

UV/sulfite-driven reductive bio-inactivation through collapse of cellular integrity and molecular functionality

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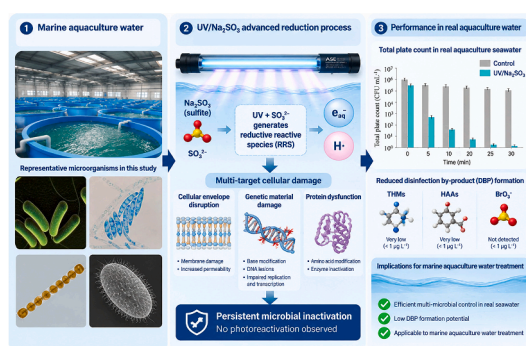
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HIGHLIGHTS

- UV/sulfite efficiently inactivates marine aquaculture microorganisms.
- UV/sulfite suppresses microbial recovery after treatment.
- Hydrated electrons dominate reductive bio-inactivation.
- Reductive reactive species damage cell envelopes, genetic material and proteins.
- Real marine aquaculture water treatment reduces pathogens with low DBPs formation.

GRAPHICAL ABSTRACT



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ABSTRACT

Oxidative disinfection is widely used in aquaculture but often generates toxic by-products (DBPs) that limit its application. Advanced reduction processes (ARPs) provide a complementary strategy for controlling DBPs and possess potential to inactivate microorganisms by disrupting nucleic acids, proteins and other bio-macromolecules, mediated by the reducing reactive species, including hydrated electrons (e_{aq}⁻) and hydrogen atoms (•H). Here, we assessed the efficacy of UV-activated sulfite (UV/sulfite, a typical ARP) against four representative microorganisms from marine aquaculture. Within 5-40 min treatment, inactivation of the bacterium *Escherichia coli*, the fungus *Fusarium oxysporum*, the alga *Heterosigma akashiwo* and the ciliate *Uronema marinum* effectively achieved 4.70-log, 4.63-log, 3.45-log and 2.44-log, respectively. Density functional theory (DFT) calculations and degradation analyses of the model compounds demonstrated that the cell wall components, nucleic acids and amino acid residues were preferentially targeted by UV/sulfite treatment. Transcriptomic analyses indicated that UV/sulfite induced systemic cellular stress that disrupted ribosome biogenesis, DNA repair, protein homeostasis and energy metabolism in the microorganisms. In real marine aquaculture water, UV/sulfite treatment achieved over 6-log reductions of total bacteria within 30 min, effectively suppressed the pathogenic bacteria such as *Vibrio* and *Salmonella*, and maintained low levels of both organic and

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inorganic DBPs. This work provides a mechanistic insight into microbial inactivation by ARPs at the cellular and molecular levels, and demonstrates a practical, low-risk strategy to eliminate pathogenic microorganisms while minimizing DBPs formation in marine aquaculture water.

1. Introduction

Water disinfection is a crucial process in safeguarding public health, particularly in sectors like marine aquaculture, where pathogens can significantly impact both aquatic ecosystems and humans (Shannon et al., 2008). Conventional oxidative disinfection technologies, such as chlorination and ozonation, are limited by insufficient microbial inactivation efficiency and the formation of hazardous disinfection byproducts (DBPs) (Mitch et al., 2023). Advanced oxidation processes (AOPs), which generate highly reactive oxygen species such as hydroxyl radicals ($\bullet\text{OH}$, redox potential $E_0 = 2.7\text{ V}$), have shown improved microbicidal performance (Liang et al., 2025; Wu et al., 2023).

However, in complex water matrices, AOPs may promote the formation of DBPs through oxidative radical-mediated halogen activation pathways (Guan et al., 2025). The strong oxidizing species generated during AOPs can react with halide ions, leading to the formation of reactive halogen species, including hypochlorous acid (HOCl), hypobromous acid (HOBr), and halogen-centered radicals ($\text{Cl}\bullet$, $\text{Br}\bullet$, $\text{Br}_2^{\bullet-}$, etc.). These reactive halogen species can subsequently react with the natural organic matter (NOM) to generate chlorinated and brominated DBPs (Čehovin et al., 2017; Ruan et al., 2021). This pathway is particularly important in bromide-rich waters, such as seawater, where bromide concentrations can reach approximately 65 mg L^{-1} . Brominated DBPs generally exhibit higher cytotoxicity and genotoxicity than their chlorinated analogues due to their greater electrophilicity and biological reactivity (Wang et al., 2014; Lei et al., 2022). Moreover, HOBr and reactive bromine radicals generated from bromide oxidation can undergo further oxidation processes, resulting in the stepwise transformation of bromine species into higher oxidation-state products. Bromide ions can ultimately be converted into bromate (BrO_3^-), a regulated inorganic DBP with recognized carcinogenic potential. Therefore, the application of AOPs in bromide-rich seawater matrices requires careful consideration of both brominated organic DBP and bromate formation risks (Fang and Shang, 2012; Morrison et al., 2023).

