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

**Taoqin Chen**
Tongji University
**Jinzhe Li**
Tongji University
**Longqian Xu**
Tongji University
**Dong Zhang**
National Engineering Rearch Center of Urban Water Resources
**Zheng Wang**
National Engineering Research Center of Urban Water Resources
**Hongbin Chen** ( bhctxc@tongji.edu.cn )
Tongji University

**Research Article**

**Keywords:** Chlorine-resistant invertebrates, Caenorhabditis elegans, Disinfection, Nematode deactivation, mechanism

**Posted Date:** February 22nd, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-157126/v1

# Abstract

The occurrence an d infestations of chlorine-resistant invertebrates in drinking water distributions have attracted concerns on water quality in China, making effective deactivation imperative. This study presents a no vel strategy for nematode ( Caenorhabditis elegans ) deactivation using peroxymonosulfate (PMS)/UV-C. The results indicated that 100% deactivation efficiency was obtained under optimal conditions. An acidic pH and 0.25 mg/L Fe(II) were beneficial to the PMS/UV-C-triggered deactivation of nematodes. A mechanism study demonstrated that  was activated by UV-C to produce · OH and · SO 4 - , which resulted in oxidative stress and stimulated the occurrence of cell apoptosis, leading to nematode deactivation. The results reveal PMS/UV-C as an alternative to chlorination in water treatment plants (WTP) or an emergency application when chlorine-resistant invertebrates breed in a second-supply water tank, is a promising strategy for disinfection. This approach afforded the advantages of avoiding the production of chlorine disinfection by-products (DBP) and greater efficacy of nematode deactivation. This work will provide ideas for on-going research efforts into chlorine-resistant invertebrate deactivation, and eventually achieve the direct drinking of municipal tap water.


# 1. Introduction

Infestations of invertebrates at waterworks and complaints by water users about the presence of invertebrates in tap water are of growing concern (van Lieverloo et al. 2012). Invertebrates are found in a water supply system due to the intensified pollution of source water and the widespread application of advanced treatment technologies, i.e., previous research showed granular activated carbon (GAC) filters provided ideal habitats for invertebrates (Schreiber et al. 1997, Wang et al. 2014, Weeks et al. 2007). Most species of invertebrates can be deactivated by conventional chlorination, but a few invertebrates such as nematodes and red worms, namely chironomid larvae, *Tubifex* worms and *Calamaria pavimentata*, are difficult to be deactivated and thus defined as chlorine-resistant invertebrates (Wu &Chen 2019). Nematodes were reported to be abundant in treated water and distribution systems (Bichai et al. 2009, Kos et al. 2019). A survey conducted by almost all water companies in the Netherlands revealed common invertebrates in treated water and in tap water were rotifers and nematodes (van Lieverloo et al. 2012). Inactivation of Chironomid larvae with chlorine (Sun et al. 2005), chlorine dioxide (Sun 2006, Sun et al. 2007) , ozone (Sun 2006) were previously reported. Chlorine inactivation of *Tubifex* in drinking water and the synergistic effect of sequential inactivation with UV irradiation and chlorine was studied by Nie et al. (2017). In addition, inactivation of *Tubifex* by copper (Huang et al. 2010) and six common disinfectants including sodium hypochlorite, liquid chlorine, chloramines, chlorine dioxide, hydrogen peroxide, and potassium permanganate composite (Chen et al. 2009) were researched before. Compared with chironomid larvae and *Tubifex* worms, there has been little research on the deactivation of nematodes.

Free living nematodes can ingest viruses and pathogenic bacteria as well as (oo)cysts (Bichai et al. 2010, Chang et al. 1960, Levy 1990), although there are no indications that free living nematodes pose a threat to public health. The associated pathogens were found to be *Escherichia coli*, *Bifidobacterium*, *Salmonella* sp., *Vibrio cholera*, *Cryptosporidium* and *Giardia* etc., and they are related to intestinal

infection, tuberculosis, typhoid fever, cholera and diarrhea (Li &Chen 2019). *Cryptosporidium* and *Giardia* often cause waterborne outbreaks of disease due to their zoonotic potential and impact on human health (Ligda et al. 2020). The estimated number of bacteria that could be associated with a single invertebrate (based on average invertebrate numbers) ranged from 10 to 4,000 bacteria per organism (Wolmarans et al. 2005). In addition, ingested (oo)cysts were found to remain intact, viable and infectious within the digestive tract of the nematode (Bichai et al. 2008). Hence, potential oral exposure to bacterial-laden nematodes, their decaying parts and endotoxins released from bacteria poses high risks to public health under the worst case scenario. (Levy 1990)

Free chlorine is widely used for the disinfection of drinking water, but the challenge remains that invertebrates often display a higher level of resistance to chlorine disinfection than bacteria and viruses (Matsumoto et al. 2002, Yan et al. 2010). Previous research attributed invalid chloride disinfection of pathogenic bacteria to the shelter provided by higher organisms such as nematodes. (Bichai et al. 2008). It was noted that nematodes have been shown to ingest, harbor and transport pathogenic bacteria and protect them against UV disinfection and chemical disinfectants (Bichai et al. 2010, Bichai et al. 2009). Smerda et al. (1971) fed *Salmonella* sp. to nematodes, surface sterilized them via 10 ppm free chlorine solution and later recovered viable *Salmonella* in the excreta. These fecal-associated bacteria may be protected from disinfection by virtue of this association, and can continue to survive in spite of residual disinfectant (Levy 1990). Thus, deactivation of chlorine-resistant nematodes is vital to ensure drinking water safety and an effective alternative to conventional chlorination is urgently needed.

Recently, peroxymonosulfate (PMS)/UV has attracted increasing attention in the deactivation of fungi and bacteria due to growing demands to control disinfection by-products (DBPs) while providing efficient microbial control (Ao et al. 2016). The oxidative environment produced by free radicals such as ·OH and  (Eq. 1) (Yang et al. 2015) played an important role in microorganism deactivation (Umar et al. 2019, Xiao et al. 2019). And the lifespan of *C. elegans* under high oxygen concentrations were shorter than those under normoxic conditions (Honda &Honda 2002). Judging from this, the strong oxidative conditions produced by PMS/UV are highly likely to cause rapid deactivation of nematodes. To the best of our knowledge, research on applying PMS/UV to the deactivation of nematodes and the potential mechanism has not been previously reported. Current research on the deactivation mechanisms of ·OH and  were mainly limited to bacteria, fungi, algae and viruses, but huge knowledge gap still existed in higher organisms in higher organisms (Xiao et al. 2019).

In this study, a PMS/UV-C system was applied to deactivate nematodes. The nematode *C. elegans* was chosen as the experimental subject (Bichai et al. 2009, Bichai et al. 2008), because it is a member of the *Rhabditidae* family, which appears ubiquitous detection in drinking water (Bichai et al. 2008, Tombes et al. 1979). This paper examined deactivation efficiency and the underlying mechanisms of nematodes by PMS/UV-C. The implications of these findings are discussed in the context of providing direct drinking water.

# 2. Materials And Methods

### *2.1. Microorganisms and chemicals*

Reagents are concisely provided in the Supporting Information (SI) **Text S1**.

*C. elegans* (strain N2, wild type) were cultured on nematode growth media (NGM) plates fed on *Escherichia coli* strain OP50 (Brenner 1974). In a typical experiment, nematodes were cultured in NGM in a 20°C constant temperature incubator, and then rinsed with deionized water.

