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Simulated sunlight-induced inactivation of tetracycline resistant bacteria and effects of dissolved organic matter

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ABSTRACT

The transmission of antibiotic resistance in surface water has attracted much attention due to its increasing threat to human health. The role of sunlight irradiation and the effect of dissolved organic matter (DOM) on the transmission of antibiotic resistance are still unclear. In this study, photo-inactivation of antibiotic resistant bacteria (ARB) was investigated using antibiotic resistant *E. coli* (AR *E. coli*) that contained the tetracycline resistance gene (Tc-ARG) as a representative. The results showed that AR *E. coli* underwent significant photo-inactivation due to the membrane damage induced by direct irradiation and by the generated reactive oxygen species. Simulated sunlight irradiation specifically suppressed the expression of tetracycline resistance, which is attributed to the destruction of tetracycline-specific efflux pump. Tetracycline inhibited the photo-inactivation of AR *E. coli* due to its selective pressure on tetracycline resistant *E. coli* and competitive light absorption effect. Suwannee River fulvic acid (SRFA), a representative DOM, promoted the inactivation of AR *E. coli* and further inhibited the expression of tetracycline resistance gene due to the generation of its excited triplet state, singlet oxygen, and hydroxyl radical. The extracellular Tc-ARG also underwent fast photodegradation under light irradiation and in the presence of SRFA, which leads to the decrease of its transformation efficiency. This study provided insight into the sunlight-induced inactivation of ARB, which is of significance for understanding the transmission of tetracycline resistance in surface water.

1. Introduction

Antibiotic resistance, as induced by the increased presence of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB), has been recognized as a worldwide environmental issue (Hvistendahl, 2012; Pruden et al., 2006). The presence of ARB, especially multiresistant bacteria, threatens the effectiveness of antibiotics against various life-threatening pathogens and causes global health concern (Pruden et al., 2012). Both ARB and their (mobile) ARGs have frequently been detected in various environments (Li et al., 2012; Luo et al., 2010; Mao et al., 2014; Zhu et al., 2013). Among these environmental media, aquatic environment represents a main reservoir of ARB and ARGs (Schwartz et al., 2003).

Different from traditional micropollutants, antibiotic resistance could be transmitted by both vertical gene transmission and horizontal gene transfer (HGT), occurring by transduction, transformation, and conjugation (Chen et al., 2019). Thus, although disinfection is commonly performed in present water treatment processes, and many advanced oxidation processes (AOPs) were developed and used to inactivate ARB (He et al., 2019; Michael-Kordatou et al., 2018), the released ARGs can induce the generation of ARB via HGT in receiving waters of the effluent as the transformation efficiencies of mobile DNA are very high (Baur et al., 1996; Stewart and Sinigalliano, 1990). The transmission of ARGs in natural waters can significantly promote the increase of ARB in terms of kinds and quantity, and subsequently increase the possibility of human exposure (Huijbers et al., 2015). There is therefore an urgent need to

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investigate the transmission behavior of ARB and ARGs in natural water.

The dissemination of ARGs in surface water was supposed to be a key pathway for the spread of ARB (Allen et al., 2010; Graham et al., 2011). Sunlight-induced degradation has been proven to be the main elimination pathway of micropollutants in surface water (Zhang et al., 2018a, 2019a). Meanwhile, sunlight-mediated inactivation of health-relevant microorganisms in surface water was also reported in previous studies (Nelson et al., 2018; Zeep et al., 2018). Once micropollutants and microorganisms have absorbed sunlight photons, especially UV-B (280–320 nm), they can undergo direct degradation and inactivation, respectively (Nelson et al., 2018; Zhang et al., 2018a) The photo-inactivation of bacteria is attributed to direct damage induced by UV irradiation (endogenous mechanism) and to photo-produced reactive intermediates (PPRIs) generated in the microorganism (indirect endogenous mechanism) (Nelson et al., 2018). The conjugative transfer frequency of ARB can be affected by simulated sunlight irradiation (Chen et al., 2019). Therefore, in surface water, sunlight irradiation can influence the antibiotic resistance of ARB.

Besides endogenous mechanisms, indirect inactivation of bacteria initiated by PPRIs from photoactive substances in natural water, i.e. exogenous mechanism, was also observed (Kohn et al., 2007; Rosado-Lausell et al., 2013). Dissolved organic matter (DOM) as a common constituent of water bodies (McKay et al., 2018; Page et al., 2011), has been indicated to be an important precursor of the PPRIs, including excited triplet state of DOM (^{3}DOM*), singlet oxygen ($^1O_2$) and hydroxyl radicals (HO$^\bullet$) (McKay et al., 2016; Wenk et al., 2011; Xu et al., 2011). In addition, DOM can be adsorbed on the outer membrane of *E. coli* (Chen et al., 2015; Tikhonov et al., 2013), and can sensitize the photodegradation of extracellular ARGs by generating PPRIs (Zhang et al., 2019b). Thus, it can be speculated that DOM can affect the sunlight-induced inactivation of ARB in surface water. Apart from this, antibiotics that coexist in natural water exhibit selective pressure towards the corresponding ARB (Zhang et al., 2015), and may also affect the sunlight-involved transmission of ARB. However, little is known about the effect of DOM and coexisting antibiotics on the expression of antibiotic resistance of ARB in sunlit surface water.

In this study, *E. coli* HB101 containing the tetracycline resistance gene was selected as model ARB (AR *E. coli*). Photo-inactivation of AR *E. coli* was investigated under simulated sunlight irradiation. Suwannee River fulvic acid (SRFA) was selected as a representative of DOM. The effect of SRFA and coexisting tetracycline on the photo-inactivation of AR *E. coli* was studied. The underlying mechanisms of the photo-inactivation were revealed by combining chemical and biological methods. Besides, the photodegradation of the extracellular tetracycline resistance gene (Tc-ARG) was also investigated for further understanding of the transmission behavior of ARGs in surface water.