As redox counterparts to AOPs, advanced reduction processes (ARPs) have emerged as promising alternatives for mitigating DBPs (Vellanki et al., 2013). ARPs rely on the generation of reducing reactive species (RRS, predominantly hydrated electrons e_{aq}^- , $E_0 = -2.9\text{ V}$ and hydrogen atom radicals $\bullet\text{H}$, $E_0 = -2.3\text{ V}$) through the activation of reductant such as sulfite (Eqs 1-2) (Zhang et al., 2023a). Due to the low negative redox potential, e_{aq}^- exhibits strong reactivity toward electrophilic contaminants such as halogenated organics, whose degradation proceeds via dehalogenation and carbon chain cleavage (Cui et al., 2020). For instance, e_{aq}^- can effectively break C-X ($\text{X} = \text{Cl}, \text{Br}$) bonds and reduce the biological toxicity of haloacetic acids, a representative class of halogenated DBPs (Yu et al., 2018). Furthermore, the reaction of e_{aq}^- with BrO_3^- to form Br^- , as well as the involvement of $\bullet\text{H}$ and sulfite radicals ($\text{SO}_3^{\bullet-}$, $E_0 = 0.63 - 0.84\text{ V}$), provides an effective pathway for reducing inorganic DBPs (Xiao et al., 2017a). Consequently, ARPs could provide a safer and more sustainable solution for water treatment compared to traditional oxidative disinfection methods by minimizing the formation of harmful DBPs.



Despite the promising potential of ARPs for water treatment, their application for microbial inactivation, particularly in high-salinity environments such as seawater, is not yet characterized. Research on

chemically induced microbial damage has predominantly focused on oxidative stress, given that most microorganisms thrive under aerobic conditions and oxidative pathways have historically been the primary target in disinfection studies (Davies and Hawkins, 2020). Reductive stress, which represents the counterpart of oxidative stress in redox reaction, has received relatively less attention. Excessive accumulation of reducing species within microbial cells disturbs redox balance, alters biomolecular structures and ultimately causes pathological damages similar to those induced by oxidative stress (Xiao and Loscalzo, 2020; Xiao et al., 2018). Radiation chemistry studies have demonstrated that e_{aq}^- and $\bullet\text{H}$ can react with biomolecules via carbonyl group reduction and unsaturated bond attack, etc (Singh and Singh, 1983). For instance, e_{aq}^- can induce reductive DNA damage through electron-transfer reactions with nucleobases, leading to the formation of transient nucleobase radical anions. The unstable intermediates can undergo subsequent protonation, bond cleavage, or other chemical transformations, resulting in nucleobase modifications and DNA lesions rather than direct DNA strand breaks (Kumar et al., 2019; Ma et al., 2019). Although isolated base lesions may be repaired by cellular enzymatic systems, clustered or multiple DNA lesions are more difficult to repair and can accumulate, ultimately impairing genetic integrity and contributing to microbial inactivation. Protein and peptide substrates are also vulnerable to reductive radical attack. e_{aq}^- and $\bullet\text{H}$ are capable of reacting with sulfur-containing amino acids including cystine and methionine, with reaction rate constants ranging from 10^7 - $10^9\text{ M}^{-1}\text{ s}^{-1}$. These reactions induce chemical transformations, such as the conversion of methionine to α -aminobutyric acid and the reduction of disulfide bonds into low-molecular-weight amino acids (Barata-Vallejo et al., 2016). These changes disrupt the tertiary structure and biological function of proteins, thereby impairing vital cellular activities. Additionally, reductive modification of membrane lipids damages cell envelope integrity. Combined with nucleic acid and protein deterioration, it further aggravates cellular dysfunction and cell death. Emerging evidence suggests that protein-lipid tandem damages under reductive stress can be more deleterious than isolated macromolecular modifications, due to cascading effects on membrane stability, transport processes, and metabolic regulation (Esterbauer et al., 1991). The biochemical evidence outlined above supports the ability of RRS to disrupt key biomolecules, highlighting their potential for microbial inactivation. Moreover, RRS can also control the formation of DBPs, making ARPs as a promising alternative for water disinfection. Nevertheless, the efficiencies and mechanisms of pathogen inactivation induced by such reduction processes particularly in complex high salinity environments such as seawater have yet to receive adequate attention.