### *2.2. Experimental procedure*

Nematodes of the same adult period length were selected and transferred to a 35mm petri dish with a pipette under a microscope (Olympus SZ61, Japan). Each individual experiment contained ~30 nematodes. Then, 5-mL aliquots of PMS at different concentrations were added to the 35-mm Petri dishes containing the nematodes. The Petri dishes were put into a homemade device (**Fig. S1**) and then irradiated at 254 nm with a UV-C lamp (8W, Cnlight, China). The UV irradiation intensity was measured to be ~50 μW/cm$^2$ by a UV radiometer (UV-B Photoelectric Instrument Factory of Beijing Normal University, China). At different time intervals, Petri dishes were removed from the device and the numbers of live nematodes were counted. Except where otherwise specified, the system temperature was maintained at 25 ± 1°C and all experiments were conducted at least in triplicate. The effects of different PMS concentrations and temperatures were investigated. To determine the influence of pH, appropriate amounts of $H_2SO_4$ (0.1 M) or NaOH (0.1 M) were added to adjust the initial pH value.

### *2.3. Analytical methods*

#### *2.3.1 Nematode dead judgement*

Nematodes were considered dead if they became straight and immobile for more than 5 seconds.

#### *2.3.2 Free radicals measurement*

To identify the major oxidants involved in the *C. elegans* deactivation by PMS/UV-C, the radicals ( and ·OH) and singlet oxygen ($^1O_2$) were detected by employing electron paramagnetic resonance (EPR, EM Xplus-10/12, Bruker, Germany) spectroscopy with 25 g/L 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) (Li et al. 2018, Nosaka &Nosaka 2017, Rao et al. 2019) and 2,2,6,6-tetramethyl-4-piperidinol (TEMP) (Li et al. 2018, Yang et al. 2018) as the spin traps. The solutions containing 1 mM PMS at pH 6.0 were irradiated for 10 min and heated for 20 min before the EPR measurement. Further details are given in **Text S2**. The ·OH production was measured using coumarin as the trapping agent. A solution containing 1 mM coumarin and 1 mM PMS was prepared and irradiated (or heated) for different duration. At given time intervals, aliquots were taken and monitored using a fluorescence spectrometer (HORIBA MAX, Japen) at an emission wavelength of 460 nm.

#### *2.3.3 Concentrations of*

A modified rapid spectrophotometric determination method was adopted to determine the concentration changes of  during the activation of PMS by UV-C or heat at 254 nm according to a reported method (Li et al. 2018, Liang et al. 2008) using a UV-1800 UV spectrophotometer (SHMADZU, Japan).

### *2.3.4 FTIR spectra and SEM test*

The changes in functional groups of the nematodes were measured by FTIR spectrometer NICOLET 5700 (Thermo, USA) in the range of 4000-400 $cm^{-1}$ using the potassium bromide (KBr) pellet method. The appearance of nematode structures before and after PMS/UV-C treatment was detected by scanning electron microscope (SEM, FEI Nova Nano SEM 450, USA).

### *2.3.5 Bioassay*

Enzyme proteins were extracted from nematode homogenates according to a previous  protocol (Zhang et al. 2017), and analyzed using a Total Superoxide Dismutase Assay Kit with WST-8 (S0101, Beyotime, China). Details of the analysis of superoxide dismutase (SOD) are presented in **Text S3**. Reactive oxygen species (ROS) and cell apoptosis were measured by a Nikon laser confocal microscope (Nikon A1R, Japan) and details were presented in **Text S4**.

### *2.3.6 Statistical Analysis*

The data obtained were averaged, and the corresponding standard deviations were determined.

ROS and cell apoptosis were expressed in relative fluorescence units (RFU) by Image J software and detailed in **Text S4**.

# 3. Results And Discussion

## *3.1. Nematode deactivation efficiency of the PMS/UV-C system*

A deactivation overview of 30 *C. elegans* nematodes at different concentrations of PMS against reaction time under UV-C irradiation (254 nm) is shown in **Fig. 1a**. The deactivation efficiency was highly correlated with the PMS concentration in the PMS/UV-C system. A deactivation efficiency of 36.7% ± 0% was found when the PMS concentration was 0 mM, which can be attributed to the UV-C irradiation alone. When the PMS concentration was increased to 1 or 2 mM, the deactivation efficiency was greatly improved and 100% ± 0% deactivation could be achieved within 90 min, which was significantly higher than that of 0 mM and 0.5 mM PMS solutions (p□0.01). During the nematode deactivation process in the PMS/UV-C system, a rapid decrease in pH was observed and became more obvious with increasing PMS concentration as shown in **Fig. S2**. When the PMS concentration was 2 mM, the pH decreased from 6.0 to 3.4. The reason for the pH decrease may be partially due to $H^+$ generation during the consumption of

(Eq. 2) (Rao et al. 2019, Sharma et al. 2015). Previous researchers found that endo- and exo-bacterial organic compounds attacked by  -derived ROS released aliphatic acids, which also contributed to the pH decrease (Rincon &Pulgarin 2004).  In this study, however, the pH variations of the PMS/UV-C system were similar with or without nematodes, so we inferred no acid was released by the nematodes during deactivation.

$$HSO_5^- + \xrightarrow{h\vartheta,\ heat,\ \Delta} \cdot SO_4^- + \cdot OH \quad (1)$$

$$\cdot SO_4^- + H_2O \rightarrow SO_4^{2-} + \cdot OH + H^+ \quad (2)$$

**Fig. 1b** shows that 1 mM PMS alone had no toxic effect on *C. elegans*. Under UV-C irradiation alone, 33% ± 4.7% deactivation efficiency was achieved, owing to the deactivating effect of UV on the organisms (Beber de Souza et al. 2015, Cadet &Wagner 2013), but the UV-C deactivation efficiency in this study was not the same as previously reported. In former report, The irradiation time required to achieve 100% elimination of nematode larvae and adults were 9 and 10 min, respectively (Dehghani et al. 2013), and this difference could possibly be attributed to a different UV reactor set-up, nematode species and whether stirring was employed. It is worth mentioning that PMS/UV-C greatly improved the deactivation efficiency compared to UV-C ($p<0.01$), and the damage to the nematodes was irreversible because there was no sign of life if the deactivated nematodes were re-cultured in NGM for 48 h at 20°C after the 90-min PMS/UV-C deactivation.

# *3.2. Factors affecting the PMS/UV-C system*

## *3.2.1 Effects of temperature on PMS activation*

The successful generation of  and ·OH and consumption of in the PMS/UV-C system were confirmed as shown in **Fig. S4**.  ·OH generation was monitored at different temperatures as shown in **Fig. 2a**. The high fluorescence intensity indicated a large amount of ·OH was produced, which was consistent with the high nematode deactivation efficiency at 35°C. Moreover, the radical generation was further verified by EPR spectroscopy. As shown in **Fig. 2b**, the obvious signals of DMPO-·OH and DMPO-  were observed at 35°C. The appearance of the TEMP signal (**Fig. 2c**) was clear evidence of $^1O_2$ production whether heated or not, which supported the view that PMS self-decomposed spontaneously to form $^1O_2$ (Zhou et al. 2015). The corresponding PMS decomposition rate was determined as shown in **Fig. S5**.

Since UV-C irradiation may cause a successive temperature rise during the reaction process, to test the possibility of PMS activation by heat, the effects of different temperatures on nematode deactivation were investigated without UV/C illumination, and the results are shown in **Fig. 2d**. Though 30.0% ± 9.4% of deactivation efficiency was obtained at 30°C in 90 min, a small amount of ·OH could be detected (**Fig. 2a**), which may mean that the generated radicals were not sufficient to overwhelm the nematode defense

systems, resulting in negligible nematode death. However, complete deactivation was found when the temperature was increased to 35°C. The effects of heating without PMS were measured as 1.67% ± 0.3% and 30.0% ± 9.4% at 30°C and 35°C, respectively. Therefore, the intrinsic heat effect at 35°C played a minor role and the nematode deactivation was mainly due to the effect of activated PMS, which also suggested that temperatures higher than 30°C would be more effective for PMS activation within 90 min ($p \leq 0.01$). Note that the temperature of the PMS/UV-C system was maintained below 30°C, so the observed PMS activation was attributed to UV-C irradiation.