2. Materials and methods

2.1. Materials and preparation of ARB

E. coli HB101 competent cells were purchased from the Takara Biotechnology Co. Ltd. (Dalian, China). pBR322 plasmid (500 ng/μL, 4361 bps, NCBI GenBank NO. J01749.1) containing tetracycline resistance gene (Tc-ARG, *tetA*, 1261 bps) was purchased from ThermoFisher Scientific Inc. Suwannee River fulvic acid (SRFA, 2S101F) was purchased from the International Humic Substances Society. Other chemicals, reagents, materials, and corresponding commercial suppliers are listed in Text S1 in the Supporting Information (SI).

The ARB used in this study (AR *E. coli*) was prepared by transforming the pBR322 plasmid into *E. coli* HB101 competent cells. The detailed methods are described in Text S2 in the SI.

2.2. Simulated sunlight irradiation experiments

A water cooled 1000 W xenon lamp equipped with 290 nm filters was used to mimic the UV-A and UV-B portions of sunlight. The irradiation experiments were performed with an XPA-7 merry-go-round photochemical reactor (Xujiang Electromechanical Plant, Nanjing, China) with quartz tubes containing the aqueous suspensions. The light intensity at the surface of the quartz tubes was detected with a TriOS-RAMSES spectroradiometer (TriOS GmbH, Germany), and the result is shown in Fig. S1 in the SI. The total light intensity from 290 to 450 nm of the light source in the position of quartz tubes was calculated to be 11.4 mW cm^{-2}, which is slightly lower than that of sunlight (12.8 mW cm^{-2}) measured at noon in mid-summer in Dalian, China. The initial concentration of AR *E. coli* is 1×10^8 CFU mL^{-1} in phosphate buffer solution (PBS, pH 7.0) with a volume of 25 mL. During the irradiation experiments, the temperature was controlled at (25 $\pm$ 1) °C using a constant-temperature liquid-circulating apparatus.

Experiments were also performed to determine the photodegradation kinetics of extracellular ARGs using plasmid pBR322 which contains the tetracycline resistance gene (Tc-ARG). The initial concentration of plasmid pBR322 was 5 μg mL^{-1} in solutions with a volume of 8 mL. SRFA was added to investigate the effect of DOM with an initial concentration of 10 mg L^{-1} (4.8 mgC L^{-1}). The concentration of SRFA was selected to be the average concentration of the determined concentration levels of the natural water samples in our previous study (Zhang et al., 2018a) and tetracycline with different initial concentrations (0.01, 0.1, 1, and 10 mg L^{-1}) was added in some solutions to investigate the effect of antibiotics on the photo-induced inactivation of AR *E. coli*. The steady-state concentrations of PPRIs, including excited triplet state of SRFA (^{3}SRFA*), $^1O_2$, and HO$^\bullet$ in the SRFA solutions (without AR *E. coli*) were determined using 2,4,6-trimethylphenol, furfuryl alcohol, and benzene as chemical probes (Zhou et al., 2018). These PPRIs have been proved to induce the inactivation of *E. coli* (Serna-Galvis et al., 2018).

2.3. Inactivation kinetics of AR E. coli and photodegradation kinetics of Tc-ARG

Throughout the irradiation experiment, 1 mL of the AR *E. coli* suspension was withdrawn periodically. The number of AR *E. coli* (N) was counted using the dilution plate counting method that was conducted on LB medium supplemented with tetracycline (selective medium). The *E. coli* with tetracycline resistance was counted using the selective medium. Meanwhile, counts of total *E. coli* (with and without tetracycline resistance) were also performed on LB medium without addition of tetracycline (regular medium). The detailed method is described in Text S3 in the SI). Both the inactivation kinetics of AR *E. coli* and total *E. coli* were determined.

As to the photodegradation kinetics of Tc-ARG, 100 μL of pBR322 solution was withdrawn periodically, real-time quantitative polymerase chain reaction (RT-qPCR) was used to determine the exact concentration of Tc-ARG, and PCR-agarose gel electrophoresis was also used to detect the degradation of Tc-ARG (details are shown in Text S4 in the SI). The photodegradation rate constant of Tc-ARG was observed by fitting the $\ln C/C_0$ versus time, with C being the concentration at a given time point and C_0 the initial concentration. Transformation experiments were performed to investigate the HGT efficiency of degraded extracellular Tc-ARG with method described in previous study (Zhang et al., 2019b). The detailed method is shown in Text S3 in the SI.

Besides, the photodegradation kinetics of tetracycline were also determined with the analysis method shown in Text S5 in the SI, and the results are shown in Fig. S2 in the SI.

2.4. Investigation of the inactivation mechanisms

The bacterial activity was determined using fluorescent staining method with a fluorescein diacetate/propidium iodide (FDA/PI) as the probes (Jones and Senft, 1985). The non-fluorescent probe FDA can enter the bacterial cell, and is subsequently decomposed by non-specific esterase, which leads to the formation of green fluorescein. On the contrary, PI can only enter bacterial cells with a seriously damaged membrane, and can be stained red. The fluorescence was obtained with a fluorescence microscope (AX8635, Olympus). After treatment with FDA/PI, the bacteria with green fluorescence are alive and with integrated cell membrane. On the contrary, the bacteria with red fluorescence have a severely damaged cell membrane, and are considered to be inactive.

The morphology of AR *E. coli* was determined using field emission scanning electron microscopy (FESEM, XL-30 ESEM FEG, FEI Company) at 20 kV, and the detailed method are shown in Text S6 in the SI. To further investigate the damage of the cell membrane of AR *E. coli* during simulated sunlight irradiation, the concentration of intracellular K^+ leaked from the bacteria was determined using ICP-OES (PerkinElmer Avio200) (details are shown in Text S6 in the SI).

