Size-dependent and shape-dependent algal toxicity of Aluminum oxide nanoparticles

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Abstract: In this work, we investigated the toxicity of Al$_2$O$_3$ particles with different size and shape to algae (*Chlorella pyenoidosa*). It was indicated that the toxicity of Al$_2$O$_3$ nanoparticles (NPs) was the strongest and the toxicity caused by bulk particles (BPs) was weaker than that of Al$_2$O$_3$ NPs. Al$_2$O$_3$ Fiber exhibited weakest toxicity to algae. Electrostatic interaction-induced physical contact could be a major mechanism to explain the difference of the observed toxicity.

1. Introduction

Al$_2$O$_3$ nanoparticles (NPs) are widely used in commercial applications such as explosive combinations, ceramics, catalyst and composite materials [1]. With the rapid increases in production and application, Al$_2$O$_3$ NPs will be released into aquatic environment. Upon exposure, they will exhibit adverse impacts on organisms. Sadiq et al. (2011) reported that Al$_2$O$_3$ NPs can inhibit the growth of *Scenedesmus sp.* and *Chlorella sp.* The EC$_{50}$ of *Scenedesmus sp.* is higher than that of *Chlorella sp.* and the entrapment ability of algal cells to Al$_2$O$_3$ played an important role [2]. Jacqueline et al. (2009) have used 0.7 μm Al$_2$O$_3$ and 1.6 μm SiO$_2$ to assess the cellular uptake and cellular toxicity of lunar dust particle analogs. According to the results, two type of cells showed minimal cytotoxicity exposure to Al$_2$O$_3$ [3]. Wang et al. (2009) characterized the toxicity and behavior of nanoparticles and bulk metal oxide ZnO, Al$_2$O$_3$ and TiO$_2$ to *C.elegans* in an aqueous medium. The results indicated that Al$_2$O$_3$ NPs induced the weakest toxicity among the three type of metal NPs and the toxicity caused by Al$_2$O$_3$ NPs is stronger than that of bulk particles (BPs) [4]. In the natural environment, the morphologies of Al$_2$O$_3$ particles are various and may occur a series of interaction. Ren et al. (2014) reported that Al$_2$O$_3$ could heteroaggregate with graphene oxide, altering the environment fate and bioavailability of graphene oxide [5]. Many articles had reported that the shape and size factors had a great effect on the toxicity of nanoparticles. Leanne et al. (2016) revealed that compared to CuO nanospheres, CuO nanosheet had higher surface reactivity and bacterial toxicity [6]. Pan et al. (2009) reported that 1-2 nm Au NPs reduced the most toxicity among all the 0.8-15nm Au NPs [7]. So, it is important to investigate the toxicity of Al$_2$O$_3$ with different morphologies on freshwater algae. Therefore, in the study, Al$_2$O$_3$ particles with different size and shape were chosen to research the toxicity on *Chlorella pyenoidosa*.

2. Materials and Method

2.1 Exposure Experiment. The three types of Al$_2$O$_3$ particles (Al$_2$O$_3$ NPs, Al$_2$O$_3$ BPs and Al$_2$O$_3$ Fiber) were purchased from Nanjing XFNANO (China). The *Chlorella pyenoidosa* was purchased from the institute of Hydrobiology, Chinese Academy of Science, China. In our study, the modified SE medium (one-tenth SE medium) was used for algal cells culture medium, in which the nutrient was sufficient for algal growth. The pH of culture medium was adjusted to 7.0 by adding negligible amounts of NaOH or HCl. The algal cells were grown under the light intensity of 12000 lux (14: 10 light: dark cycle) in a light growth chamber (GXZ, China) at 24°C. For algal growth inhibition assay, algal cells (1×10$^6$ cells/mL) were cultured two days to achieve the exponential growth phase. Al$_2$O$_3$ were pre-suspended in algal medium and then added to exponentially growing algal cells to reach the final concentrations. The algal suspensions were shaken twice every day and the algal cells were counted under a light microscope (JNOEC, China) after two days.
2.2 Statistical Analysis. The inhibition rate of Al\textsubscript{2}O\textsubscript{3} particles was calculated by the equation as follows:

\[
\text{Inhibition Rate (\%) = (1 - A/A_0) \times 100\%}
\]

\(A\): the algae cells concentration of treatment group
\(A_0\): the algae cells concentration of control group

The median effective concentration (EC\textsubscript{50}) was calculated by nonlinear regression using the cumulative distribution function of SPSS Statistics. All data included three replicates and standard error was statistically analyzed (p<0.05).