## 3.2.2 Effects of pH, Fe(II) and common anions

The nematode deactivation efficiency in the PMS/UV-C system was found to be highly dependent on pH values. Acidic reaction condition exhibited a better deactivation performance, and a significant decline in deactivation efficiency was observed with increasing pH values. When the pH value increased to ~9.0, the deactivation efficiency of the nematodes dropped significantly to 55.0% ± 2.4% ($p \leq 0.01$) (**Fig. 3a**). A possible reason is that  ions are stable in neutral and acidic solutions, but will apparently be converted to ·OH at pH > 8.5 (Eq. 2) (Huang &Huang 2009). Compared with ·OH,  exhibits a higher oxidation-reduction potential and longer half-life (Chen et al. 2019b, Rastogi et al. 2009). Therefore, the role of  was more important than that of ·OH for nematode deactivation (Wang et al. 2019). In addition, the pH was monitored to remain rather stable under acidic and neutral conditions, while decreased significantly from 9.0 to 6.4 in an alkaline environment (**Fig.S6a**). This result was consistent with previous studies, which showed that when the pH was ~9.0, PMS exhibited the poorest stability as  decomposed to  and $H^+$ (Wang &Wang 2018). Efficiencies at different pH values without PMS in the dark were determined to examine the impacts of sole pH on nematode deactivation, and no nematode deactivation was observed in the pH range of 5.0–9.0 (**Fig. S6b**).

The effects of common ions in drinking water on the PMS/UV-C system were investigated. Bicarbonate, which can quench radicals to inhibit the oxidation process (Wu et al. 2015), is the representative of inorganic carbon in drinking water. As shown in **Fig. S6c**, 1 mg/L $NaHCO_3$ exhibited a slight inhibition; with further addition of $NaHCO_3$, the deactivation efficiency decreased greatly with the presence of 10 mg/L and 20 mg/L of $NaHCO_3$. Bicarbonate could quench  and ·OH to generate , which has a much lower redox potential, see Eq.3, 4 (Sharma et al. 2015) In addition, the variation of pH was monitored during the reactions (**Fig. S6d**). The results showed pH dropped over time and the terminal pH values dropped more gently with increasing concentration of $NaHCO_3$. Therefore, both the relatively higher pH and the quenching of  and ·OH might produce detrimental effects on nematode deactivation.

As shown in **Fig. 3b**, $Cl^-$ was found to only slightly reduced the nematode deactivation. This could be due to the scavenging of  by $Cl^-$ to produce the less reactive chlorine species ·Cl and , see Eq. 5, 6 (Fang et al. 2012, Sharma et al. 2015). The deactivating ability of reactive chlorine species was expected to compensate for the loss of , leading to the insignificant influence of $Cl^-$, which implied that much higher concentrations (0.01M) than conventional applied doses might reach the same efficiency as PMS. As for $CH_3COO^-$, a significant decline (30.0% ± 4.7%) in deactivation efficiency was observed with the addition of $CH_3COO^-$, which could be explained by competition for free radicals. Apart from these anions, it is well-known that very low concentrations of Fe(II) are usually present in drinking water. Fe below 0.3 mg/L meets the drinking water standard of China, the deactivation performance of 0.25 mg/L of Fe(II) in the PMS/UV-C system was investigated (**Fig. 3b**). Rather more effective than pure PMS/UV-C, PMS/UV-C/Fe(II) achieved 100% deactivation in a shorter time (**Fig. 3b**). This was because ferrous ions catalyzed PMS to generate sulfate radicals, see Eq. 7 (Anipsitakis &Dionysiou 2004, Ghanbari &Moradi 2017, Rao et al. 2019). Therefore, PMS/UV-C was an effective method for nematode deactivation, especially when the drinking water contained trace Fe(II), although this was not expected to be the

$$HCO_3^- + \cdot SO_4^- \rightarrow \cdot CO_3^- + SO_4^{2-} + H^+ \quad (3)$$

$$\cdot SO_4^- + Cl^- \rightarrow SO_4^{2-} + \cdot Cl \quad (5)$$

$$\cdot Cl + Cl^- \rightarrow \cdot Cl_2^- \quad (6)$$

$$Fe(II) + HSO_5^- \rightarrow Fe(III) + \cdot SO_4^- + OH^- \quad (7)$$

# 3.3 Mechanism of nematode deactivation

Various factors, inside or outside an organism, can result in ROS production, which induce oxidative stress. In response to such oxidative stress, organisms can deploy superoxide dismutase (SOD) to scavenge ROS to maintain cellular homeostasis (Balaban et al. 2005). Therefore, a higher SOD activity indicates the cells are encountering more severe oxidative stress from the environment. As shown in **Fig. 4a**, in the initial 60 min, SOD activity increased rapidly with irradiation time, which indicated there were increasing numbers of ROS attacking the nematodes at the beginning of the deactivation process, with defense systems displaying a higher SOD activity to protect the cells. This observation is consistent with the results of **Fig. 1a** (1 mM PMS/UV-C), that the killing efficiency was 0% before 60 min because the anti-oxidative enzymes protected the nematodes up to 60 min. After 60 min, the increasing deactivation efficiency in **Fig. 1a** (1mM PMS/UV-C) could be reasonably explained by SOD activity decreased as the deactivation process progressed. It was probable that the increasing amount of radicals eventually overwhelmed the self-defense system and led to the downregulation of SOD activity and the

accumulation of ROS in vivo, which induced severe impairment in nematode cells (**Fig. 4c**). In contrast, negligible SOD activity was observed under light control (without PMS) and slight SOD activity under dark control (without UV-C irradiation) conditions. This result further confirmed the radical-induced oxidative deactivation of nematodes in the PMS/UV-C system.

To further confirm the hypothesis raised above, the ROS level and degree of cell apoptosis were evaluated after different operating durations. The intracellular ROS level was tested using a H2DCFDA probe, which can be hydrolyzed to non-fluorescent dichlorofluorescein (DCFH) by esterases in the cells and can subsequently be oxidized to the fluorescent dichlorofluorescein (DCF) by cellular ROS. **Figure. 4b** showed significant increases in ROS production were detected after exposure to PMS/UV-C for more than 20 min; **Fig. 4c** reveals that significant cell apoptosis was discovered (Chen et al. 2019a, Zhou et al. 2016) after exposure to PMS/UV-C for 40 min. It is inferred that the ROS accumulated as the time passed, and then caused significant cell apoptosis.

SEM spectra was applied to suggest that the nematodes suffered from cuticle wrinkling and cracking after PMS/UV-C treatment (**Fig. 5b**) compared with the intact and smooth cuticles of untreated nematodes (**Fig. 5a**). FTIR indicated that the effect of PMS/UV-C on the cuticle was an important prerequisite for nematode deactivation. **Fig. 5c** shows that vibrations at 1660, 1548 and 3469 $cm^{-1}$ were assigned to amide I, amide II and asymmetric stretching N–H bonds of proteins (Movasaghi et al. 2008, San-Blas et al. 2011). Vibrations at 2925 and 2856 $cm^{-1}$ were assigned to asymmetric $CH_2$ and symmetric $CH_3$ stretches of the lipids (Bouyanfif et al. 2018), conforming to literature reports that the main biochemical components of the cuticle are proteins, lipids and water (Xue et al. 2012). The smooth and dense cuticle provided a protective effect for the nematodes (Zhan et al. 2007, Zhou et al. 2010). But after 90 min of PMS/UV-C processing, substantial changes took place. The –C=O of the phosphodiester group stretching vibration of the triglycerides band was seen at 1727 $cm^{-1}$(Wu et al. 2001); 1282 $cm^{-1}$ could be assigned to collagen $CH_2$ wagging, nucleic acids and amide III (Ci et al. 1999). The band at 1124 $cm^{-1}$ was due to C–O, C–C stretching, C–O–H, C–O–C deformations of glycogen (Chiriboga et al. 1988, Diem et al. 2004). The region around 1074 $cm^{-1}$ was assigned to C–C, C–O, C–O–C stretching vibrations of carbohydrates (San-Blas et al. 2011). This evidence indicated external oxidative stress damaged the nematode cuticle. Therefore, the deactivation process was confirmed to damage both the outer cuticle and *in vivo* components of the nematodes.

