The study of the biotoxicity effect of alumina nanoparticle on soil microbes

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Abstract. Due to the unique properties, nanoparticles (NPs) have been widely used both in industrial products and individual life. However, with the huge benefits, NPs also possess potential toxicity to human health and ecosystem. Aluminum oxide NPs is so significant in industry that it occupies the second important position in US nanomaterials market. Aluminum oxide NPs belong to metal oxide nanoparticles which may have strong antimicrobial properties. As there is limited prior research on the toxicity of alumina NPs, the main goal of present research is to study the effects of alumina NPs on microorganisms in soils by combination methods of microcalorimetry and urease. Power-time curves and thermo-kinetic parameters (such as total heat evolution QT, growth rate constant k and maximum power output Pmax) were applied to evaluate the microbial community activities of the soil samples. A range of concentration of Aluminum oxide NPs gradient from 0 mg/L to 1000 mg/L was applied. The results showed that the concentration under 500 mg/L has negligible inhibitory effects on soil microorganisms while concentrations at 500mg/L and 1000 mg/L only have mild inhibition on microbes in soil. Urease experiments verified this result, therefore, it suggests that combination of microcalorimetry and urease may provide practical method for microorganism activity evaluating.

1. Introduction

Nano-materials refer to particles which three dimensions are less than 100 nm. When the dimension of a material reaches to nano-meter level, it gains unique properties due to larger reaction surface area, quantum effect and so on [1]. Nano-materials have been increasingly used in various applications. According to prior research, the total values of nano-materials relevant production can reach a trillion dollars by 2015. Meanwhile, the fast development of nanotechnology might also lead to the release of a large amount of nanomaterials pollutions into natural environment that have potential toxicity to ecological conditions [2]. As the imperative interest for toxicity research of nanomaterials, relevant reports are booming these years.

Nano-Al2O3 is one of engineered nanomaterials commonly used in various applications such as abrasive, microelectronics and catalyst support. Nano-Al (the elemental form of Nano-Al2O3) also served as an important military material for superior fuel of space vehicles [3]. These applications can largely increase the chances that Nano-Al2O3 release into the terrestrial environment, which raises public concerns about potential threaten to soil system. In the soil ecosystem, soil microbes play an imperative role in translocation of soil nutrients, environmental pollution purification, cycles of biogeochemistry, biological health maintenance [4]. The toxicity of nano-Al2O3 has been studied on
various model bacteria and in different media before [3, 5]. The effect of alumina nanoparticles on model bacteria Escherichia coli has been studied and it showed slight inhibition by nano-Al$_2$O$_3$[6]. However, soil ecosystem is a very complex system containing huge amount of microbe species. Merely experiments to model organism cannot represent the influence to the entire soil system. There are very limited prior published researches on interaction between soil microorganisms and Nano-Al$_2$O$_3$. It is very necessary to gain further information about interactions between soil microbes and Nano-Al$_2$O$_3$.

Thermal on-line Activity Monitor (TAM III multi-channel microcalorimetric system) could be applied to explain the effects of contaminations on metabolic heat release and soil microbial growth rate [7]. Our main goal in this study was to quantify the toxicity of Nano-Al$_2$O$_3$ on the microbial metabolism by measuring the heat production from the soil microbes using microcalorimetric system. Mass specific heat rate, total heat production, microbial growth rate constant was applied to evaluate soil microbial activity as real-time parameters. The study aims to evaluate the biota tolerance to Nano-Al$_2$O$_3$, the evolution of microbial population and activity in polluted soil.

2. Materials and Methods

2.1. Characterization of alumina nanoparticles

Dry alumina nanoparticles were bought from Beijing Boyu Technology Company. The supplier’s data can be summarized as follows: alpha phase alumina nanopowder, particle size is around 13nm, melting point 2040°C (from the literature).

2.2. Soil samples collection

Soil samples for this study were collected from farmland in Huangtuo village, Hebei province. The soil type is moisture soil with loamy texture. After collection work, all soil samples were transferred to the lab. Then they were air-dried and processed by a sieve (mesh size 2 ×2 mm$^2$) to remove root fragments and large particles, then they were placed into polyethylene bags and kept at 4°C in refrigerator [8].

