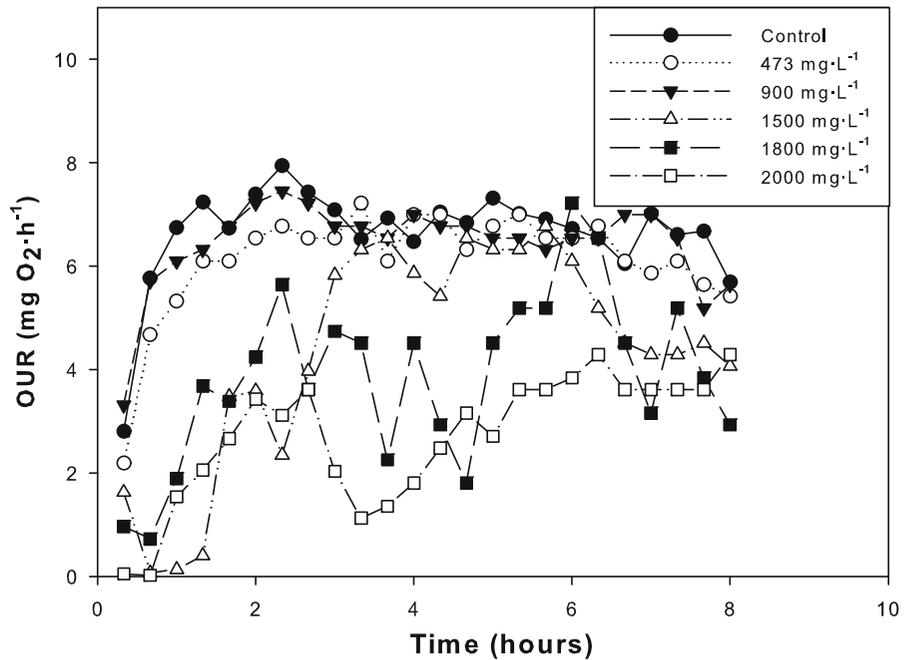


Fig. 4 Oxygen uptake rate on the main concentrations of ZnO NPs



Remarkable reduction in the OUR was detected in presence of 1500, 1800 and 2000 mg L⁻¹ of ZnO NPs, suggesting a possible decay of the heterotrophic microorganisms that carry out a slow oxygen uptake, and consequently a slow organic biodegradation of the

compounds present in wastewater. However, in presence of 1500 mg L⁻¹ of ZnO NPs was detected an increase in the OUR in the period from the third to the sixth hour, showing resilience of some aerobic microorganisms such as *Haliscomenobacter hydrossis*, *Zoogloea*

ramigera, and *Methyloversatilis universalis* which are species commonly present in wastewater treatment and have been reported as tolerant to ZnO NPs (Chen et al. 2014), reducing the OUR when has been exhausted the easily biodegradable compounds in wastewater. In the case of 1800 and 2000 mg L⁻¹ of ZnO NPs, the time-course variability, from 4 to 8 h, showed a slight increase in the OUR, reflecting recuperation of the system. This phenomenon is commonly observed when the substrate biodegrades slowly and hydrolyzes into a readily biodegradable form, although it could also indicate that the microorganisms have adapted to the presence of the ZnO NPs as was mentioned in the long-term experiments (Hou et al. 2012; Puay et al. 2015; Tan et al. 2015). Regarding to the exposure time, the tendency observed in this study about stabilization of OUR after 4 h of experimentation has been also reported by Zheng et al. (2011).