Despite the potential role of UV-C in the deactivation of nematode **(Fig. 1b)**, oxidative radicals generated from PMS/UV-C were believed to play the major role in deactivating nematode. The deactivation mechanism in the PMS/UV-C system was proposed as shown in **Fig. 6**.At first, was activated by UV-C to produce and ·OH. These oxidative radicals damage the outer cuticle and induced oxidative stress in nematode, including the accumulation of ROS and altered activities of antioxidant enzymes. Cell

apoptosis was further confirmed to occur in the nematode, which stimulated the cuticle wrinkling and cracking and finally resulted in the death of nematode.

# 4. Environmental Implications And Future Investigative Studies

The challenge of being able to directly drink municipal tap water calls for new technologies to ensure the biosafety of drinking water. This study provides the first research on applying PMS/UV-C to nematode deactivation by investigating its efficiency and mechanism, finding  and ·OH were efficient in deactivating chlorine-resistant nematodes. This suggested that PMS/UV-C was an alternative to chlorination in WTPs or an emergency application when chlorine-resistant invertebrates breed in a second-supply water tank. PMS/UV-C offers the following advantages: first, a superior nematode deactivation effect compared to chlorination; second, a reduction in chlorinated DBPs (and it must be stated that the detection, characterization and quantification of other DBPs was outside of the scope of this study); third, prevention of the dissemination of pathogens associated with nematodes. This work will offer consideration to on-going research efforts to achieve chlorine-resistant invertebrate deactivation.

# 5. Conclusion

This is the first report applying PMS/UV-C to nematode deactivation by investigating its efficiency and mechanism. The results showed that 100% deactivation efficiency was obtained under optimal conditions. The acidic pH and 0.25 mg/L Fe(II) promote the PMS/UV-C-triggered nematode deactivation. The mechanism study demonstrated that  was activated by UV-C to produce  and ·OH, which induced oxidative stress in nematode, including the accumulation of ROS and altered activities of antioxidant enzymes. Cell apoptosis was further confirmed to occur in the nematode, which stimulated the cuticle wrinkling and cracking and finally resulted in the death of nematode. PMS/UV-C has the potential to be an alternative to chlorination in a WTP or an emergency application, for example, chlorine-resistant invertebrates breed in a second-supply water tank.

# Declarations

**Acknowledgements** We thank Sheng Wei, Haixin Jiang, Xueli Jiang for helping with the experiment.

**Authors' contributions** HC and TC conceived and designed research. TC and JL conducted the chemical analysis. TC and LX wrote the manuscript. All the authors read and approved the manuscript.

**Funding** This study was financially supported by the National Major Science and Technology Project of China (2017ZX07207004-6).

**Data availability** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

# Compliance with ethical standards

**Competing interests** The authors declare that they have no competing interests.

**Ethical approval and consent to participate** Not applicable.

**Consent to publish** Not applicable.

# References


Anipsitakis GP, Dionysiou DD (2004): Transition metal/UV-based advanced oxidation technologies for water decontamination. Applied Catalysis B: Environmental 54, 155-163

Ao X, Li H, Liu W, Yu J (2016): Characteristics of disinfection by-products and genotoxicity during drinking water disinfection with potassium monopersulfate compound powder. Environmental Science 37, 4241-4246

Balaban RS, Nemoto S, Finkel T (2005): Mitochondria, oxidants, and aging. Cell 120, 483-95

Beber de Souza J, Queiroz Valdez F, Jeranoski RF, Vidal CMdS, Cavallini GS (2015): Water and wastewater disinfection with peracetic acid and UV radiation and using advanced oxidative process PAA/UV. International Journal of Photoenergy 2015, 1-7

Bichai F, Payment P, Barbeau B (2008): Protection of waterborne pathogens by higher organisms in drinking water: a review. Canadian Journal of Microbiology 54, 509-24

Bichai F, Barbeau B, Payment P (2009): Protection against UV disinfection of *E. coli* bacteria and *B. subtilis* spores ingested by *C. elegans* nematodes. Water Res 43, 3397-406

Bichai F, Barbeau B, Dullemont Y, Hijnen W (2010): Role of predation by zooplankton in transport and fate of protozoan (oo)cysts in granular activated carbon filtration. Water Res 44, 1072-81

Bouyanfif A, Liyanage S, Hequet E, Moustaid-Moussa N, Abidi N (2018): Review of FTIR microspectroscopy applications to investigate biochemical changes in *C. elegans*. Vibrational Spectroscopy 96, 74-82

Brenner S (1974): The DNA of *Caenorhabditis elegans*. Genetics 77, 71

Cadet J, Wagner JR (2013): DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. Cold Spring Harbor Perspective in Biology 5

Chang SL, Woodward RL, Kabler PW (1960): Survey of free-living nematodes and amoebas in municipal supplies. Journal - AWWA 52, 613-618

Chen H, Guo S, Li H, Zhou D, Cao X, Wang C, Liu Y, Xiang M, Li L, Yu Y (2019a): Multi-generational effects and variations of stress response by hexabromocyclododecane (HBCD) exposure in the nematode Caenorhabditis elegans. J. Environ. Manage. 245, 216-222

Chen X, Gao W, Zhu L, Li T (2009): Inactivation of Tubifex (Monopylephorus limosus) in Water by Six Common Disinfectants, international conference on bioinformatics and biomedical engineering, pp. 1-4

Chen X, Hu X, Gao Y (2019b): Removal of NO in simulated flue with aqueous solution of peroxymonosulfate activated by high temperature and Fe(II). Chemical Engineering Journal 359, 419-427

Chiriboga L, Xie P, Yee H, Vigorita V, Zarou D, Zakim D, Diem M (1988): Infrared spectroscopy of human tissue. I. Differentiation and maturation of epithelial cells in the human cervix. Biospectroscopy 4, 47-53

Ci YX, Gao TY, Feng J, Guo ZQ (1999): Fourier transform infrared spectroscopic characterization of human breast tissue: implications for breast cancer diagnosis. Applied spectroscopy 53, 312-315

Dehghani MH, Jahed G, Zarei A (2013): Investigation of low-pressure ultraviolet radiation on inactivation of Rhabitidae nematode from water. Iranian Journal of Public Health 42, 314-319

Diem M, Romeo M, Boydston-White S, Miljkovic M, Matthaus C (2004): A decade of vibrational micro-spectroscopy of human cells and tissue (1994-2004). Analyst 129, 880-5

Fang GD, Dionysiou DD, Wang Y, Al-Abed SR, Zhou DM (2012): Sulfate radical-based degradation of polychlorinated biphenyls: effects of chloride ion and reaction kinetics. Journal of Hazardous Materials 227-228, 394-401

Ghanbari F, Moradi M (2017): Application of peroxymonosulfate and its activation methods for degradation of environmental organic pollutants: Review. Chemical Engineering Journal 310, 41-62

Honda Y, Honda S (2002): Oxidative stress and life span determination in the nematode Caenorhabditis elegans. Annals of the New York Academy of Sciences 959., 466-474.