Intracellular ROS were responsible for the oxidative damage of enzymes and DNA, which will lead to the inactivation of bacteria (Rahmanto et al., 2012). Thus, the intracellular ROS (mainly $HO^{\bullet}$ and H_2O_2) level in the simulated sunlight treated AR *E. coli* was determined with 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) as a fluorescent probe (Sun et al., 2014, 2016). The detailed method is described in Text S7 in the SI. Briefly, the non-fluorescent DCFH-DA could enter bacterial cells. It is then hydrolyzed by intracellular esterase, and subsequently oxidized by intracellular ROSs. This generates fluorescent 2′,7′-dichlorodihydrofluorescein (DCF), the fluorescence intensity of which was determined using a microplate reader (Synergy HTX, BioTek, USA).

The concentrations of extracellular, intracellular and total Tc-ARG of AR *E. coli* during the simulated sunlight irradiation were determined using RT-qPCR and PCR-agarose gel electrophoresis. The DNA was extracted using the Ezup Column Bacteria Genomic DNA Purification Kit (Sangon Biotech), followed by the DNA extraction procedures. The detailed detection method of Tc-ARG is described in Text S4 in the SI. *Re*-sequencing analysis of Tc-ARG in AR *E. coli* during the simulated sunlight irradiation was performed on an Illumina HiSeq2000™ by Personal Gene Technology Co., Ltd. (Shanghai, China) to further reveal the inactivation mechanism. The proteins in AR *E. coli* were detected using SDS-PAGE gel electrophoresis (Kinoshita-Kikuta et al., 2019).

2.5. Statistical analysis

To ensure the accuracy of the experiment results, each experiment was performed at least thrice. The error bars represent 95% confidence intervals for $n = 3$. The relative error bars represent the standard deviation from the mean value. The Student's *t*-test (two-tailed) was used to determine the significance of the differences between treatments. Differences were considered to be significant at the 95% confidence level ($p < 0.05$).

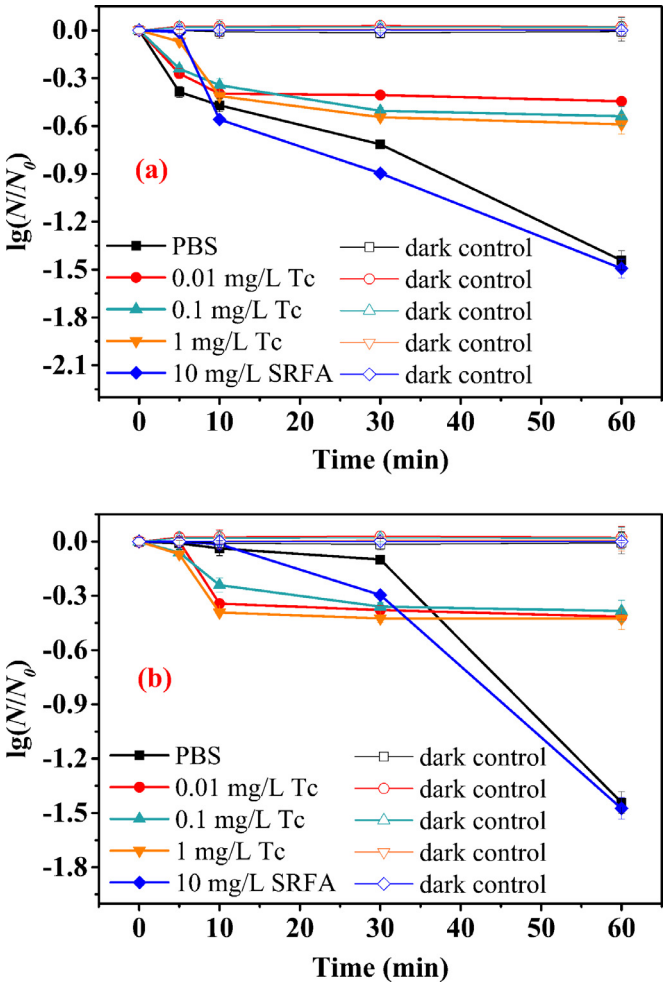

Fig. 1. Photo-inactivation kinetics AR *E. coli* in PBS solutions with different concentration of tetracycline (Tc) and in SRFA solution at pH 7.0 (N is the colony forming units at a given time point and N_0 (1×10^8 CFU mL^{-1}) is the initial colony forming units of the *E. coli* that counted on (a) selective medium; (b) regular medium).

3. Results and discussion

3.1. Photo-inactivation of AR E. coli

Significant inactivation of AR *E. coli* was observed during simulated sunlight irradiation (Fig. 1), and few *E. coli* were alive after 60 min of treatment (Fig. S3 in the SI). No obvious loss of AR *E. coli* was observed in the dark controls, indicating that light irradiation is the driving force for the inactivation of AR *E. coli*. In PBS, the AR *E. coli* underwent fast inactivation, especially at the initial 10 min (Fig. 1(a)), indicating that sunlight irradiation can directly induce the photo-inactivation of *E. coli* with tetracycline resistance. The inactivation rate of total *E. coli*, which was counted in regular LB medium, is lower compared to the inactivation rate of AR *E. coli* (Fig. 1(b)). Sunlight mediated inactivation of non-antibiotic resistant *E. coli* was also reported in previous studies and was mainly attributed to the DNA damage initiated by UVA and UVB portions of sunlight (Kadir and Nelson, 2014; Probst-Rüd et al., 2017).