Water disinfection prior to seedling release is critical for inactivating pathogenic microorganisms in marine aquaculture (Deng et al., 2024). Due to the high concentrations of Br^- , oxidative disinfection technologies are unsuitable for marine aquaculture, as they inevitably generate toxic brominated organic DBPs and BrO_3^- . Considering that e_{aq}^- and $\bullet\text{H}$ can inactivate microorganisms through biomolecular damage, this study evaluates the UV/sulfite system (the most mature and extensively studied ARP) for its application in marine aquaculture water disinfection (Ren et al., 2023). Specifically, we investigate the inactivation efficiency and kinetics of representative pathogenic microorganisms, including bacteria (*Escherichia coli*), fungi (*Fusarium oxysporum*), algae (*Heterosigma akashiwo*) and protozoa (*Uronema marinum*). We also characterize the formation of RRS in the UV/sulfite system and quantify their contributions to microbial inactivation. To understand the underlying mechanisms, we analyze cell integrity under UV/sulfite treatment,

focusing on the degradation of key cell membrane and wall component peptidoglycan, which is further elucidated through degradation product identification and density functional theory (DFT) simulations (Egan et al., 2020). Additionally, the reduction of biomolecules including proteins and DNA is studied using model compounds (Bröer et al., 2017; Ma et al., 2019). Transcriptomic analysis is employed to investigate the molecular biological responses of microorganisms to reductive stress. Finally, we evaluate the disinfection efficiency of the UV/sulfite system in real marine aquaculture water and assess the generation of DBPs. Overall, this work develops an innovative disinfection technology suitable for marine aquaculture water. It achieves efficient pathogenic microorganism inactivation and effectively controls DBPs formation simultaneously.

2. Materials and methods

2.1. Microorganisms and water matrices

Four representative microbial surrogates including *F. oxysporum*, *E. coli*, *H. akashiwo* and *U. marinum* were selected to evaluate disinfection performance. Sources of reagents and microbial surrogates are provided in Text S1, and preparation of microbial stock cultures is detailed in Text S2. Synthetic seawater was prepared to mimic the ionic composition of natural seawater, with major inorganic cations and anions added to deoxygenated water (Table S1) (Kester et al., 1967). The initial concentration of the four microbial surrogates was adjusted to approximately 5×10^5 cells mL⁻¹ before UV/sulfite treatment to ensure reliable quantification of microbial inactivation kinetics. Real mariculture water was collected from a storage-pond of a coastal aquaculture facility in Guangxi, China, and transported to the laboratory at 4°C. The storage pond was not aerated during sampling, and the on-site dissolved oxygen (DO) concentration was approximately 2–3 mg/L. Prior to experiments, water was prefiltered through a 100 µm mesh to remove large debris while retaining indigenous microbial communities. The collected water was further purged with N₂ before UV and UV/sulfite treatment.

2.2. UV/sulfite disinfection experiments

Experiments were conducted in a cylindrical glass reactor equipped with a low-pressure mercury lamp ($\lambda = 254$ nm, 14W) (Bolton and Linden, 2003). Reaction solutions (20 mL), containing target microorganisms and sulfite at the desired concentration, were placed in tubes (inner diameter 2.4 cm) positioned perpendicular to the incident UV light at a distance of 8 cm from the lamp. The UV fluence rate was 1.32 µEinstein L⁻¹ s⁻¹, corresponding to an apparent average UV irradiance of 2.75 mW cm⁻² based on the reaction volume and projected irradiation area. The cumulative UV dose was calculated by multiplying the apparent average UV irradiance by the irradiation time, resulting in UV doses of 825–6596 mJ cm⁻² for irradiation times of 5–40 min. The UV lamp was preheated for ≥30 min before experiments, and solutions were continuously stirred using a magnetic stirrer to ensure homogeneity. UV irradiation commenced immediately after sulfite addition, and aliquots were withdrawn at predetermined intervals from ~2 cm below the solution surface for microbial analysis. Before each UV/sulfite experiment, the reaction solution was purged with high-purity nitrogen (N₂) to remove DO, because DO is known to react rapidly with e_{aq}⁻ ($k = 1.9 \times 10^{10}$ M⁻¹ s⁻¹) (Buxton et al., 1988). The oxygen concentration was subsequently measured, and the DO level was below the detection limit of the oxygen meter before initiating the reaction. After confirming oxygen depletion, the reactor was immediately sealed to minimize oxygen intrusion during UV irradiation. The UV/sulfite reactions were therefore conducted under sealed deoxygenated conditions. These procedures ensured that oxygen interference was minimized. pH 8.0 was selected as the experimental condition to represent realistic seawater aquaculture environments. All microbial inactivation experiments in

this study were conducted at a salinity of 12‰.

Microbial quantification methods are provided in Text S3. Total plant counts in real seawater were determined using standard plate counting to assess practical disinfection performance (Allen et al., 2004). Microbial surrogates were inactivated using nitrite (NO₂⁻) and nitrate (NO₃⁻) as scavengers. The concentration of the scavengers was set at 1 mM, which was sufficient to ensure effective scavenging of e_{aq}⁻ and •H (Text S4). The addition of the relatively low concentration of NO₂⁻ and NO₃⁻ alone did not enhance microbial inactivation in the control groups. Organic DBPs were analyzed by gas chromatography, while inorganic DBPs were quantified via ion chromatography (Xie, 2001; Creed et al., 1997).

