***-Supporting Information-***

# Deactivation of *Caenorhabditis elegans* nematodes in drinking water by PMS/UV-C: Efficiency and mechanisms

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**References**

# Texts

**Text S1.** A concise listing of reagents

Peroxymonosulfate (KHSO5，PMS) was prepared from Oxone® (2KHSO5∙KHSO4∙K2SO4), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), coumarin and levamisole were purchased from Aladdin (Shanghai, China). 2,2,6,6-tetramethyl-4-piperidinol (TEMP) was purchased from Sigma-Aldrich (Shanghai, China). Tert-butyl alcohol (TBA) and methanol were obtained from Macklin (Shanghai, China). Benzoic acid (H2DCFDA, C24H16Cl2O7) of 99.14% purity was purchased from MedChemExpress (USA). Acridine orange (Basic orange 14) was obtained from CNW Technologies GmbH (Germany). All the chemicals were of analytical grade or better and used without further purification. Ultrapure Milli-Q deionized water ( > 18 MΩ·cm, Millipore) was used throughout the experiments.

**Text S2.** Details of electron paramagnetic resonance (EPR) analysis

EPR spectra were recorded on a Bruker EM Xplus|| ||10/12 EPR spectrometer (Bruker, Germany) at ambient temperature. DMPO was used as the spin-trapping agent of and **·**OH, and TEMP as the spin-trapping agent of 1O2 (Li et al. 2018, Nosaka &Nosaka 2017, Rao et al. 2019, Yang et al. 2018). 250 mg DMPO was firstly dissolved in 10 mL of ultrapure water, magnetically stirred for 1 h, divided into 50 μL each and packed into a 1.5 mL centrifuge tube, and finally saved in −20° C for use.

(1) To detect the effect of UV-C irradiation on free radical production, a certain amount of 1 mM PMS solution (pH ≈ 6.0) after 10 minutes of irradiation was added to a centrifuge tube containing DMPO. Correspondingly, control experiment was carried out that 1 mM PMS solution (pH ≈ 6.0) without irradiation was added to a centrifuge tube containing DMPO.

(2) To detect the effect of temperature (25, 30, 35°C) on free radical production, a certain amount of 1 mM PMS solution (pH ≈ 6.0) after 20 minute water bath heating was transferred to a centrifuge tube containing DMPO.

The mixed sample in the centrifuge tube was quickly transferred into a 100 μL capillary tube, which was then inserted into the cavity of the EPR spectrometer.

TEMP was followed the same experimental operation as DMPO.

**Text S3.** Detailed SOD activity assay kit protocols for *C. elegans*

The deactivated nematodes were first rinsed with ice-cold PBS (pH ≈ 7.0), and then collected in 1.5 mL centrifuge tubes, finally discard the supernatant PBS carefully with pipette. They were heated in an ice-water bath using an electric homogenizer (TGrinder electric tissue mill set, Tiangen Biochemical, China).

Wash the pestle in ice-cold PBS, and clean it with the absorbent paper. Push the pestle tightly against the pellets in the 1.5 ml centrifuge tube. Keep the centrifuge tube in an ice-water bath. Start the motor-driven tissue grinder, grind the nematodes for 10 seconds with a 2-sec cooling down followed by another 10-sec grinding. Then, use 200 μL ice-cold PBS to wash the residual liquids on the pestle back to the centrifuge tube before taking it out of the tube. Centrifuge the tubes at 5,000 x *g* for 5 min at 4 °C. Pipette out 200 μL supernatants from each sample, which was the crude enzyme solution (Zhang et al. 2017).

Each crude enzyme solution was analyzed by Enhanced BCA Protein Assay Kit (P0010S, Beyotime, China) and Total Superoxide Dismutase Assay Kit with WST-8 (S0101, Beyotime, China) to test the enzyme activity. SOD activity of each sample was expressed as SOD activity per unit of protein. In order to eliminate the difference caused by different biomass between samples, the samples of each treatment group were repeated 3 times. A multi-purpose microplate reader (Synergy™4, BioTek, USA) was used.