The activity of *E. coli* during light irradiation was also detected using the FDA/PI fluorescent staining method (Tawakoli et al., 2013), and the results are shown in Fig. 2. Almost all *E. coli* were alive and with integrated cell membrane within 30 min of treatment. All the bacteria were stained red after 60 min of irradiation, implying that both inner and outer membranes were damaged and few bacteria were survived. These results are in accordance with

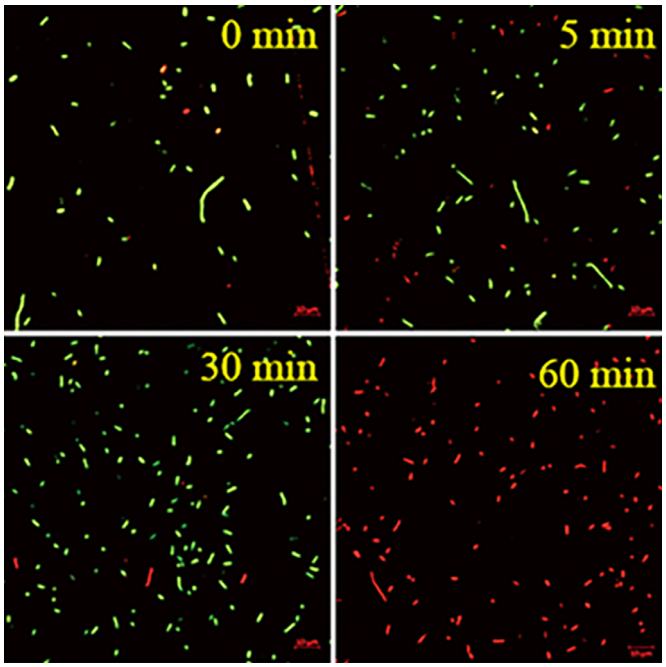

Fig. 2. Fluorescent microscope images obtained with FDA/PI staining during simulated sunlight irradiation of AR *E. coli* (N_0: 1×10^8 CFU mL^{-1}) at pH 7.0.

the results obtained using the plate counting method (Fig. S3 in the SI). The total *E. coli* that counted in the regular medium included *E. coli* with antibiotic resistance (i.e. AR *E. coli*) and *E. coli* that lost the antibiotic resistance. Although many *E. coli* are alive in regular medium, only little *E. coli* are alive in selective medium after light treatment from 5 to 30 min (Fig. 2 and Fig. S3). Thus, simulated sunlight irradiation can not only induce the death of *E. coli* but also preferentially inhibits the expression of antibiotic resistance. It can be concluded that sunlight irradiation exhibits specific effects on ARB.

In the presence of tetracycline, the inactivation of AR *E. coli* was significantly inhibited (Fig. 1(a)). However, no significant difference of the inhibitory effect was observed with the increase of tetracycline concentration (Fig. 1(a)). On the contrary, tetracycline promoted the inactivation of total *E. coli* during the first 30 min (Fig. 1(a)). Contrary to the observation in PBS without tetracycline, the inactivation rate of total *E. coli* showed no obvious difference compared with that of AR *E. coli* in the presence of tetracycline. These findings can be attributed to the selective pressure of antibiotics on the proliferation of ARB (Rysz et al., 2013) as antibiotics exert selective pressure, which leads to the increase of the proportion of plasmid-bearing ARB (Löser, 1995). Besides, the competitive light absorption effect between tetracycline and AR *E. coli* can also inhibit the photo-inactivation of AR *E. coli*. Thus, the presence of tetracycline inhibited the inactivation of AR *E. coli*.

3.2. Inactivation mechanisms of AR E. coli induced by simulated sunlight irradiation

Three endogenous mechanisms were proposed for sunlight-induced inactivation of bacteria, including membrane damage, genome damage, and cytosolic protein damage (Nelson et al., 2018; Xiao et al., 2019). For the first mechanism, the morphologies of AR *E. coli* were observed with SEM. As can be seen in Fig. 3(a), the *E. coli* cells were deformed significantly under simulated sunlight irradiation, indicating that the damage of the outer membrane for AR *E. coli* occurred indeed.

It was considered that K^+ leakage would be caused by any damage in the cell membrane structure, especially inner membrane (Liu et al., 2019). Significant K^+ leakage of AR *E. coli* was indeed observed during simulated sunlight irradiation, whereas no obvious leakage of K^+ was detected in the dark controls (Fig. 3(b)). Thus, solar irradiation can induce the damage of the membrane of ARB in surface waters. The leakage rate of K^+ was especially high in the first 10 min, which is supposed to be the cause of the fast inactivation of AR *E. coli* at the initial 10 min (Fig. 1(a)). The ROSs generated in the cells are important driving forces for the observed membrane damage (Xiao et al., 2019). During simulated sunlight irradiation, a significant increase of levels of ROSs (mainly $HO^\bullet$ and H_2O_2) was observed, especially in the last 30 min (Fig. 4). The intercellular $HO^\bullet$ can induce DNA damage and protein damage in the bacteria due to its high reactivity (Nelson et al., 2018). This leads to the fast inactivation of total *E. coli* from 30 to 60 min (Fig. 1).

The pBR322 plasmid containing Tc-ARG was extracted and detected using RT-qPCR. The results showed that the concentration of extracellular Tc-ARG (e-ARG), intercellular Tc-ARG (i-ARG), and total Tc-ARG (total-ARG) are all maintained steady no matter whether under light irradiation or in dark controls (Fig. 5). This finding was confirmed by the results obtained using PCR-agarose gel electrophoresis (Fig. S4). These results are in accordance with a previous study which showed that no obvious damage or leakage of intracellular ARG was observed even though there are heavy membrane damage and even though inactivation of *E. coli* occurred during ozone treatment (Czekalski et al., 2016).