2.3. Model compounds and molecular level analyses

N-acetylmuramic acid (MurNAc), uridine 5'-diphospho-N-acetylglucosamine (UDP-GlcNAc), ribothymidine, histidine, and aspartic acid (Sigma-Aldrich) were used without further purification. Reaction intermediates and transformation products were identified by LC-MS/MS. DFT calculations were performed to evaluate electronic structures, reactive sites, and thermodynamic feasibility of proposed reductive pathways (Pucci and Angilella, 2022). The obtained Gibbs free energy changes represent intrinsic aqueous-phase thermodynamic tendencies of the investigated transformation pathways. Orbital distribution and NPA were used to identify electron-accepting regions susceptible to attack by reductive reactive species. Detailed experimental conditions, analytical procedures, and computational parameters are provided in Texts S5–S7.

2.4. Transcriptomic and microbial community analyses

Total RNA was extracted from microbial samples (*F. oxysporum*, *E. coli*, *H. akashiwo* and *U. marinum*) using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) (Text S8). RNA integrity was assessed using an Agilent 2100 Bioanalyzer, with RIN ≥ 7.0 (eukaryotes) or RIN ≥ 6.0 (bacteria) considered acceptable (Schroeder et al., 2006). For eukaryotes, mRNA was enriched using Oligo(dT) magnetic beads (New England Biolabs, Ipswich, MA, USA); for *E. coli*, rRNA was removed using the Ribo-Zero™ Magnetic Kit (Epicentre, Madison, WI, USA) (Wahl et al., 2022). Strand-specific cDNA libraries were prepared following the manufacturer's protocol (Illumina, San Diego, CA, USA) and sequenced on an Illumina NovaSeq 6000 platform (PE150) (Grabherr et al., 2011). Raw reads were quality-filtered using fastp (Chen et al., 2018). For non-model eukaryotes, clean reads were assembled de novo using Trinity (Haas et al., 2013); for *E. coli*, reads were aligned to the reference genome (K-12 MG1655) using HISAT2 (Kim et al., 2015). Gene expression levels were quantified using RSEM and normalized as FPKM and TPM (Li and Dewey, 2011). DEG between UV/sulfite-treated and control groups were identified using edgeR, with thresholds of FDR < 0.05 and |log₂FC| > 1. KEGG pathway enrichment and GO analyses were performed using clusterProfiler (Schroeder et al., 2006).

Microbial biomass was collected by filtering water samples (500 mL) through 0.22 µm polycarbonate membranes. Total genomic DNA was extracted using the PowerWater DNA Isolation Kit (Qiagen, Venlo, The Netherlands). Bacterial 16S rRNA V3–V4 region was amplified and sequenced on an Illumina NovaSeq 6000 platform (Caporaso et al., 2012). Raw reads were processed using QIIME2, and OTUs were clustered at 97% similarity using UPARSE (Edgar, 2013). Taxonomic classification was performed using the SILVA database (release 138) (Quast et al., 2013). Alpha diversity (Sobs index) and community composition were analyzed using Mothur and R packages.

3. Results and discussion

3.1. Microbial inactivation performance in the UV/sulfite system

The time-dependent inactivation performances of the four microbial surrogates were comparatively evaluated under sulfite, UV irradiation and the integrated UV/sulfite process. To ensure consistent experimental conditions among the four microbial surrogates, the UV irradiation dose, sulfite concentration and salinity of waters were selected based on the response of *U. marinum* (Fig. S1). As illustrated in Fig. 1a, compared with the control groups, sulfite as a mild reducing agent exerted negligible inactivation toward the tested microbial surrogates, indicating that inactive sulfite lacks sufficient reactivity to cause appreciable loss of microbial viability. Upon 40 min of UV irradiation alone, the inactivation of *F. oxysporum*, *E. coli* and *H. akashiwo* were 3.21-log, 2.06-log and 3.29-log, respectively. The inactivation of

U. marinum was 0.87-log within 5 min. Comparatively, after 40 min of treatment in the UV/sulfite system, the inactivation increased to 4.63-log for *F. oxysporum*, 4.70-log for *E. coli* and 3.45-log for *H. akashiwo*, respectively. For *U. marinum*, the UV/sulfite system significantly enhanced inactivation to 2.44-log within 5 min. Subsequently, no viable cells of *U. marinum* were detected in any of the treated groups. *H. akashiwo* showed slight additional inactivation in the combined system, due to its high susceptibility to direct UV photodamage (Chen et al., 2023).