2.3. Preparation of nano-alumina contamination samples

Appropriate amount of nano-alumina was weighed and dissolve into 50mL volumetric flasks. The concentration range was from 0 to 1000 mg/L, namely 0, 10, 50, 100, 500, 1000 mg/L. Then, 1g soil was put on containers and added 1mg solution from each flask to make the concentration gradients from 0 mg/kg to 1000 mg/kg. Then the sample was air-dried for 1 day before calorimetry test.

2.4. Determination of soil physicochemical properties

Several soil physicochemical properties, such as soil pH, total nitrogen (N), organic matter, total phosphorus (P), were determined by modified methods. Soil total phosphorus (P) and total nitrogen (N) were determined by HF-HClO$_4$ digestion and by Kjeldahl digestion respectively. Soil organic matter was measured by titrating the soil samples with a redox indicator [9]

2.5. Microcalorimetric measurements

The microcalorimetric system (TAM III multi-channel thermal activity microcalorimeter) was used to study microbial community activity. The 4.0 mL stainless steel ampoules were washed with deionized and sterilized in a sterilizing oven at 120°C. Soil samples with different concentrations of Nano-Al$_2$O$_3$ were settled in an incubator at 37°C for 24 hours. Before the microcalorimetric system began, 1g soil were weighed from each sample and put into the stainless steel ampoules. Then, aqueous solutions of glucose (5.0 mg) and 0.2 mL of ammonium sulphate (5.0 mg) was added into each ampoule [10]. Ampoules were sealed with stainless caps and load into microcalorimeter. The thermal power-time curves were recorded by the TAM III machine and total heat production was gained from the integration of each power-time curve of different experiment. The temperature in workroom was controlled at 28°C throughout the experiment.
Metabolic parameters such as $J_{Q_S}$ (The mass specific heat rate), $Q_T$ (total heat production), $\Delta H_{\text{met}}$ (metabolic enthalpy) and $k$ (microbial growth rate constant) were gained from power-time curves. From integration of each curve, $Q_T$ (the total heat production) was calculated for each sample. Meanwhile, according to the modified measurement method of Monod, constant $k$ (microbial growth rate) could be calculated from the semilogarithmic alteration of heat flow rate [11].

$$\ln P_t = \ln P_0 + kt$$

where $P_t$ is the power yield at time $t$, $P_0$ is the power output at the initial point of the microbial exponential growth phase, $t$ is the time and $k$ is the growth rate constant. The parameters $t_{\text{max}}$ (time of the peak heat) and $P_{\text{max}}$ (the peak of heat evolution) could be directly obtained from the power-time curves.

2.6. Determination of urease activity in soil

According to prior research, soil urease activity was measured by modified methods [12]. Diploid soil samples (2.5g) were placed into 25ml volumetric flasks and added with 0.5 ml toluene for 15min. After that, 10 mL of 10% urea and 20 mL of citrate buffer (pH 6.7) were added into each volumetric flask. Then, soil samples were transferred into the incubator at 37°C for 1d. Thereafter, 37°C distilled water was added into samples and mixed thoroughly.

The mixtures were immediately filtered, 3ml filtrate was transferred into a 50 ml volumetric flask, to which was added 10 mL distilled water, 4 mL sodium phenate (1.35 M) and 3 mL sodium hypochlorite (active chlorine 0.9%). The soil samples were filtered immediately and 3 ml filtrate was moved into a 50 ml volumetric flask. Then, 3 mL sodium hypochlorite (chlorine 0.9%), 4 mL 1.35 M sodium phenate and 10 mL distilled water were added into the flask and mixed thoroughly. The flask was settled for 20 min and then diluted to the labelled volume.

2.7. Statistical analysis

All the data were stated as “mean ± standard error mean”. Graphs were prepared using Origin 9.0 (OriginLab, MA, USA). Arithmetic mean of two independent measurements was calculated for physical and chemical properties.

3. Results and Discussion

3.1 Physicochemical properties of soil samples

The physicochemical properties of all soil samples are listed in the table 1 below

| C (μg/g) | OM (g/kg)$^a$ | Total N (g/kg)$^a$ | Total P (mg/kg)$^a$ | Total K (mg/kg)$^a$ |
|---------|------------|-----------------|-----------------|-----------------|
| 0-1000  | 16.0 ± 0.8 | 0.8 ± 0.02      | 13.2 ± 0.06     | 96.7 ± 3.5      |

$a$. Mean values of soil organic matter (OM); total N, total nitrogen; total P, total phosphorus; total K, total potassium.