Sunlight irradiation can directly induce damages to DNA (Giannakis et al., 2016). Thus, re-sequencing analysis of Tc-ARG during the simulated sunlight irradiation was performed to investigate its potential mutation and deletion induced by light irradiation and the generated ROSs. The results showed that only one base mutation at position 1129 (C was replaced by T, Fig. S5) was observed. However, the coding amino acids of CTG and TTG are both leucine. Therefore, the mutation of Tc-ARG induced by light irradiation does not affect the transcription process. This was also proven by the RNA expression analysis results as the expression levels showed no obvious change during the simulated sunlight irradiation (Fig. 6). Thus, it can be concluded that the damage of Tc-ARG in bacteria is not the main reason for the inactivation of AR *E. coli* under simulated sunlight irradiation.

The cytosolic protein of AR *E. coli* was detected during simulated sunlight irradiation, and the results are shown in Fig. 7. No obvious damage was observed for the proteins of AR *E. coli*. Thus, cytosolic protein damage is also not the dominant reason for the inactivation of AR *E. coli* during the light irradiation.

According to the above analysis, it can be concluded that the inactivation of AR *E. coli* induced by simulated sunlight irradiation is mainly attributed to membrane damage. As reported in previous studies, the resistance to tetracycline in gram-negative bacteria (e.g. *E. coli* used in this study) is usually attributed to three different mechanisms: tetracycline-specific efflux pumps (mainly *tetA, tetB, tetC, tetG, tetH*), ribosomal protection proteins (mainly *tetM, tetO, tetQ, tetT* and *tetW*), and enzymatic inactivation of tetracycline (*tetX*) (Alekshun and Levy, 2007; Nolivos et al., 2019; Zhao et al., 2018). Thus, in this study, efflux by tetracycline-specific pumps is the dominant mechanism for tetracycline resistance as AR *E. coli* with *tetA* was used as target ARB. A specific membrane-associated efflux protein plays important role in the efflux system (Møller et al., 2020). Therefore, the severe damage of the cell membrane of AR *E. coli* induced by simulated sunlight irradiation depressed the expression of tetracycline resistance gene by damaging the efflux system.

As shown above, the presence of tetracycline inhibited the photo-inactivation of AR *E. coli* via two mechanisms: selective pressure and competitive light absorption. For the former, the synthesis

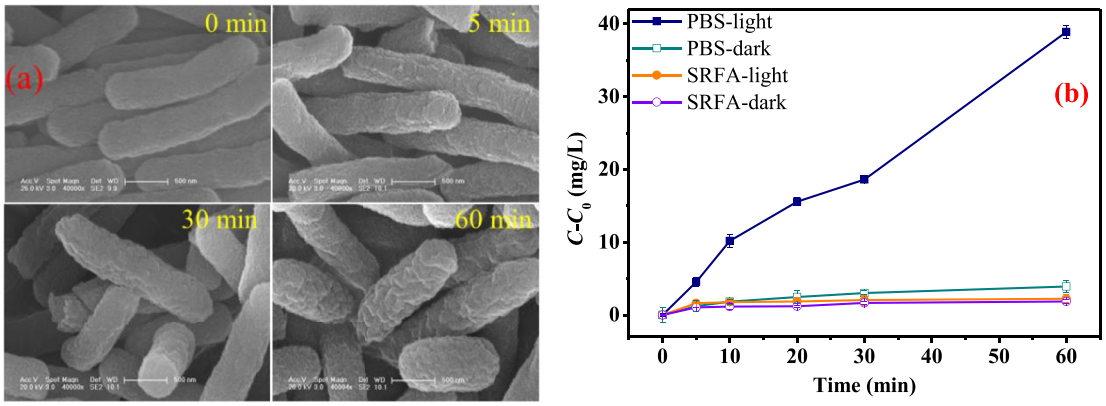

Fig. 3. (a) SEM images of AR *E. coli* treated by simulated sunlight irradiation in PBS (pH 7.0); (b) the leakage of K^+ in PBS and in SRFA solutions (10 mg L^{-1}) at pH 7.0.

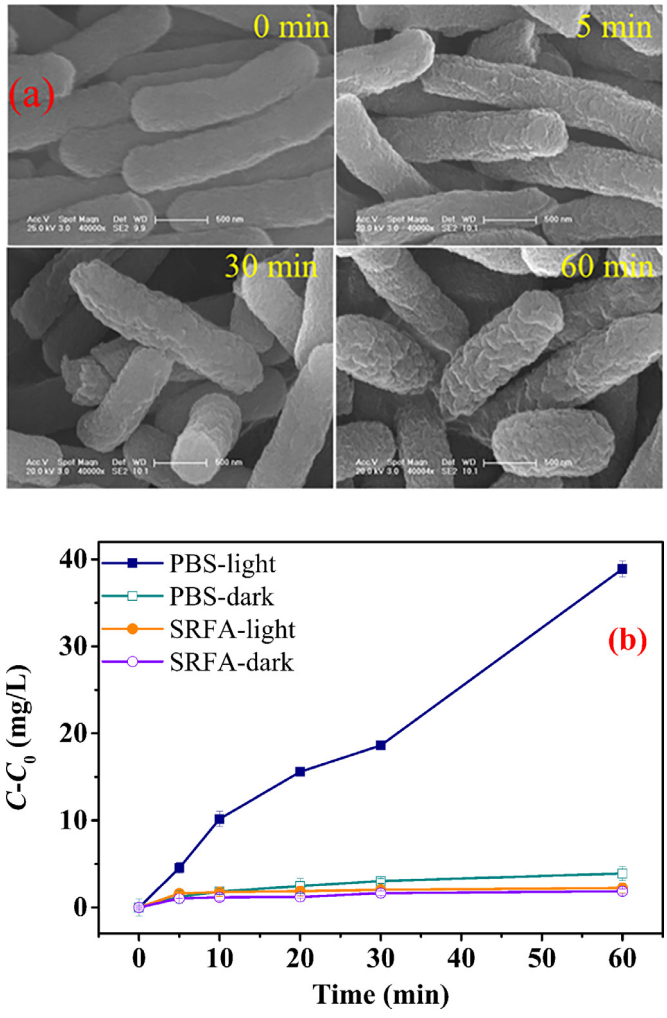

Fig. 4. Relative concentrations of ROSs in AR *E. coli* (N_0: 1 × 10^8 CFU mL^{-1}) during simulated sunlight irradiation in PBS and in SRFA solutions (10 mg L^{-1}) at pH 7.0.