A comparison of the inactivation kinetics between UV and UV/sulfite revealed a clear microorganism-dependent contribution of sulfite. For *F. oxysporum* and *E. coli*, UV/sulfite consistently achieved higher inactivation than UV alone at all tested time points. In particular, after approximately 30 min, the inactivation by UV alone gradually reached a plateau, whereas that by UV/sulfite continued to increase, indicating additional inactivation mediated by RRS beyond direct UV effects. For

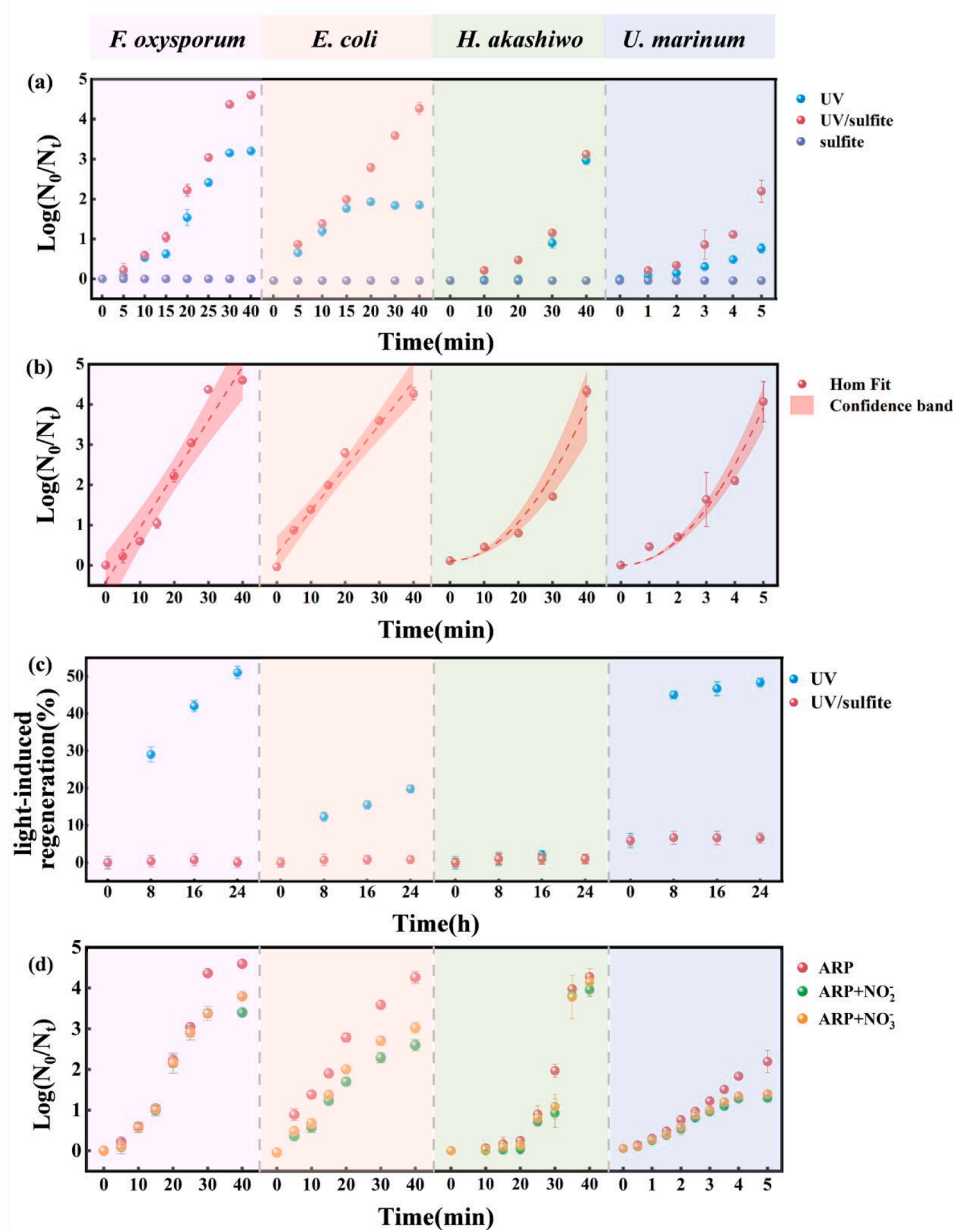


Fig. 1. Inactivation of microbial surrogates under different systems. (a) UV, sulfite and UV/sulfite systems. (b) Inactivation kinetics fitting by Hom model. (c) light-induced regeneration of microbial surrogates. (d) inactivation efficiency of adding nitrate and nitrite as active species quenchers. ([microbial surrogates] = 5×10^5 cells mL⁻¹, [sulfite] = 1 mM, UV fluence rate = 2.75 mW·cm⁻², [nitrate] = 1 mM, [nitrite] = 1 mM, salinity = 12 ‰, T = 298.15 K, pH = 8).