Bacterial diversity

The bacterial diversity was evaluated at the end of the respirometry assays with 473, 900, 1500, and 2000 mg L⁻¹ of ZnO NPs. After data treatment, the TRFs were normalized and each one was considered as a taxonomic unit (OTU). These OTUs are illustrated by circles in Fig. 5—the amplitude of each circle indicates the relative abundance of TRFs detected. The three restriction enzymes revealed a clear reduction in the bacterial population abundance in microcosms exposed to ZnO NP as compared to control ones. For instance, with HaeIII 28 OTUs were detected in the control microcosms, while in the samples exposed to ZnO NPs around 15 OTUs were observed (Fig. 5a). Interestingly, some populations, which were apparently absent in the controls, emerged in the systems exposed to the NPs—in other words, had their size augmented. This was observed for the OTUs 39, 180, and 300 with HaeIII; 270, 300, and 332 with HinfI; and 152, 236, and 373 with AluI. On the other hand, some major populations present in the controls, were not detected in the systems treated with ZnO NPs, such as the TRF 323 with HinfI and 75 with AluI. This behavior is typically observed when a reduction in the competition occurs, i.e., one population grows up while several others disappear. Despite the fact that the respirometry assays have yielded a negative result, these results suggest that the ZnO NP acted over the populations that carry out the aerobic

decomposition of organic carbon, and apparently, the reduction of these populations seems to have favored the production of those not participating in such a process. This can be seen, for instance, in the T-RFLP profile for 2000 mg L⁻¹ ZNO NPs, in which a small number of dominant populations remained, while others almost disappeared.

Similar results were observed by Chen et al. (2014) using T-RFLP which detected a decrease of some OTUs exposed to Ag NPs and ZnO NPs, and it was found that microorganism populations involved in organic matter decomposition and nitrogen removal were affected. The absence of these species is related to the reduction in the OUR, and as a consequence, in the development of the biological processes such as organic matter decomposition and nitrogen removal, as was mentioned in some reports exposing activated sludge to ZnO NPs for 10 h in a laboratory experiment (Hou et al. 2013; Chen et al. 2014).

Another study on the long-term supply of ZnO NPs for 242 days (Sun et al. 2013) demonstrated that microorganisms related with organic matter decomposition, nitrification, and denitrification process were affected. In other hand, the development of bacteria tolerant to ZnO NPs has permitted recover wastewater treatment capability in long term experiments (Tan et al. 2015). The persistence of some microorganisms species that can develop activities related to the wastewater treatment has been linked to the mitochondrial function (Sirelkhatim et al. 2015). ZnO NPs can induce mitochondrial function mainly through two ways: the generation of ROS for bacteria as a defense mechanism and the use of Zn ions as a cofactor in some enzymes, which regulate metabolic activities. Thereby, as the microorganisms have the ability to normalize the presence of Zn ions and ROS, they can be tolerant to ZnO NPs (Yu et al. 2013).

TEM imaging

The samples for TEM imaging were collected at the end of the respirometry assays. The control imaging allows us to detect morphologies of algae, helminths, and protozoa, which are common in the aerobic system for wastewater treatment. In activated sludge reactors, the microorganisms form conglomerates called flocs, which are suspended in the mixed liquor, and they are responsible for the removal of contaminants in the biological wastewater treatment.