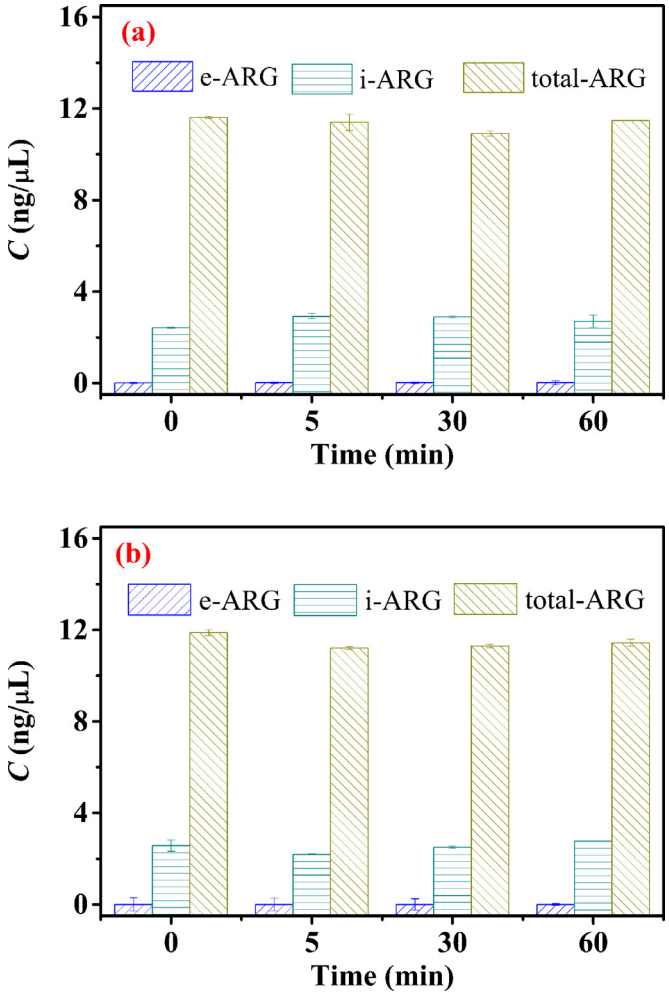

Fig. 5. Concentrations of extracellular Tc-ARG (e-ARG), intercellular Tc-ARG (i-ARG), and total Tc-ARG (total-ARG) extracted from AR *E. coli* (N_0: 1 × 10^8 CFU mL^{-1}) in PBS (pH 7.0) during simulated sunlight irradiation (a) and in dark controls (b).

of the specific membrane protein in the efflux system is inducible by tetracyclines (Alekshun and Levy, 2007). Thus, the presence of tetracycline can induce the synthesis or repair of the specific membrane protein due to its selective pressure on AR *E. coli*, which is beneficial for the expression of tetracycline resistance gene. For the latter, tetracycline can competitively absorb photons with AR *E. coli*, which decreases the photo-inactivation rate of AR *E. coli*.

3.3. *Effects of SRFA on the inactivation of AR* E. coli

The presence of SRFA obviously inhibited the inactivation of AR *E. coli* in the first 10 min, and promoted the inactivation in the following 50 min (Fig. 1(a)). For the total *E. coli*, SRFA exhibited no obvious influence on its inactivation at the first 10 min and subsequently enhanced the inactivation of *E. coli* (Fig. 1(b)). The

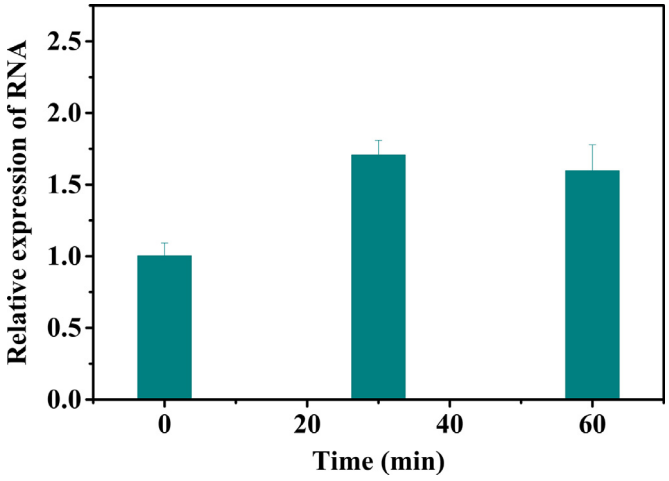

Fig. 6. Relative expression of RNA from Tc-ARG that extracted from AR *E. coli* (N_0: 1×10^8 CFU mL^{-1}) during simulated sunlight irradiation at pH 7.0.