Huang T, Nie XB, Zhang JS, Li W, Li XY (2010): Rapid inactivation mechanism of copper to Tubifex tubifex and its application analysis in water plant. Environmental Sciences 31, 331

Huang YF, Huang YH (2009): Identification of produced powerful radicals involved in the mineralization of bisphenol A using a novel UV-$Na_2S_2O_8$/$H_2O_2$-Fe(II,III) two-stage oxidation process. Journal of Hazardous Materials 162, 1211-6

Kos J, Brmež M, Markić M, Sipos L (2019): The mortality of nematodes in drinking water in the presence of ozone, chlorine dioxide, and chlorine. Ozone: Science & Engineering 42, 120-127

Levy RV (1990): Invertebrates and associated bacteria in drinking water distribution lines, Drinking water microbiology. Springer, New York, pp. 224-248

Li H, Shan C, Pan B (2018): Fe(III)-Doped g-C3N4 mediated peroxymonosulfate activation for selective degradation of phenolic compounds via high-valent iron-oxo species. Environ. Sci. Technol. 52, 2197-2205

Li J, Chen H (2019): Safety risks of invertebrates in water supply systems. Water & Wastewater Engineering 45, 119-124

Liang C, Huang CF, Mohanty N, Kurakalva RM (2008): A rapid spectrophotometric determination of persulfate anion in ISCO. Chemosphere 73, 1540-3

Ligda P, Claerebout E, Kostopoulou D, Zdragas A, Casaert S, Robertson LJ, Sotiraki S (2020): Cryptosporidium and Giardia in surface water and drinking water: Animal sources and towards the use of a machine-learning approach as a tool for predicting contamination. Environmental Pollution 264, 114766

Matsumoto N, Aizawa T, Ohgaki S, Hirata T, Toyooka K, Kanbayashi T, Tsutsumi Y, Hasegawa T (2002): Removal methods of nematoda contained in the effluent of activated carbon. Water Science and Technology: Water Supply 2, 183-190

Movasaghi Z, Rehman S, ur Rehman DI (2008): Fourier transform infrared (FTIR) spectroscopy of biological tissues. Applied Spectroscopy Reviews 43, 134-179

Nie X, Li Z, Long Y, He P, Xu C (2017): Chlorine inactivation of Tubifex tubifex in drinking water and the synergistic effect of sequential inactivation with UV irradiation and chlorine. Chemosphere 177, 7-14

Nosaka Y, Nosaka AY (2017): Generation and detection of reactive oxygen species in photocatalysis. Chemical Reviews 117, 11302-11336

Rao D, Sun Y, Shao B, Qiao J, Guan X (2019): Activation of oxygen with sulfite for enhanced removal of Mn (II): The involvement of $SO_4^{-}$•. Water Res 157, 435-444

Rastogi A, Al-Abed SR, Dionysiou DD (2009): Sulfate radical-based ferrous–peroxymonosulfate oxidative system for PCBs degradation in aqueous and sediment systems. Applied Catalysis B: Environmental 85, 171-179

Rincon A-G, Pulgarin C (2004): Effect of pH, inorganic ions, organic matter and $H_2O_2$ on *E. coli* K12 photocatalytic inactivation by $TiO_2$ Implications in solar water disinfection. Applied Catalysis B: Environmental 51, 283-302

San-Blas E, Guerra M, Portillo E, Esteves I, Cubillán N, Alvarado Y (2011): ATR/FTIR characterization of Steinernema glaseri and Heterorhabditis indica. Vibrational Spectroscopy 57, 220-228

Schreiber H, Schoenen D, Traunspurger W (1997): Invertebrate colonization of granular activated carbon filters. Water Research 31, 743-748

Sharma J, Mishra IM, Dionysiou DD, Kumar V (2015): Oxidative removal of bisphenol A by UV-C/peroxymonosulfate (PMS): Kinetics, influence of co-existing chemicals and degradation pathway. Chemical Engineering Journal 276, 193-204

Smerda SM, Jensen HJ, Anderson AW (1971): Escape of Salmonellae from chlorination during ingestion by *Pristionchus lheritieri* (Nematoda: Diplogasterinae). Journal of Nematology 3, 201-204

Sun X (2006): Inactivation of Chironomid larvae from drinking water by ozone and its removal using ozone-GAC process. Journal of Chemical Industry and Engineering

Sun X, Cui F, Zhang J, Xu F, Liu L (2007): Inactivation of Chironomid larvae with chlorine dioxide. Journal of Hazardous Materials 142, 348-353

Sun XB, Cui FY, Zhang JS, Guo ZH, Xu F, Liu LJ (2005): Toxicity and Influencing Factors of Liquid Chlorine on Chironomid Larvae. Environmental Sciences 26, 95-99

Tombes AS, Abernathy AR, Welch DM, Lewis SA (1979): The relationship between rainfall and nematode density in drinking water. Water Research 13, 619-622

Umar M, Roddick F, Fan L (2019): Moving from the traditional paradigm of pathogen inactivation to controlling antibiotic resistance in water - Role of ultraviolet irradiation. Science of the Total Environment 662, 923-939

van Lieverloo JH, Hoogenboezem W, Veenendaal G, van der Kooij D (2012): Variability of invertebrate abundance in drinking water distribution systems in the Netherlands in relation to biostability and sediment volumes. Water Res 46, 4918-32

Wang J, Wang S (2018): Activation of persulfate (PS) and peroxymonosulfate (PMS) and application for the degradation of emerging contaminants. Chemical Engineering Journal 334, 1502-1517

Wang Q, You W, Li X, Yang Y, Liu L (2014): Seasonal changes in the invertebrate community of granular activated carbon filters and control technologies. Water Res 51, 216-27

Wang W, Wang H, Li G, An T, Zhao H, Wong PK (2019): Catalyst-free activation of persulfate by visible light for water disinfection: Efficiency and mechanisms. Water Research 157, 106-118

Weeks MA, Leadbeater BS, Callow ME, Bale JS, Holden JB (2007): Effects of backwashing on the prosobranch snail Potamopyrgus jenkinsi Smith in granular activated carbon (GAC) adsorbers. Water Res 41, 2690-6

Wolmarans E, Du Preez HH, De Wet CME, Venter SN (2005): Significance of bacteria associated with invertebrates in drinking water distribution networks. Water Science and Technology 52, 171-175

Wu JG, Xu YZ, Sun CW, Soloway RD, Xu DF, Wu QG, Sun KH, Weng SF, Xu GX (2001): Distinguishing malignant from normal oral tissues using FTIR fiber-optic techniques. Biopolymers 62, 185-192

Wu L, Chen H (2019): Progress occurrence and control strategy of chlorine-resistant invertebrates in urban water supply system. Water Purification Technology 38, 19-24

Wu Y, Bianco A, Brigante M, Dong W, de Sainte-Claire P, Hanna K, Mailhot G (2015): Sulfate Radical Photogeneration using Fe-EDDS: Influence of critical parameters and naturally occurring scavengers. Environmental Science & Technology 49, 14343-14349

Xiao R, Liu K, Bai L, Minakata D, Seo Y, Kaya Göktaş R, Dionysiou DD, Tang C-J, Wei Z, Spinney R (2019): Inactivation of pathogenic microorganisms by sulfate radical: Present and future. Chemical Engineering Journal 371, 222-232

Xue M, Liang Q, Huang Z, Nie H, Liu W, Wang H (2012): Effect of l-menthol on skin keratoderma structure by ATR-FTIR changes. Chinese Traditional and Herbal Drugs 43, 2474-2477

Yan K, Zhang M, Zhou W, Cai Y, Zou L, Ding G (2010): Inactivation kinetics of Plectus sp. in application of disinfection with sodium hypochlorite. Water Purification Technology 29

Yang Y, Jiang J, Lu X, Ma J, Liu Y (2015): Production of sulfate radical and hydroxyl radical by reaction of ozone with peroxymonosulfate: A novel advanced oxidation process. Environmental Science & Technology 49, 7330-7339

Yang Y, Banerjee G, Brudvig GW, Kim J-H, Pignatello JJ (2018): Oxidation of organic compounds in water by unactivated peroxymonosulfate. Environmental Science & Technology 52, 5911-5919

Zhan J, Ding G, Xu K (2007): Study on inactivation effect of chlorine on *Rhabditis* sp. in drinking water. Water Purification Technology 26, 42-44

Zhang J, Chen R, Yu Z, Xue L (2017): Superoxide dismutase (SOD) and catalase (CAT) activity assay protocols for Caenorhabditis elegans. Bio-Protocol 7