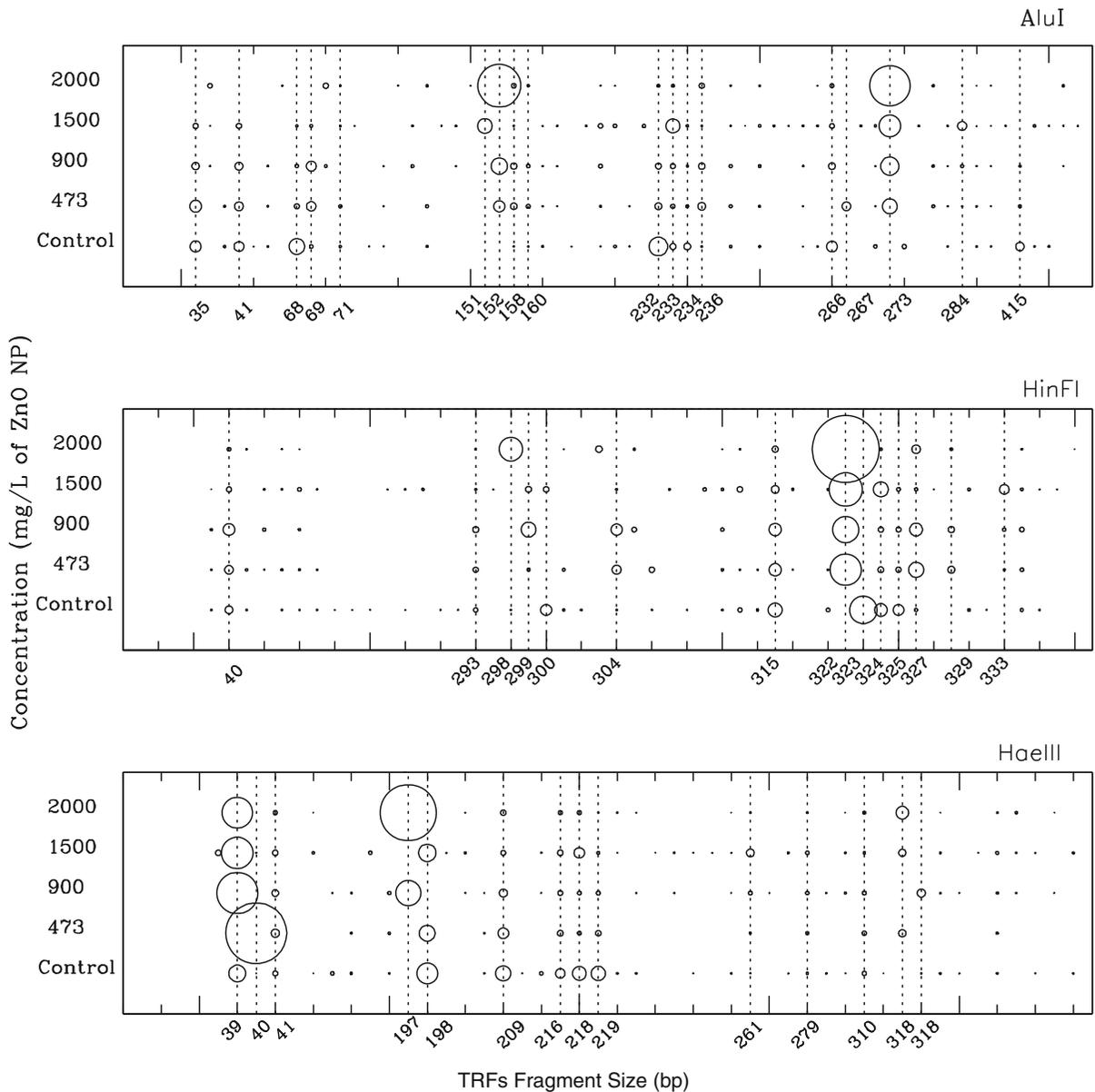


Fig. 5 T-RFLP bacterial population profile of activated sludge exposed to ZnO NPs with the three restrictive enzymes: HaeIII, HinfI, and AluI. The TRFs fluorescence (*x axis*) were

normalized and expressed by the size of the circles—representing the relative abundance of the respective bacterial population. The *y axis* represents the ZnO NPs concentration

In samples exposed to 473 mg L⁻¹ of ZnO NPs, the disruption of the flocs was detected (Fig. 6b), which was compared with the typical floc structure of the control (Fig. 6a). This disruption of the flocs can have an impact on the settling rate of the biomass and hence the quality of the treated water. For concentration of 900 mg L⁻¹ of ZnO NPs, an electrodense particulate material bound to the membrane cells (Fig. 6c)

without internalization inside the cells of electrodense material was detected, similar to that detected when CeO₂ NPs was supplied in activated sludge system (Limbach et al. 2008). At concentrations of 1500 mg L⁻¹ of ZnO NPs an electrodense material inside the cells was detected (Fig. 6d), while in samples exposed to 2000 mg L⁻¹ of ZnO NPs, invaded cells of electrodense material and a