3. Results and Discussion

3.1 Characterization of different types of Al\textsubscript{2}O\textsubscript{3}. Characterization of Al\textsubscript{2}O\textsubscript{3} particles was analyzed by transmission electron microscopy (TEM, H-7650, Hitachi, Japan). The TEM images showed that the size of Al\textsubscript{2}O\textsubscript{3} NPs was 8-12 nm (Fig. 1A). The size of Al\textsubscript{2}O\textsubscript{3} BPs was 100-300 nm (Fig. 1B). The length of Al\textsubscript{2}O\textsubscript{3} Fiber was 100-200 nm and the width was 8-10 nm (Fig. 1C). Different with Al\textsubscript{2}O\textsubscript{3} BPs and Al\textsubscript{2}O\textsubscript{3} Fiber, Al\textsubscript{2}O\textsubscript{3} NPs formed the biggest homoaggregate. It was possibly attributed to the huge surface area of Al\textsubscript{2}O\textsubscript{3} NPs.

![Fig. 1 TEM images of Al\textsubscript{2}O\textsubscript{3} particles. (A) Al\textsubscript{2}O\textsubscript{3} NPs, the ruler line is 50 nm; (B) Al\textsubscript{2}O\textsubscript{3} BPs, the ruler line is 200 nm; (C) Al\textsubscript{2}O\textsubscript{3} Fiber, the ruler line is 200 nm.](image-url)

At pH 7.0, the zeta potentials of Al\textsubscript{2}O\textsubscript{3} and algae were measured by Zetasizer (Nano ZS90, Malvern, UK) and showed in Table 1. The zeta potentials of Al\textsubscript{2}O\textsubscript{3} NPs, Al\textsubscript{2}O\textsubscript{3} BPs and Al\textsubscript{2}O\textsubscript{3} Fiber were 35.04 mV, 28.87 mV and 19.16 mV, respectively. However, algae cells were highly negatively charged.

<table>
<thead>
<tr>
<th>Type</th>
<th>Al\textsubscript{2}O\textsubscript{3} NPs</th>
<th>Al\textsubscript{2}O\textsubscript{3} BPs</th>
<th>Al\textsubscript{2}O\textsubscript{3} Fiber</th>
<th>algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeta potential (mV)</td>
<td>35.04±3.56</td>
<td>28.87±0.87</td>
<td>19.16±0.48</td>
<td>-33.43±0.65</td>
</tr>
</tbody>
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3.2 The toxicity of Al\textsubscript{2}O\textsubscript{3} particles. Fig. 2 showed the toxicity of Al\textsubscript{2}O\textsubscript{3} particles on 48 h. Al\textsubscript{2}O\textsubscript{3} NPs had the strongest toxicity among the three type of Al\textsubscript{2}O\textsubscript{3} particles, while the toxicity of Al\textsubscript{2}O\textsubscript{3} Fiber was weakest. The EC\textsubscript{50} of Al\textsubscript{2}O\textsubscript{3} NPs, Al\textsubscript{2}O\textsubscript{3} BPs and Al\textsubscript{2}O\textsubscript{3} Fiber were 64.57 mg/L, 95.50 mg/L, 147.91 mg/L, respectively. The order of toxicity was completely same with that of zeta potential. The three types of Al\textsubscript{2}O\textsubscript{3} particles were both positive charged at pH 7.0, while algae was highly negatively charged. Zhao et al. (2015) reported that GO with negatively charged and goethite with positive charged could happen heteroaggregation\[8\]. Heteroaggregation may occur between Al\textsubscript{2}O\textsubscript{3} and algae, too. So we assume heteroaggregation caused by electrostatic interaction may be a major reason to explain the toxicity of Al\textsubscript{2}O\textsubscript{3} particles.
Fig. 2 The toxicity of Al\textsubscript{2}O\textsubscript{3} particles to algal cells on 48 h. The red, blue and purple column indicated respectively the toxicity of Al\textsubscript{2}O\textsubscript{3} NPs, Al\textsubscript{2}O\textsubscript{3} BPs and Al\textsubscript{2}O\textsubscript{3} Fiber. Error bars indicated one standard deviation of at least three measurements.

4. Conclusion

Al\textsubscript{2}O\textsubscript{3} particles were toxic to algae. Among the three types of Al\textsubscript{2}O\textsubscript{3} particles, Al\textsubscript{2}O\textsubscript{3} NPs showed the strongest toxicity, while, the toxicity of Al\textsubscript{2}O\textsubscript{3} Fiber was weakest. Electrostatic interaction-induced physical contact could be a major mechanism for the toxicity.

Acknowledgments

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References