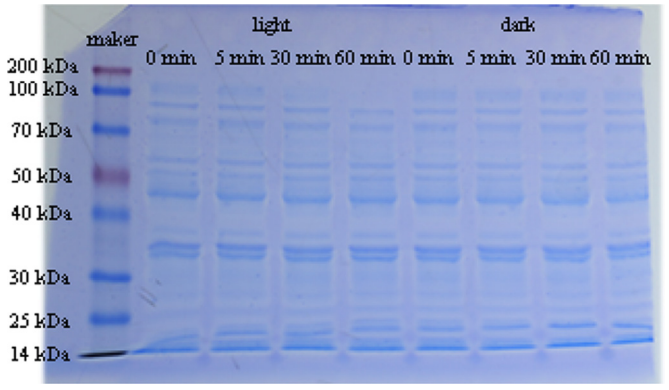

Fig. 7. Cytosolic protein extracted from AR *E. coli* (N_0: 1×10^8 CFU mL^{-1}) during simulated sunlight irradiation at pH 7.0.

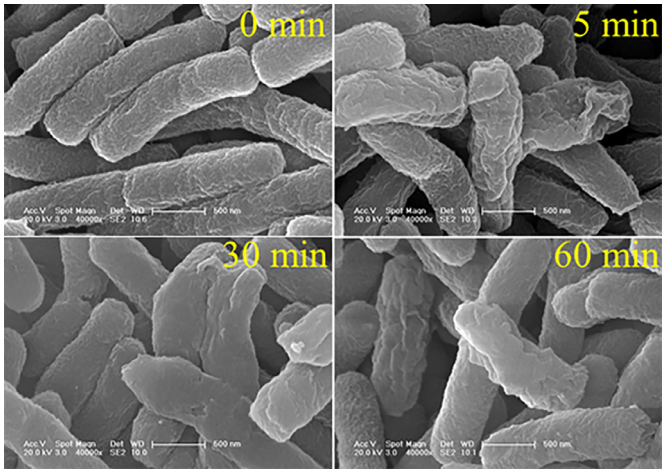

Fig. 8. SEM images of AR *E. coli* (N_0: 1×10^8 CFU mL^{-1}) in SRFA suspension (10 mg L^{-1}) under simulated sunlight irradiation at pH 7.0.

leakage of K^+ of AR *E. coli* in SRFA solutions was determined, and the results showed that different from the observation in PBS, the K^+ leakage of AR *E. coli* is completely inhibited in SFRA solutions (Fig. 3(b)), which is indicative of a specific effect of SRFA on the membrane damage of AR *E. coli*.

As can be seen in the SEM images (Fig. 8), the morphologies of AR *E. coli* were significantly affected even before light irradiation (at 0 min), and deeper sinking and shriveled cell membranes were observed compared with the membranes in PBS (Fig. 3(a)). As reported in a previous study, DOM can be adsorbed on the cell membrane of AR *E. coli*, which inhibited the tetracycline diffusion into the cells (Chen et al., 2015). The adsorption of SRFA on the AR *E. coli* cell membrane was evaluated by determining the dissolved concentration of SRFA. The results showed that the freely soluble concentration of SRFA decreased and reached an adsorption equilibrium within 3 h (Fig. S6). The adsorption of SRFA severely inhibited the leakage of K^+ as DOM exhibits a negative charge in suspensions at pH 7.0 due to the deprotonation of carboxyl and phenolic groups (Mensch et al., 2017).

Dual roles of DOM on the photo-inactivation of *E. coli* were reported in previous (Serna-Galvis et al., 2018). SRFA can inhibit the direct photo-inactivation of *E. coli* through light shielding, and can promote the photo-inactivation by generating reactive species. The generation of intercellular ROS was promoted in the presence of SRFA compared with irradiation in PBS (Fig. 4). The generation of PPRIs from SRFA under simulated sunlight irradiation has been shown in previous studies (Shang et al., 2017; Wenk et al., 2011; Zhang et al., 2014). The steady-state concentrations of $^3SRFA^*$, $^1O_2$, and $HO^\bullet$ in the SRFA solutions were determined to be $(2.41 \pm 0.05) \times 10^{-13}$ M, $(5.66 \pm 0.07) \times 10^{-13}$ M, $(5.14 \pm 0.03) \times 10^{-17}$ M, respectively. These PPRIs are the main reason for the increased shriveling of cell membrane as shown in Fig. 8.

Besides, $^3DOM^*$, $^1O_2$, and $HO^\bullet$ can induce the photo-inactivation of bacteria through both endogenous and exogenous pathways (Nelson et al., 2018; Serna-Galvis et al., 2018). Among these PPRIs, $^1O_2$ is of the highest concentration, and was proven to be an effective reactive species that induce the photo-inactivation of bacteria via exogenous mechanisms (Nelson et al., 2018). Thus, the promotional effect of SRFA on the photo-inactivation of AR *E. coli* is supposed to be mainly attributed to $^1O_2$.

The generated PPRIs from the adsorbed SRFA by AR *E. coli* can enter the cells as the cell membrane was damaged during light irradiation, which leads to the increase of intracellular ROS level. However, the increase of ROS level in the cells did not promote the damage of Tc-ARGs as no obvious changes of e-ARG, i-ARG, and total-ARG was observed in SRFA solutions during simulated sunlight irradiation (Fig. S7). Thus, similar to simulated sunlight irradiation induced direct inactivation, SRFA promoted the inactivation of AR *E. coli* also via a membrane damage mechanism initiated by the generated PPRIs.

Similar with the findings in PBS solutions, the inhibitory effect of SRFA on the expression of tetracycline resistance gene is also attributed to the damage of the efflux system induced by the generated ROS. Besides, there is a larger hydrophilic cytoplasmic region in the middle of the specific protein in the efflux system (Levy, 1992), which is beneficial to the occurring of protein-damage as initiated by the generated ROS from SRFA in the aqueous phase. Therefore, the presence of SRFA further inhibited the expression of tetracycline resistance gene. These findings demonstrate that DOM plays a negative role in the transmission of tetracycline resistance in surface water.