**Text S4.** Evaluation of ROS and cell apoptosis

ROS production was determined as described previously (Chen et al. 2019, Zhou et al. 2016). The washed nematodes were pre-incubated in 10 μM H2DCFDA solution at 20° C for 2 h. It's noted 10 μM H2DCFDA solution was prepared by dissolving H2DCFDA in M9 buffer (3 g KH2PO4, 6 g Na2HPO4, 5 g NaCl, 1 mL 1M MgSO4, DI water to 1 L). Next, the examined nematodes were put on agar pads containing 60 μg/mL levamisole, which worked as an anesthetic for nematodes, and the levamisole-treatment was used to take pictures of the live nematodes. The fluorescent images were collected using a confocal laser scanning microscope (Nikon A1R, Japan). The ROS production was expressed as relative fluorescent units (RFU) by Image J software (30 nematodes for each treatment, 3 independent experiments).

Degree of cell apoptosis was analyzed by acridine orange vital staining as described (Chen et al. 2019, Zhou et al. 2016). Briefly, examined nematodes were pre-incubated for 2 h at 20 °C in M9 buffer containing OP50 and acridine orange (25 mg/mL). The OP50 in the buffer facilitated the nematodes taking up the dye. And then nematodes were mounted on agar pads with 60 mg/mL levamisole in M9, and fluorescent photos were taken.The ROS production was expressed as relative fluorescent units (RFU) by Image J software (30 nematodes for each treatment, 3 independent experiments).

# Figures



**Fig. S1** - **Illustration of the setup using PMS/UV-C for *C. elegans* deactivation.**



**Fig. S2** - **Variation of pH values during the deactivation in the PMS/UV-C system. (Experimental conditions: Number of nematodes: 30; [PMS] =1 mM; T = 25 ± 1°C; [pH]0 ≈ 6.0)**

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**Fig. S3** - **(a) Nematodes deactivation efficiency in the different conditions; (b) nematode deactivation efficiency in the presence of different scavengers under the dark (Number of nematodes: 30; [PMS] = 1 mM; T = 25 ± 1°C; [pH]0 ≈ 6.0)**



**Fig. S4** - **(a) EPR spectra obtained with DMPO after (1) 10 min UVC irradiation, (2) dark, and (b) EPR spectra obtained with TEMP after (1) 10 min UVC irradiation, (2) dark；(c) fluorescence spectra of PMS solution in the presence of 1 mM coumarin under different time** **of UV-C irradiation; (d) fluorescence intensity under UV-C irradiation and concentration variation during the nematodes deactivation in PMS/UV-C system. (Experimental conditions: nematodes numbers = 30; [PMS] = 1 mM; T = 25 ± 1°C; [pH]0 ≈ 6.0).**

To understand the high deactivation efficiency of PMS/UV-C, different scavengers were used to trap potential reactive species in the PMS/UV-C system, including methanol for and tert-butyl alcohol (TBA) for **·**OH (Duan et al. 2016, Wang et al. 2019). In the control experiment without PMS, no nematode loss was observed by adding 1mM of each scavenger, indicating no toxicity of these chemical scavengers to the nematodes within 90 min (**Fig. S3a**). It is known that methanol can effectively scavenge both and **·**OH, while TBA is more efficient to scavenge **·**OH than that of (k·OH = (1.6–7.7) × 108 M–1 s–1; k·SO4=(4–9.1) × 105 M–1 s–1) (Anipsitakis &Dionysiou 2004). As shown in **Fig. S3b**, with the addition of methanol, the deactivation of nematodes was almost completely quenched (similar to the deactivation efficiency of UV-C system), indicating the major reactive species in the PMS/UV-C system were or **·**OH. In contrast, the addition of TBA only partially decreased the deactivation efficiency (**Fig. S3b**), suggesting both and **·**OH were involved in the nematode deactivation process. Previous studies had proposed that PMS could be activated by homogenous/heterogeneous transition metals, metal-free heterogeneous catalysts, UV irradiation, conduction electron, heat, ultrasound and so on (Eq.1) (Rodriguez-Chueca et al. 2019, Xiao et al. 2019). PMS self-decomposition without explicit activation could generate singlet oxygen (1O2) (Zhou et al. 2015) (Eq. 2). All these reactive oxygen species (ROSs), including , **·**OH and 1O2 were potential active oxidants contributing nematode deactivation in PMS/UV-C system.

