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Effects of dispersible MoS_2 nanosheets and Nano-silver coexistence on the metabolome of yeast

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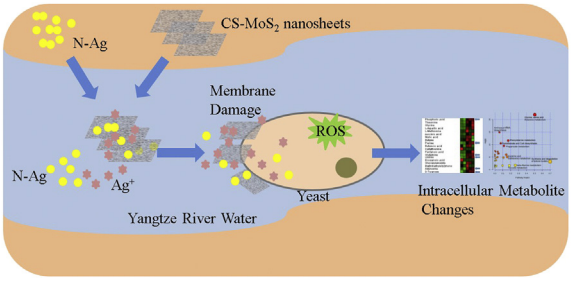
HIGHLIGHTS

- Coexistence of CS-MoS_2 nanosheets and N-Ag perturbed the metabolome of yeast.
- CS-MoS_2 nanosheets absorbed the released Ag^+ and attenuated oxidative stress.
- CS-MoS_2 nanosheets caused more membrane damages in yeast cells.

GRAPHICAL ABSTRACT



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ABSTRACT

As a new rising star in the post-graphene two-dimensional materials (2DMs), molybdenum disulfide (MoS_2) attracts increasing attentions and is widely applied. However, the chemical and toxicological interaction between MoS_2 and other co-contaminants is still poorly understood. Nano-silver (N-Ag) is the most commonly used nanomaterial in commercial products and distributed widely in the environment. Herein, we investigated the effects of chitosan functionalized MoS_2 (CS-MoS_2) nanosheets, a water-dispersible form of MoS_2, on the microbial toxicity of N-Ag. We found that the incorporation of CS-MoS_2 nanosheets attenuated the oxidative stress induced by N-Ag on yeast cells, while caused more membrane stress. In addition, the inhibition of N-Ag on the metabolic activities of yeast cells could be attenuated by CS-MoS_2 nanosheets as well. The coexistence of N-Ag and CS-MoS_2 nanosheets mainly perturbed the amino acid-related metabolic pathways in yeast cells, and phosphoric acid was a potential nanotoxicity biomarker. We further found that CS-MoS_2 nanosheets dramatically absorbed the Ag ion released from N-Ag, which might be responsible for its attenuation effect on the microbial toxicity of N-Ag. Our findings provide more new insights for the ecotoxicity evaluation of MoS_2 and other 2DMs.

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1. Introduction

The rapid pace of progress and fruitful achievements in graphene encourage the research developments in other two-dimensional materials (2DMs)(Mak et al., 2010, 2012; Zeng et al., 2012). In the post-graphene 2DMs, molybdenum disulfide (MoS_2) has become a rising star due to its intriguing physicochemical properties and promising potentials in a wide range of applications, such as dry lubrication, hydrogen evolution, photovoltaic, and sensing(Lee et al., 2010; Puthussery et al., 2011; Laursen et al., 2012; Li et al., 2012; Kurapati et al., 2016).

The increasing applications of MoS_2 will inevitably give rise to the concern of assessing the ecotoxicity of MoS_2. In fact, MoS_2 and other engineered nanomaterials (ENMs) will undergo environmental re-processing after their release into the environment. MoS_2 and other ENMs probably exist in a complex mixture containing various ENMs or other contaminants(Tong et al., 2015). The interaction between ENMs and other contaminants might cause new unprecedented toxicological outcomes, which may be defined as "indirect" nanotoxicity(Hu et al., 2014). Although some efforts have been made to uncover the toxicity of MoS_2 and other 2DMs (Qureshi et al., 2014; Shah et al., 2015; Wu et al., 2016), it is still very poorly explored and understood (Kurapati et al., 2016), let alone to decipher the chemical and toxicological interactions between MoS_2 and its co-contaminants. Moreover, due to its hydrophobic and inert nature, the ecotoxicity of MoS_2 is often hard to be unveiled by investigating the native MoS_2 because it is insoluble in water(Wang et al., 2015). Although it could be argued that this will represent the real world situation, it is important to recall that in the environment these materials will encounter turbulent waters in flowing rivers and a variety of chemicals, such as detergents and natural organic matters, which may act as dispersants(Klaine et al., 2008). Therefore, preparing MoS_2 material in its well-dispersible form is critical for further addressing the ecotoxicity of MoS_2.

Nano-silver (N-Ag) is the most commonly used nanomaterial in commercial products and distributed widely in the environment(Benn and Westerhoff, 2008; Zhang et al., 2016). Although N-Ag has been widely used for more than 100 years, it is still a great concern that N-Ag might impair the ecosystem due to its wide usage and strong antimicrobial property(Colman et al., 2014; Zhang et al., 2016). It has been demonstrated that the effects of N-Ag on environmental microbes depend on many factors, such as the dose, the time period applied, the property of N-Ag (size, shape and coating, etc.) and the co-contaminants(Wilke et al., 2016; Zhang et al., 2016).