3.2 Effects of Nano-Al$_2$O$_3$ on microbial metabolic heat rate

The values of $P_{\text{max}}$, $t_{\text{max}}$, $k$, and $Q_{\text{total}}$, obtained from the power–time curves, are listed in Table 2. The microbial metabolism in the soils containing different concentration of Nano-Al$_2$O$_3$ is illustrated in Figure 1.
Figure 1. The power-time curves of the soil samples with different concentrations of Nano-Al₂O₃ at 28°C

As illustrated, Figure 1 shows a representative procedure of microbial metabolic activity, of which growth curves can be divided into four phases, namely lag, exponential, stationary and death phase. From the growth curves, it is clearly that microbial activity decreases generally with the increased concentration of Nano-Al₂O₃. In detail, for Pₚₘₓ, control sample shows the highest value and Pₚₘₓ of the other five samples is caused varying degrees of inhibition by Nano-Al₂O₃. There is little difference among samples concentration of 10, 50, 100 μg/mL which reveal that concentration below 100 μg/mL may have negligible toxicity on soil microbes. When Nano-Al₂O₃ levels reaches 500 μg/mL, Pₚₘₓ of the two concentration samples are lower than the others, which showed mild inhibitory effects on microorganisms in soil. Total heat released indicates little difference below 500 μg/mL while sample of 1000 μg/mL is lower than the other groups. Growth rate k also shows decreasing trend which verify the argument above.

Table 2. Microcalorimetric parameters

| C (μg/g) | Qᵃ (J/mL) | Pₘᵇ (μW) | Tₘ (hour) | kᶜ (hour) x 10⁻³ | R² ᵈ |
|---------|-----------|----------|-----------|-----------------|-----|
| 0       | 38.60±0.19| 675.12±5.32| 12.35±0.32| 0.59±0.019      | 0.9991 |
| 10      | 38.29±0.15| 630.07±4.63| 13.25±0.21| 0.58±0.009      | 0.9998 |
| 50      | 37.92±0.10| 609.55±7.22| 12.85±0.19| 0.58±0.011      | 0.9997 |
| 100     | 36.95±0.21| 633.33±5.92| 14.24±0.25| 0.57±0.018      | 0.9998 |
| 500     | 35.32±0.28| 564.87±8.37| 11.11±0.15| 0.51±0.011      | 0.9996 |
| 1000    | 31.18±0.18| 532.73±4.15| 11.98±0.17| 0.50±0.008      | 0.9994 |

ᵃ Total heat output  
ᵇ Maximum heat flow rate  
ᶜ Microbial growth rate constant  
ᵈ Correlation coefficient
3.3 Effects of Nano-Al₂O₃ on soil urease activity

The urease activity of soil samples with various nanoparticle concentration treat are shown in Figure 2. In general, Nano-Al₂O₃ inhibited urease activity in soils at certain extent. Urease activity values range from 3.4 to 4.2. With the lowest amount of nanoparticles in soil, urease activity is highest among all the soil samples. As the concentration increases, urease activity drops sharply before 200μg/mL, then decreases mildly from 200μg/mL to 1000μg/mL. So, this indicates that nano-Al₂O₃ may have mild toxicity effect on soil urease activity.

![Graph showing urease activities of soil samples with different concentrations of nano-Al₂O₃](image)

Figure2. Urease activities of soil samples with different concentrations of nano-Al₂O₃

4. Conclusion

In this research, the toxicity of alumina nanoparticle on soils is studied using combination method of microcalorimetry and urease method. The results of microcalorimetric and urease method presents consistence in the study. It is concluded that nano-Al₂O₃ may only have mild effect on microorganisms in soil. Nano-Al₂O₃ exerts little influence on microbes below 100μg/mL, when the concentration is above 100μg/mL, toxicity increases, but even the concentration reaches 1000μg/mL, there is still not serious toxicity. Meanwhile it also indicates that the combination method of microcalorimetry and urease may be an effective tool for microorganism toxicity monitor.

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