 + + **·**OH (1)

 + → 1O2 + + (2)

 + → + **·**OH + (3)

The key step was the radicals generation, therefore further experiments for the generation of , **·**OH and 1O2 by EPR was also carried out. Because signal intensity was subject to loading volume and instrument tune up time, signals were only used for identification purposes (Yang et al. 2018). **Fig. 4Sa** shows the obvious signals of both DMPO-·OH (1:2:2:1) and DMPO- (1:1:1:1:1:1) (Ouyang et al. 2017, Wang et al. 2019), indicating both **·**OH and radicals were generated immediately in the PMS/UVC system. The appearance of the 1:1:1 peaks (**Fig. S4b**) was clear evidence for 1O2 production (Li et al. 2018) in both PMS self-decomposition and PMS/UV-C system but did not demonstrate its effectiveness in nematodes deactivation, because deactivation didn’t occur in the control experiment (**Fig. S3b**). 1O2 has received attention for its potential to deactivate pathogens (Kohn &Nelson 2007, Moor et al. 2015), while Li et al. (2018) found its efficient quenching by water. In addition, former researches found nematodes are highly resistant to disinfectant, which may be attributed to the smooth and dense cuticle (Zhan et al. 2007, Zhou et al. 2010). So we inferred that 1O2 played a minor role in deactivating nematodes because of special epidermal structure of nematodes and rapid quenching of 1O2 by water. Thus, EPR and radical scavenging studies demonstrated that and ·OH were the key ROSs responsible for nematodes deactivation in PMS/UV-C. As well known, reacts with hydroxide or water to produce **·**OH (Eq. 3) (Gao et al. 2016, Wang et al. 2019). Therefore, to further confirm the mechanism of radical production during PMS/UV-C, **·**OH production was assayed using coumarin as the trapping agent. Coumarin is believed to quantitatively react with **·**OH to produce highly fluorescent 7-hydroxylcoumarin with emission at 460 nm (Guan et al. 2008, Nosaka &Nosaka 2017, Wang et al. 2019). **Fig. S4c** showed the fluorescence spectra of the coumarin solution (1mM) in the presence of PMS under UVC irradiation. It was clearly seen that the fluorescence intensity of 7-hydroxylcoumarin increased with irradiation time, suggesting the conversion of to **·**OH (Wang et al. 2019). This result matched well with the nematode deactivation efficiency, suggesting the nematodes were deactivated as a result of PMS activation to produce reactive species. As shown in **Fig. S4d**, the generation of fluorescent 7-hydroxycoumarin was linearly proportional to irradiation time, obeying a pseudo-zero-order reaction rate equation in kinetics based on previous literatures (Guan et al. 2008), the apparent rate constant (k) for **·**OH generation was calculated as k = 6840.49 min–1 (R2 = 0.963). The consumption of was performed and ~60 mg/L of PMS was decomposed under UV-C irradiation shown in **Fig. S4d**, further confirming the nematode deactivation was caused by the consumption of caused by UV-C irradiation.

These results above confirmed the successful activation of by UV-C to produce and **·**OH, which then caused nematode deactivation.



**Fig. S5** - **HSO5- decomposition at different temperatures (Experimental conditions: [PMS] = 1 mM; [pH]0 ≈ 6.0).**



**Fig. S6** - **(a) Nematode deactivation efficiency without PMS/UV-C in the dark at different pH values; (b) pH value variations during the PMS/UV-C process with different initial pH; (c) nematode deactivation efficiency in the PMS/UV-C system: Effect of 1–20 mg/L bicarbonate; (d) variations of pH values during the reactions with different initial concentrations of bicarbonate at [pH]0 ≈ 6.0. (Experimental conditions: Number of nematodes: 30; T = 25 ± 1°C.)**

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