Zhou D, Yang J, Li H, Lu Q, Liu YD, Lin KF (2016): Ecotoxicological evaluation of low-concentration bisphenol A exposure on the soil nematode *Caenorhabditis elegans* and intrinsic mechanisms of stress response in vivo. Environ. Toxicol. Chem. 35, 2041-7

Zhou W, Zhang M, Li N, Zou L, Ni Q, Shi J, Ding G (2010): Inactivation effect and kinetics of *Plectus* sp. inactivated by hydrogen peroxide. Technology of Water Treatment 36, 19-21

Zhou Y, Jiang J, Gao Y, Ma J, Pang S-Y, Li J, Lu X-T, Yuan L-P (2015): Activation of peroxymonosulfate by benzoquinone: A novel nonradical oxidation process. Environmental Science & Technology 49, 12941-12950

# Figures

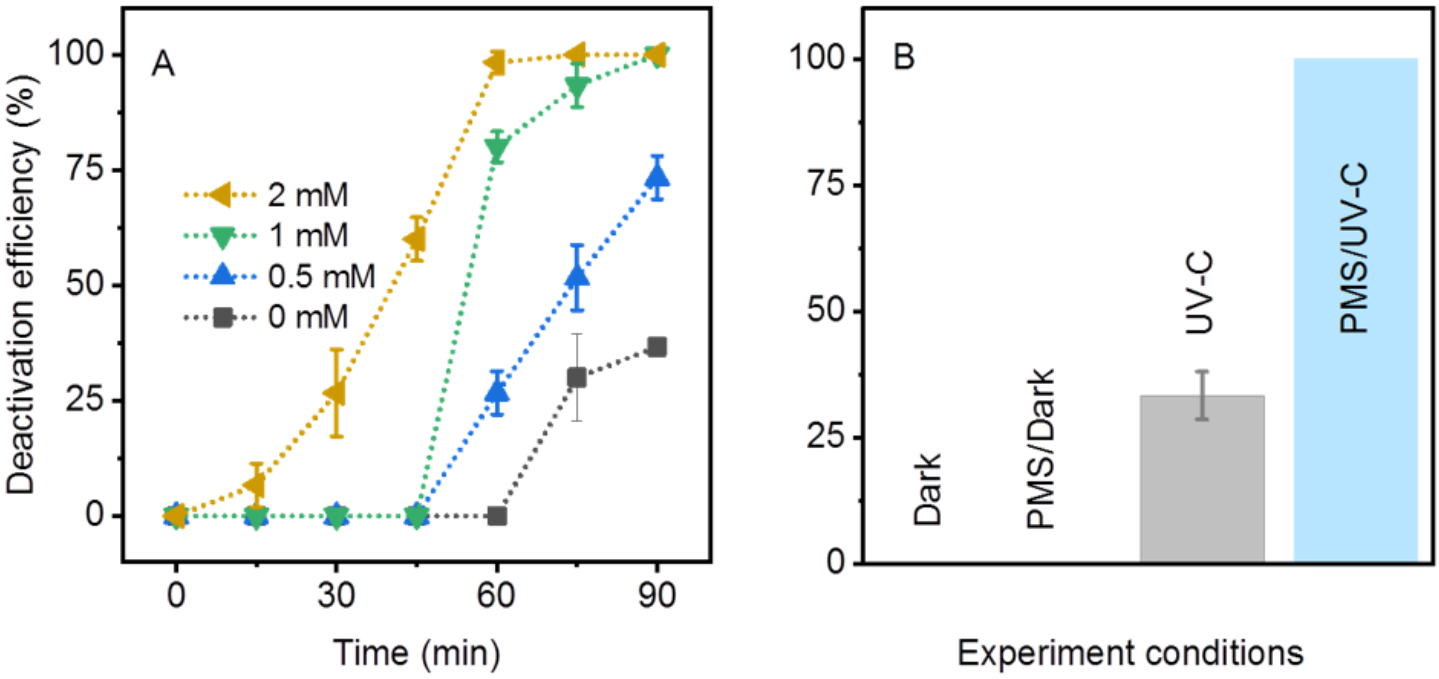
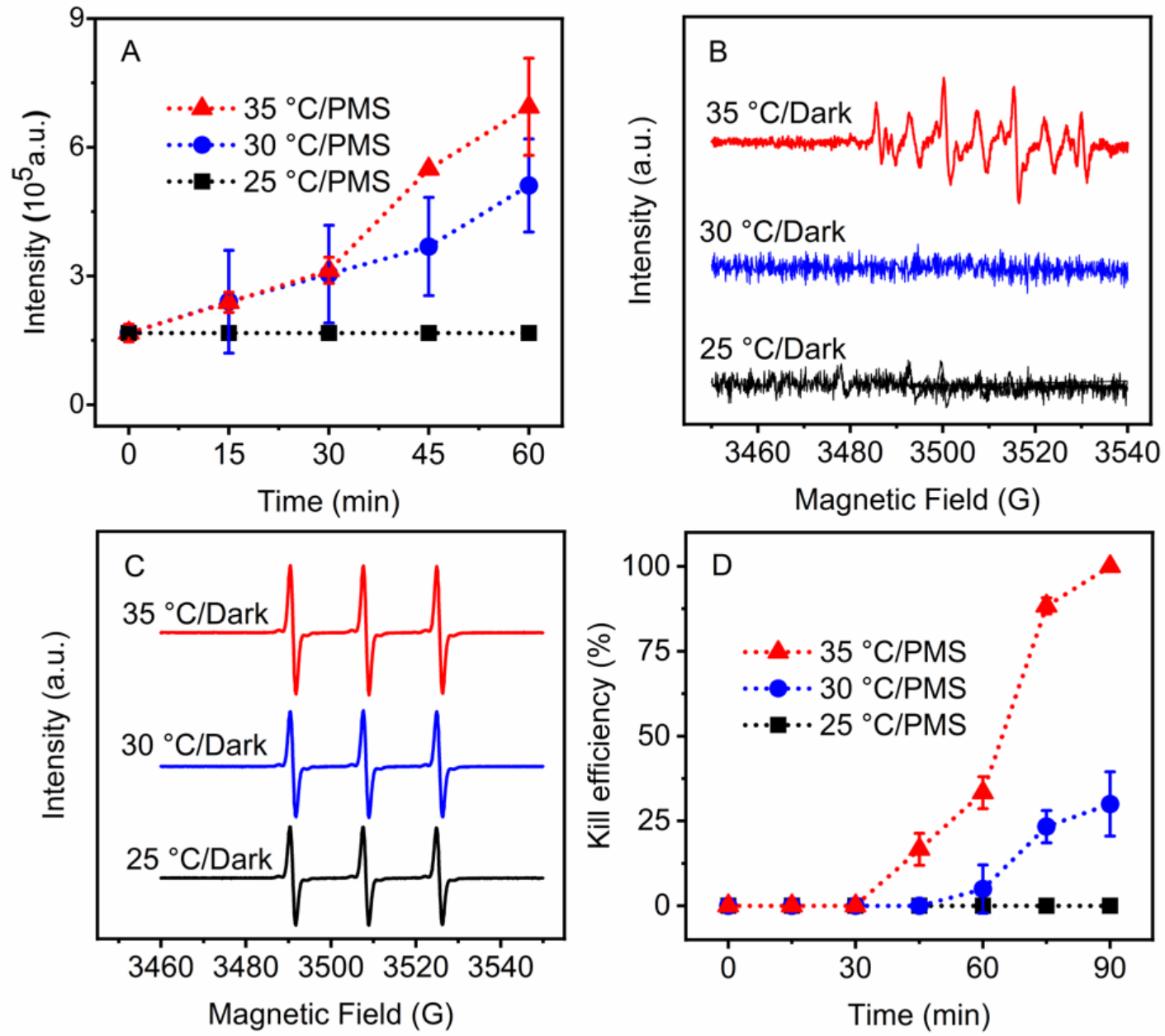



**Figure 1**

(a) C. elegans deactivation efficiencies in the PMS/UV-C system with different PMS concentrations; (b) nematode deactivation efficiencies under different conditions within 90 min. (Experimental conditions: number of nematodes: 30; [PMS] = 1 mM ; T = 25 ± 1 °C; [pH]0 ≈ 6.0.)