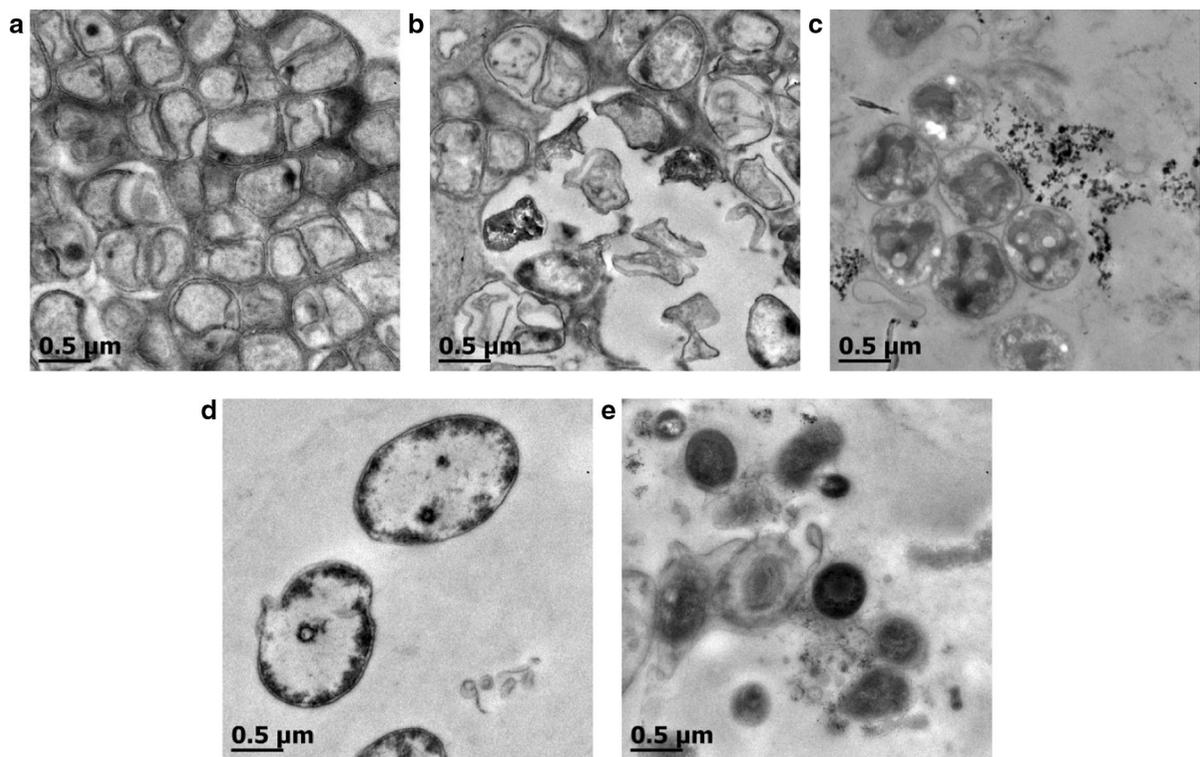


Fig. 6 TEM images at 50,000 \times . Samples of activated sludge exposed to different concentrations of ZnO NPs: **a** control, **b** 473 mg L⁻¹, **c** 900 mg L⁻¹, **d** 1500 mg L⁻¹, and **e** 2000 mg L⁻¹

remarkable fraction of cellular detritus for lysis of some microorganisms present were detected (Fig. 6e). To determine whether the electron-dense materials inside the cells correspond to Zn compounds, HAADF-STEM was performed and images were analyzed.

The mapping of Zn inside the cells is shown in Fig. 7. The abundance and distribution of Zn-K and Zn-L can demonstrate that the electron-dense material observed in TEM images could correspond to Zn compounds (Zhou et al. 2013). However, it is important to consider that a fraction of ZnO NPs can be soluble and can enter inside the cell by diffusion (Liu et al. 2011). Studies related to biological wastewater treatment in the presence of NPs have not recorded any reports on the internalization of NPs inside the microorganisms involved. However, when studied using isolated microorganisms, two possible mechanisms have been associated with NPs entering into the bacteria of environmental samples. The first mechanism is the disruption of membrane cells for