With regard to this, it will be imperative and meaningful for us to detail the interactions between the MoS_2 material and the "oldest" and most widespread nanomaterial - N-Ag. Therefore, we herein synthesized a kind of chitosan functionalized MoS_2 (CS-MoS_2) nanosheets, which showed a satisfying water-dispersible performance. Then different approaches including metabolomics technology were utilized to decipher the CS-MoS_2 nanosheets - N-Ag interaction and their combined effects on a typical environmental microorganism - yeast.

2. Materials and methods

2.1. Preparation and characterization of CS-MoS_2 nanosheets and N-Ag

Chitosan functionalized MoS_2 (CS-MoS_2) nanosheets were prepared according to a previously reported method(Zhang et al., 2015). Briefly, 250 mg of MoS_2 (99.995%, Sigma-Aldrich, USA) and 100 mg of chitosan (CS) (Sigma-Aldrich, USA) were ground with an agate mortar and a pestle for 10 min. Then 0.5 mL of ionic liquid (≥97.0%, 1-butyl-3-methylimidazolium hexafluorophosphate, Shanghai Chengjie Chemical Company, China) was added and then the mixture was ground for another 50 min. The finely grounded mixture was transferred to a centrifuge tube and washed three times with acetone, *N,N*- Dimethylformamide (DMF) and 0.5% acetic acid, respectively, in order to remove excess chitosan and residual ionic liquid. This washing cycle was repeated triply. The precipitate was re-suspended by double-distilled water (ddH_2O) and then centrifuged (1000 × g, 20 min). The supernatant was collected and stored at 4 °C. A scanning electron microscopy (EVO® MA, ZEISS, Germany) was used to observe the morphological characteristics of the CS–MoS_2 nanosheets. A HR800 Raman Microscope (Horiba Jobin Yvon, France) which focalizes by a 40 × objective at an excitation wavelength of 514 nm was utilized to analyze the CS–MoS_2 nanosheets sample. To conduct the high-resolution transmission electron microscope (HRTEM) experiment, the samples were dispersed in acetone and then the acetone solution containing the CS-MoS_2 nanosheets samples were dropped onto a mesh with a carbon-coated copper. After being stored in a desiccator for three days, the mesh was observed under a JEM-1011 transmission electron microscope (JEOL, Japan) at 200 kV. A Fourier transform infrared (FT-IR) spectroscopy (Vetex70, Bruker Corp. Germany) was used to record the FT-IR spectrum. The UV–vis spectra were recorded with a spectrophotometer (UV-2550, Shimadzu, Japan) at room temperature. The as-prepared CS–MoS_2 nanosheets and native MoS_2 powders were added to Yangtze River water (YRW), Phosphate Buffer Solution (PBS) and ddH_2O, respectively, to determine their dispersible performances.

After the ultrasonic dispersion was carried out, 1 μL N-Ag solution (0.02 mg mL^{-1}, Sigma-Aldrich, USA) was added to the copper mesh. After drying at room temperature, the morphology of the N-Ag was observed by a transmission electron microscopy (JEM-2000EX, JEOL, Japan). Image J software (Image J 1.43, NIH) was used to analyze the particle size. The UV–vis spectra of N-Ag were recorded with a spectrophotometer (UV-2550, Shimadzu, Japan) at room temperature.

2.2. Strain cultivation

The Yangtze River water (YRW) was filtrated and then sterilized before being used as the test medium for the following toxicity experiments. According to a previously reported method (Wilke et al., 2016), the chemical composition of YRW was measured and presented in Table A.1. Yeast cells (Saccharomyces cerevisiae, No. 2.3871, purchased from the China General Microbiological Culture Collection Center) were maintained on Yeast Extract Peptone Dextrose Medium (YPD) containing 20 g L^{-1} peptone (OXIOD, UK), 10 g L^{-1} yeast extract (OXIOD, UK) and 22 g L^{-1} glucose, at 28 °C with a constant orbital shaking speed of 220 rpm for up to 48 h. Then, cells were harvested for the following toxicity tests.

2.3. Determination of oxidative stress

2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) (Sigma-Aldrich, USA) was mixed with 50 mL of yeast suspension with a density of 10^7 cells mL^{-1} as a 10 mM stock in DMSO. Then the mixture was incubated at 28 °C under constant orbital shaking at 220 rpm for 15 min, and after which the cells were washed twice with the YRW and dispersed in the YRW containing N-Ag (0, 5, 10, 20, 30 and 40 μg L^{-1}) and CS-MoS_2 nanosheets (0, 1, 10 mg L^{-1}). Subsequently, the YRW solutions containing the cells, N-Ag and CS-MoS_2 nanosheets were incubated at 28 °C with a shaking speed of 220 rpm for another 4 h, after which the supernatants were transferred into the wells of a 96-well plate and evaluated on a