## Figure 2

(a) Nematode deactivation efficiencies at different temperatures; (b) fluorescence intensities of PMS solution with 1 mM coumarin at different temperatures; (c) EPR spectra obtained with DMPO and (d) EPR spectra obtained with TEMP after 20 min heating at different temperatures in the dark. (Experimental conditions: [PMS] = 1 mM; [pH]0 ≈ 6.0).

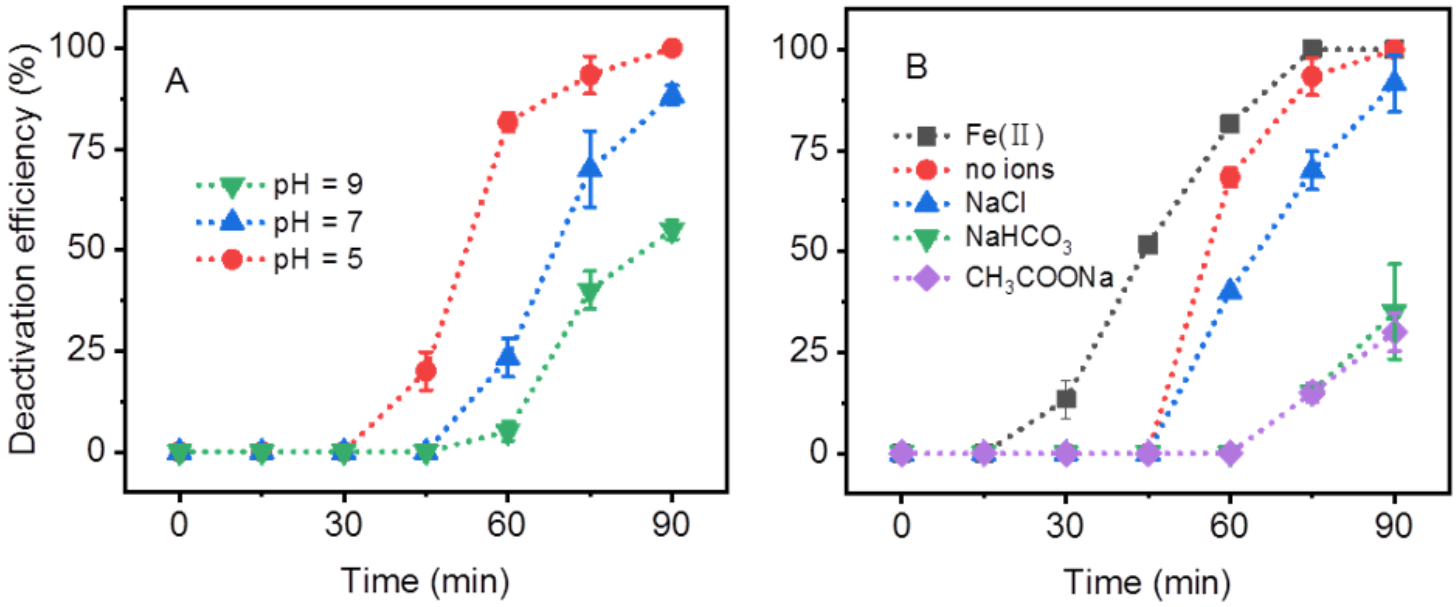



**Figure 3**

(a) Nematode deactivation efficiencies in the PMS/UV-C system: effects of different initial pH values; (b) effects of different ions on the bacterial deactivation efficiency in the PMS/UV-C system. (Experimental conditions: nematode numbers = 30; [PMS] = 1 mM; T = 25 ± 1°C; [pH]0 ≈ 6.0; [Fe2+] = 0.25 mg/L; [inorganic anions] = 0.01M.)

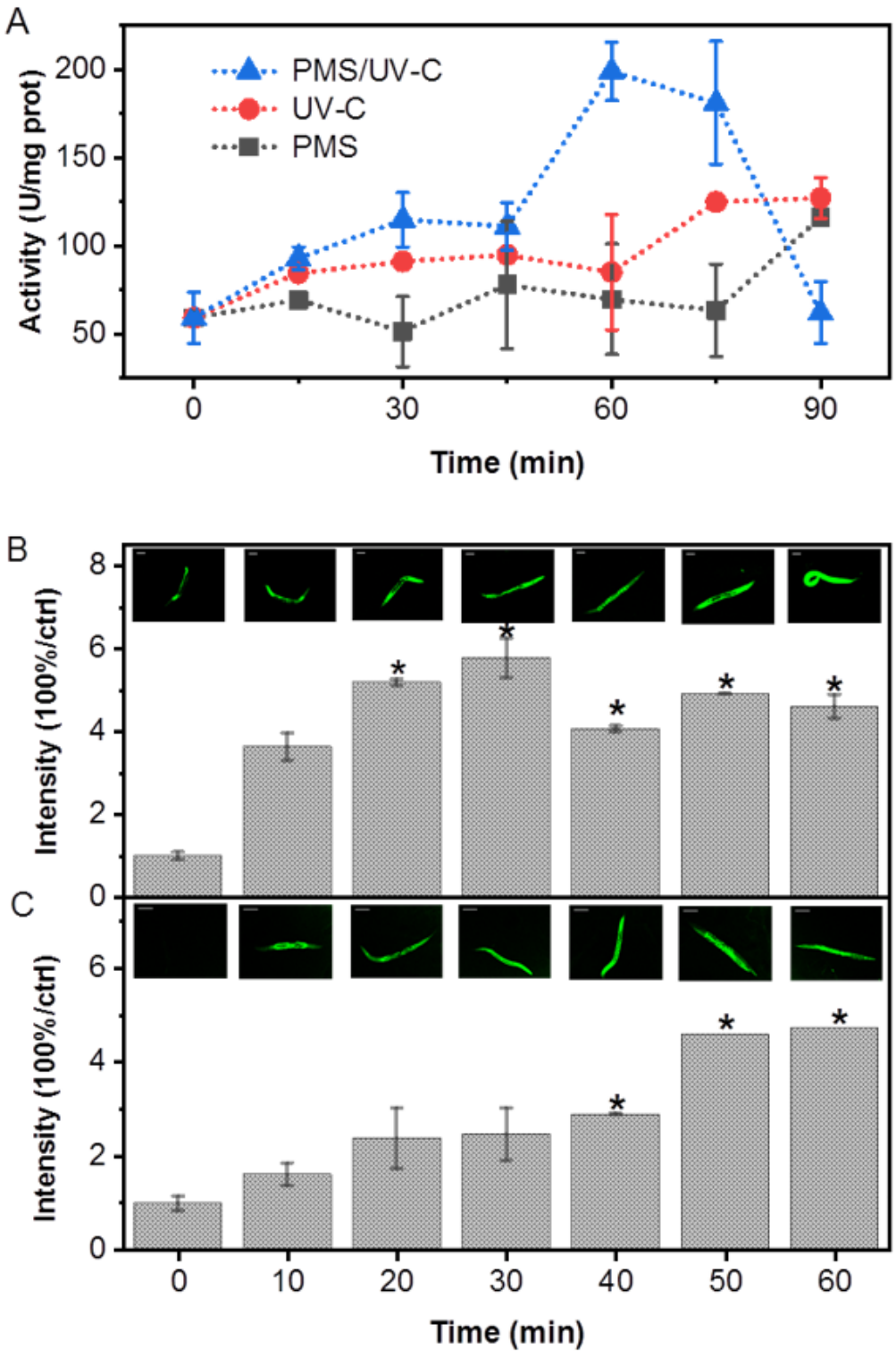



**Figure 4**

(a) SOD activity after different treatments; (b) effects of PMS/UV-C treatment on ROS production; (c) effects of PMS/UV-C treatment on the degree of cell apoptosis and were quantified by fluorescence intensity. The asterisks indicate significant differences between the treatment and control groups, *p < 0.05. (Experimental conditions: [PMS] = 1 mM; T = 25 ± 1°C; [pH]0 ≈ 6.0.)