electrostatic and physical interactions with NPs (Sinha et al. 2011). The second mechanism describes the entry of NPs inside the cell due to the coating of NPs by proteins and other biomolecules that correspond to extracellular polymeric substances (EPS) and the type of coating determines how the NPs interact with a cell—active (receptor-mediated) or passive transport across the cell membrane (Shang et al. 2014). The last mechanism is the presence of electron-dense particulated material inside the cells without apparent membrane damage, which has been demonstrated in this experiment through the TEM images (Fig. 6). The decay of aerobic bacteria present during wastewater treatment could be attributed to the excess of Zn⁺² in the cells, which can atrophy ribosomes and then affect metabolic activities (Jiang et al. 2009). The presence of ion Zn⁺² could be attributed to the chemical mechanism reported by Lv et al. (2012), where ZnO NPs are hydroxylated forming Zn(OH)₂ which present high solubility. The mechanisms about how ZnO NPs damage to microorganisms involved in aerobic

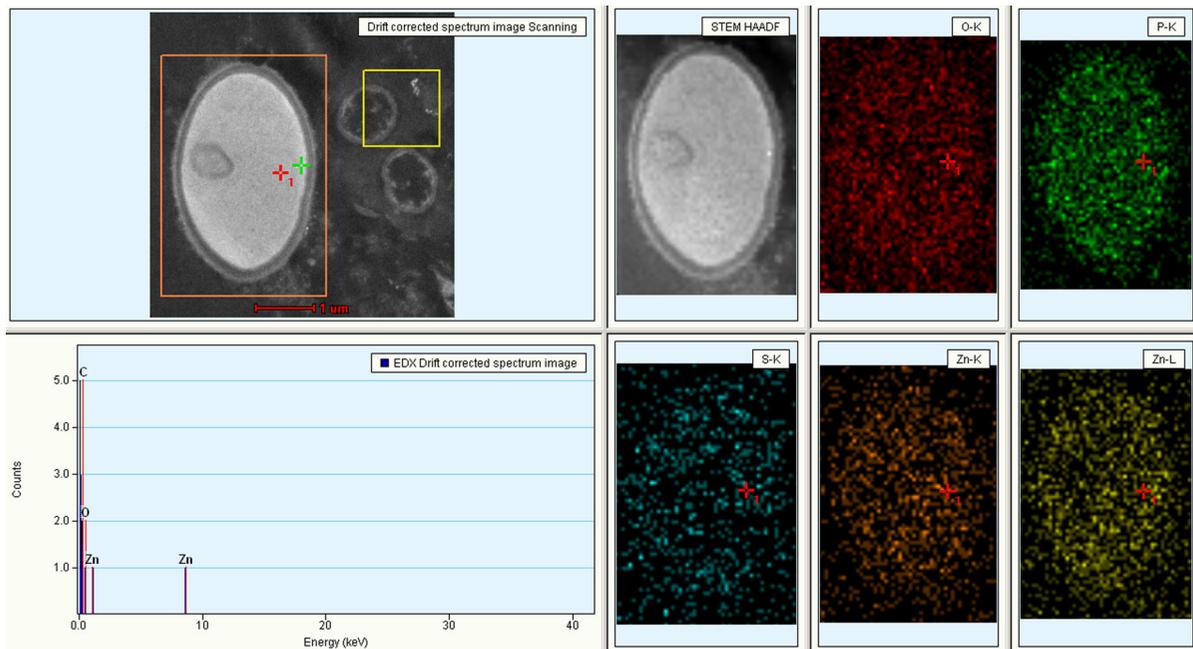


Fig. 7 Z-contrast image of a sample of activated sludge exposed to 2000 mg L⁻¹ of ZnO NPs using HAADF detector in STEM mode

reactors is still unknown due to this phenomenon is related to the chemical and biological complexity of the activated sludge reactors.

Conclusions

This study demonstrates that ZnO NPs in activated sludge inhibited the OUR of aerobic microorganisms at different concentrations and exposure times. The inhibition of oxygen uptake was related to the bacterial diversity, which is reduced as increase the concentration of ZnO NPs. The presence of Zn compounds inside the cell indicates that the main toxic effect of ZnO NPs on aerobic microorganisms occurs through the alteration of metabolic activities. The inhibition in the oxygen uptake due to presence of ZnO NPs can affect the aerobic biological processes and hence the removal of pollutants present in wastewater.

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