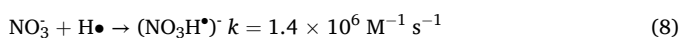
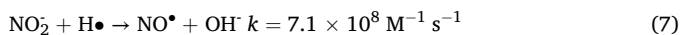
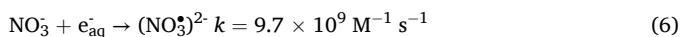
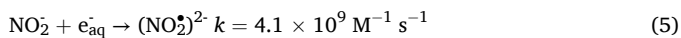
U. marinum, UV/sulfite also consistently exhibited higher inactivation than UV alone throughout the 5-min treatment period. In contrast, *H. akashiwo* showed relatively similar inactivation kinetics under UV and UV/sulfite within 40 min, indicating a limited additional contribution of sulfite to its apparent inactivation. To quantitatively describe the inactivation kinetics, four classical disinfection models (Hom, Rational, Hom-Power law and delayed Chick-Watson; Text S9) were evaluated (Fig. S2-S9) (Dunkin et al., 2017). Shapiro-Wilk tests on model residuals confirmed that the Hom model provided the best fitting performance for the four microbial surrogates, with the highest coefficients of determination and the lowest sum of squared errors (SSE) (Fig. 1b, Table S2) (Silva Goulart et al., 2024). The 95% confidence bands around the fitted curves further validated the reliability and statistical robustness of the Hom model, as most experimental data points fell within the predicted confidence intervals (Huang et al., 2026). It should be noted that the units of the fitted kinetic parameter (k) in the Hom model are dependent on the power parameter (m), according to the equation $\ln(N/N_0) = -kC^m t^m$. Therefore, k values derived from different microorganisms with different m values cannot be directly compared as conventional first-order rate constants. The differences in m values reflect the distinct non-linear inactivation behaviors among microorganisms, which may result from variations in cellular structure, resistance and repair capacity. The kinetic parameters were therefore compared only within the same microorganism under UV and UV/sulfite treatments. Sulfite addition increased the fitted kinetic parameter compared with UV treatment alone, including *F. oxysporum* (from 0.088 to 0.121 min⁻¹), *E. coli* (from 0.076 to 0.129 min⁻¹), *H. akashiwo* (from 0.002 to 0.003 min⁻²), and *U. marinum* (from 0.036 to 0.054 min⁻²). The increase in the rate constants indicates that sulfite plays a critical role in enhancing the microbial inactivation process when activated by UV irradiation. The UV/sulfite system significantly enhanced the microbial inactivation, suggesting the involvement of additional reactive species generated in the system. The formation of RRS such as e_{aq}^- and $\bullet H$ likely contributes to the increased inactivation efficiency. These species target and degrade key cellular components such as DNA and proteins, ultimately causing irreversible damage and loss of cell viability.

F. oxysporum, *E. coli* and *U. marinum* exhibited obvious light-induced recovery after single UV exposure, with regeneration rates of 51.10%, 19.79% and 38.9%, respectively (Fig. 1c). The results are consistent with the previous reports and can be explained by enzymatic repair of UV-damaged DNA (Zhang et al., 2023b; Raesizadeh and Taghipour, 2021). Interestingly, *H. akashiwo* exhibited limited photoreactivation after UV treatment compared with the other tested microorganisms. This difference may be attributed to the distinct cellular damage patterns and repair capacities between prokaryotic and eukaryotic microorganisms. UV irradiation mainly induces DNA photolesions in bacteria which can be partially repaired through photoreactivation and dark repair pathways. In contrast, UV-induced damage in microalgae may extend beyond DNA lesions to photosynthetic apparatus, including photosystem II and associated electron transport components. Such damage may impair photosynthetic activity and cellular recovery even when DNA repair occurs, resulting in limited photoreactivation of *H. akashiwo*. However, none of the microbial surrogates treated with the UV/sulfite system showed signs of recovery, as they could not be subcultured, indicating the irreversible inactivation. Even when the photoreactivation experiment was extended to 7 days, no detectable recovery was observed. The phenomenon further supports the conclusion that the RRS generated in the UV/sulfite system induce cellular damage beyond repair capacity. To further evaluate the cellular status and extent of damage in microorganisms following UV/sulfite treatment, excitation-emission matrix (EEM) fluorescence spectroscopy was employed as a complementary characterization (Fig. S10). Although the detailed fluorescence responses varied among microorganisms, UV/sulfite treatment generally induced more alterations in protein-associated fluorescence, particularly in the tryptophan-like

(Region T) and tyrosine-like (Region B) components. The overall magnitude of these changes was greater than that observed under UV irradiation alone. These findings indicate that UV/sulfite causes substantial alterations in protein-like and other fluorescent organic components, thereby reducing the recovery potential of the treated microbial surrogates.