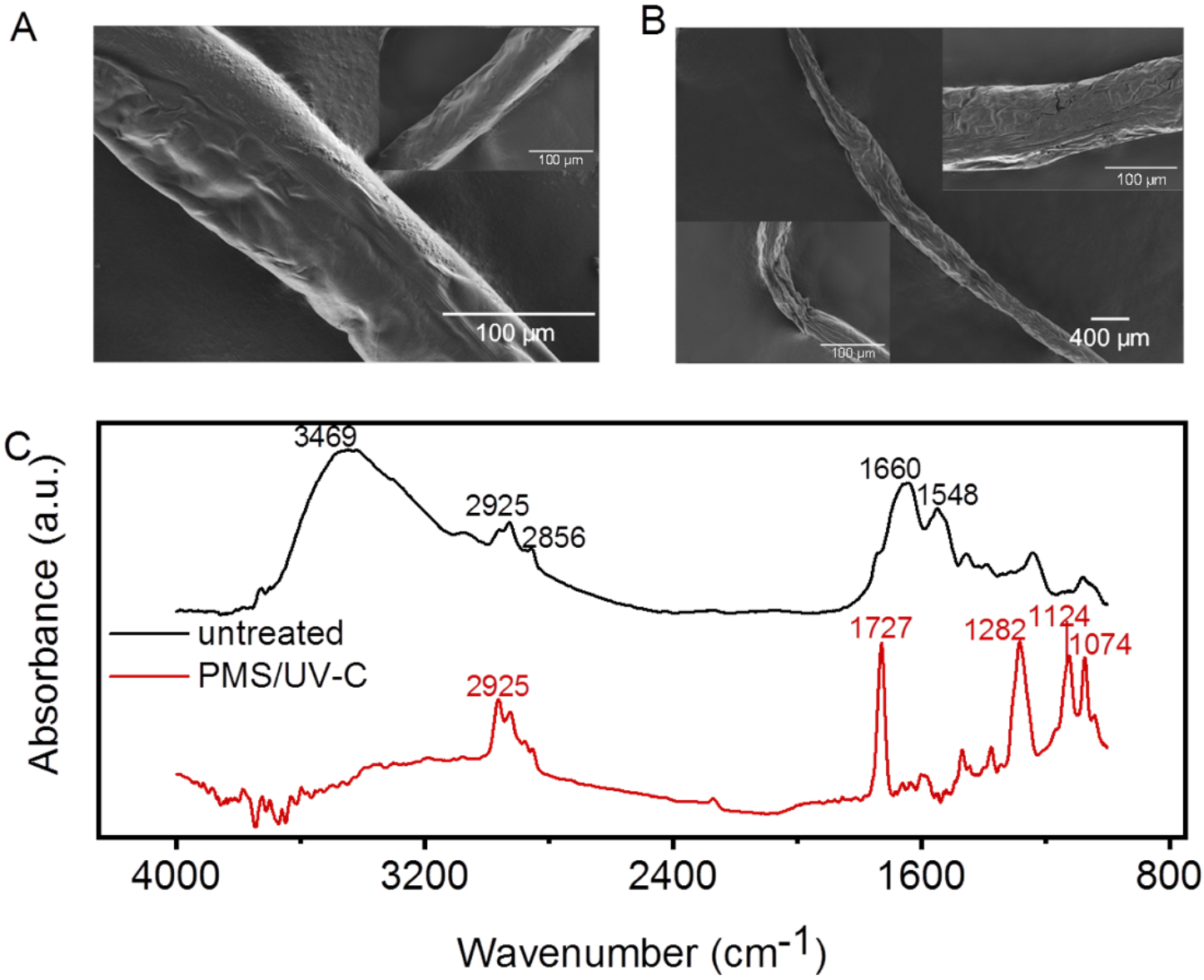



**Figure 5**

SEM images of nematodes: (a) untreated; (b) 90 min treatment of PMS/UV-C; (c) FTIR spectra of nematodes untreated or treated by PMS/UV-C for 90 min. (Experimental conditions: nematode numbers = 300; [PMS] = 1 mM; T = 25 ± 1°C; [pH]0 ≈ 6.0.)

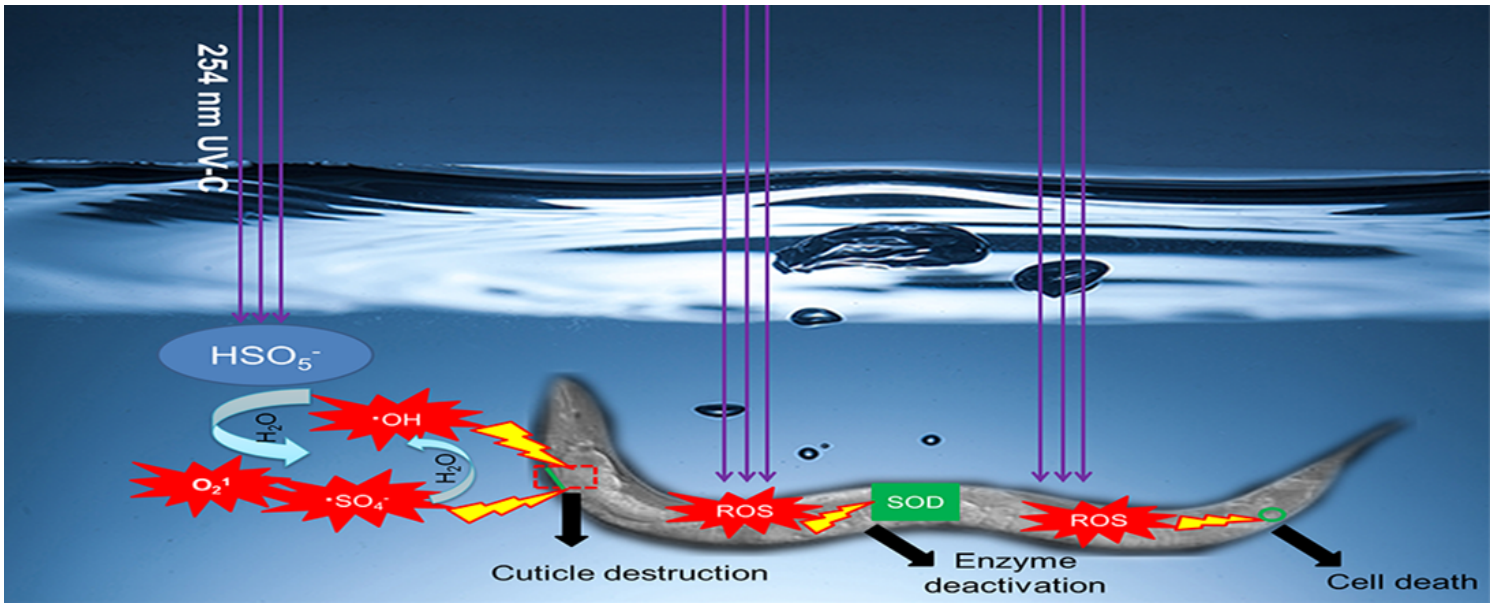



**Figure 6**

Schematic diagram of the nematode deactivation mechanism in the PMS/UV-C system.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supportinformation.docx
- Fig.S1.tif
- Fig.S2.tif
- Fig.S3.tif
- Fig.S4.tif
- Fig.S5.tif
- Fig.S6.tif