3.4. Photo-induced degradation of cell-free Tc-ARG

The inactivation of ARB will eventually lead to the leakage of ARGs. Cell-free ARGs, especially plasmid-coded ARGs, play important roles in the transmission of antibiotic resistance through HGT (Mao et al., 2014; Zhang et al., 2018b). Thus, to further investigate the transmission behavior of ARGs in surface waters, simulated sunlight irradiation induced degradation and HGT efficiency change of extracellular Tc-ARG was studied. The results shown in Fig. 9(a) indicate that Tc-ARG undergoes fast photodegradation in PBS with an observed photolysis rate constant (k_{obs}) of (0.030 ± 0.005)

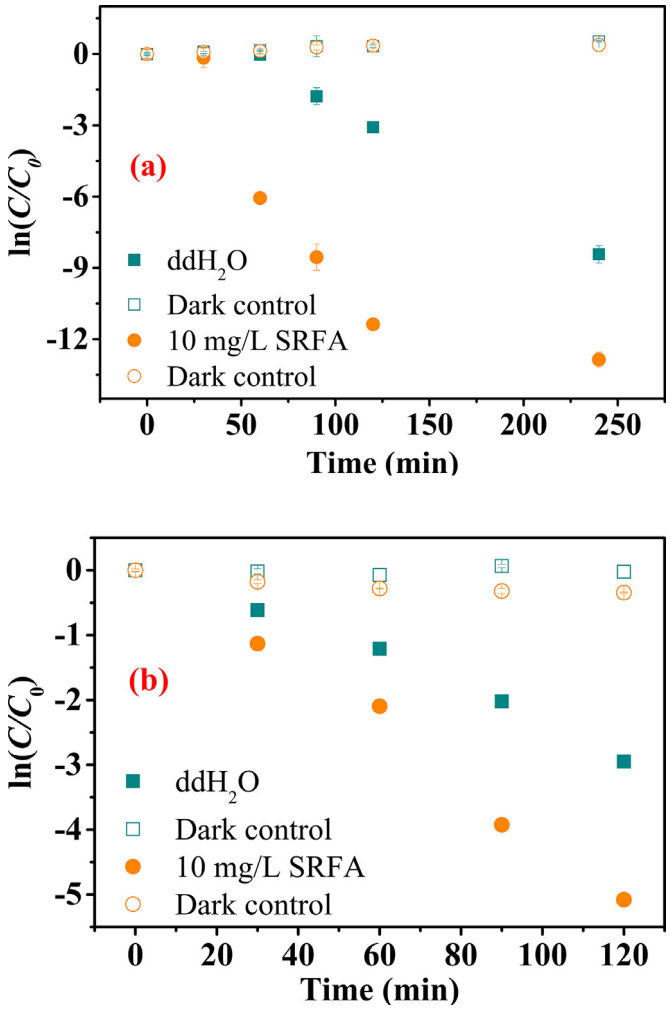

Fig. 9. Photodegradation kinetics of Tc-ARG (C_0: 5 μg mL^{-1}) (a) and changes in transformation efficiencies (b) at pH 7.0.

min^{-1}, which is different from in AR *E. coli* that no significant degradation of Tc-ARG was observed (Fig. 5). This is attributed to the complex components in the bacteria. The direct irradiation and generated ROSs firstly act on the cell membrane of *E. coli*, leading to its severe damage as shown above.

The presence of SRFA (10 mg L^{-1}) significantly promoted the photodegradation of Tc-ARG (Fig. 9(a)) and the k_{obs} increased to (0.067 ± 0.009) min^{-1}. These results are in accordance with the results obtained using a 500 W medium mercury lamp as illuminant in a previous study with the promotional effect of SRFA proposed to be attributed to photo-generated HO$^{\bullet}$ and $^1O_2$ (Zhang et al., 2019b).

The transformation efficiencies of Tc-ARG that coded on pBR322 plasmid were significantly inhibited and the inhibitory effects are enhanced by an increasing time of irradiation (Fig. 9(b)). Similar with the photodegradation of Tc-ARG, SRFA also promoted the decrease of transformation efficiencies (Fig. 9(b)). It is therefore to be concluded that the photodegradation of Tc-ARG in surface water could leads to the decrease of HGT ability and subsequently inhibits the spread of ARGs in natural waters.

However, the presence of SRFA also decreased the transformation efficiencies (about 50% decrease) of Tc-ARG in the dark controls although no obvious degradation of Tc-ARG was observed without light irradiation (Fig. 9). This can be attributed to the adsorption of SRFA on the cell membrane of *E. coli* as proven above. The adsorbed SRFA on the membrane inhibits the transformation of pBR322 into the competent cells of *E. coli*.

4. Conclusions

Simulated sunlight irradiation can induce significant photo-inactivation of AR *E. coli*. Light irradiation induced direct damage and the intracellular ROSs initiated damage of cell membranes is the main reason for the photo-inactivation of AR *E. coli*. Specific inhibitory effect of simulated sunlight irradiation on the expression of tetracycline resistance gene is attributed to a mechanism involving the tetracycline-specific efflux pump. The presence of tetracycline inhibited the photo-inactivation of AR *E. coli* and protected the expression of tetracycline resistance gene due to its selective pressure on tetracycline resistant bacteria and competitive light absorption effect. On the contrary, DOM promoted the photo-inactivation of AR *E. coli* at high treatment time and further inhibited the expression of tetracycline resistance gene due to the generation of PPRIs. Although no significant degradation of Tc-ARG in the AR *E. coli* was observed, the cell-free Tc-ARGs can be degraded by light irradiation and its photodegradation was proven to be facilitated by DOM. These findings can contribute key scientific information and build understanding to control the transmission and spread of ARB and ARGs in surface waters and are also of significance for understanding the transmission behavior of tetracycline resistant ARB during water treatment based on UV-irradiation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at *****.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2020.116241.

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