The formation of RRS was confirmed by electron spin-resonance spectroscopy (ESR) using 2,2,6,6-Tetramethylpiperidin-1-oxyl (TEMPO) and 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) as probes (Fu et al., 2022; Liu et al., 2022). A characteristic three-line ESR signal (1:1:1 intensity ratio) was observed, which was rapidly attenuated in the UV/sulfite system due to reduction of TEMPO to ESR-silent TEMPOH by e_{aq}^- and $\bullet H$ ($k_{e_{aq}^-} = 7.8 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, $k_{\bullet H} = 4.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$). In addition, distinct ESR signals corresponding to $\bullet H$ - DMPO adduct further confirmed the generation of $\bullet H$ (Fig. S11). To elucidate the roles of e_{aq}^- and $\bullet H$ in microbial inactivation, microbial surrogates were inactivated using NO_2^- and NO_3^- as scavengers (Liu et al., 2022; Dong et al., 2023). The potential consumption of $\text{NO}_2^-/\text{NO}_3^-$ by seawater matrix components was considered. Although seawater contains high concentrations of Cl^- , Br^- , SO_4^{2-} , HCO_3^- , and dissolved organic matter (DOM), these components generally do not undergo rapid direct reactions with NO_2^- and NO_3^- under the experimental conditions. Cl^- and Br^- mainly exist as stable halide ions in seawater. Their transformation generally requires strong oxidative species, such as $\bullet \text{OH}$ or $\text{SO}_4\bullet^-$, through oxidation or halogen activation pathways. However, the UV/sulfite system is an ARP dominated by e_{aq}^- and $\bullet H$, rather than oxidative radicals. Therefore, the formation of reactive halogen species is not favored, Cl^- and Br^- are unlikely to significantly consume NO_2^- and NO_3^- . Similarly, SO_4^{2-} and HCO_3^- are highly stable inorganic anions and have no efficient rapid reaction pathways with $\text{NO}_2^-/\text{NO}_3^-$ under neutral to weakly alkaline conditions. For DOM, although some electron-accepting functional groups, such as quinone-like structures, may participate in redox reactions, DOM mainly competes for transient reducing species rather than directly consuming NO_2^- or NO_3^- . Moreover, both NO_2^- and NO_3^- possess UV absorption capability, therefore, their potential photolysis under 254 nm irradiation and the possible formation of nitrogen-centered radicals should be considered. Previous studies have demonstrated that NO_2^- and NO_3^- possess photochemical activity and can generate reactive nitrogen/oxygen species under UV irradiation. However, these photochemical processes are strongly wavelength-dependent. NO_3^- photolysis producing $\bullet \text{OH}$ and $\bullet \text{NO}_2$ has been mainly investigated under short-wavelength UV irradiation (e.g., 205–220 nm), where NO_3^- exhibits strong charge-transfer absorption. Although NO_3^- photolysis can occur under 254 nm irradiation, its efficiency is substantially lower, with NO_2^- and peroxyxynitrite being the major photoproducts rather than sustained accumulation of oxidative radicals (Goldstein and Rabani, 2007). NO_2^- exhibits absorption maxima around 350–360 nm and a weaker absorption region below 250 nm. Therefore, 254 nm irradiation does not correspond to its most efficient photochemical excitation region (Jankowski et al., 1999). More importantly, the addition of 1mM $\text{NO}_2^-/\text{NO}_3^-$ in the UV-alone system caused no obvious change in microbial inactivation performance (Fig. S12). These results confirm that the primary role of $\text{NO}_2^-/\text{NO}_3^-$ in this study was the selective scavenging of transient RRS generated in the UV/sulfite system, rather than direct participation in photochemical reactions or generation of additional inactivation-active species. Both NO_2^- and NO_3^- effectively quenched e_{aq}^- , whereas only NO_2^- scavenged $\bullet H$ due to the slower reaction between $\bullet H$ and NO_3^- (Eqs. 5–8) (Buxton et al., 1988). For instance, as shown in Fig. 1d, the inactivation of *E. coli* decreased to 2.88-log and 3.35-log upon addition of NO_2^- and NO_3^- to the UV/sulfite system, respectively. The other three microorganisms showed similar responses following the addition of NO_2^- and NO_3^- . The smaller decrease in microbial inactivation observed upon $\bullet H$ scavenging than that observed upon e_{aq}^- scavenging indicates that $\bullet H$ may play a

secondary role relative to e_{aq}^- in the UV/sulfite system. This observation is consistent with the solution chemistry of the UV/sulfite system under circumneutral pH conditions, where sulfite predominantly exists as SO_3^{2-} and the protonation-dependent formation of $\bullet H$ is relatively limited (Liu et al., 2022).



3.2. Loss of cellular integrity in the UV/sulfite system

To elucidate the intrinsic microbial inactivation mechanism induced by the UV/sulfite process, cellular morphological changes of four microbial surrogates in the control group, single UV treatment and UV/sulfite treatment were observed using transmission electron microscopy (TEM, Fig. 2). As shown in the TEM images, all microbial surrogates in the control group exhibited intact cellular morphology, clear cell

boundaries, and well-organized intracellular constituents. Under sole UV irradiation, *F. oxysporum* (fungus) and *E. coli* (bacterium), which have rigid cell walls, maintained relatively intact cell envelopes with only mild intracellular disturbance. Similarly, the protozoan *U. marinum* showed negligible structural damage with an intact cell membrane. In contrast, *H. akashiwo* which lacks a cell wall suffered severe intracellular destruction, including organelle disassembly, cytoplasmic cavitation and cellular content leakage.

In the UV/sulfite system, all the four microbial surrogates suffered visibly greater structural damage than those exposed to UV alone. *F. oxysporum*, *E. coli*, and *U. marinum* exhibited severe cell wall/membrane rupture, cytoplasmic shrinkage, leakage of intracellular contents and overall structural collapse. Although *H. akashiwo* already sustained severe damage under UV irradiation, its cellular destruction was further exacerbated in the combined system. The RRS preferentially attacked cell membranes and triggered extensive, irreversible and lethal structural disruption, thereby achieving microbial inactivation without observable recovery. These findings are consistent with previous studies demonstrating that free radicals generated in AOPs can cause extensive damage to microbial cell walls and membranes, leading to loss of viability (Wen et al., 2017). Notably, these results demonstrate that the RRS possess comparable capacity toward microbial cell envelope destruction as conventional oxidative radicals in AOPs.

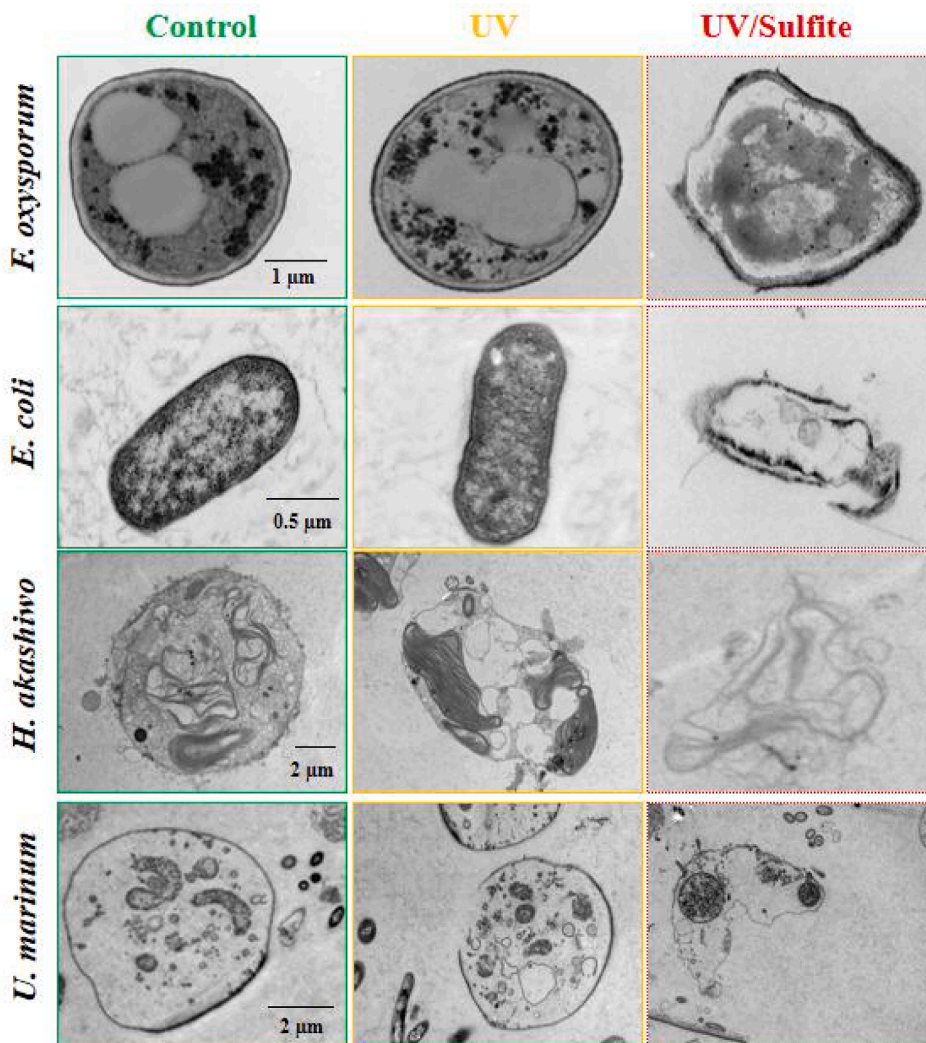


Fig. 2. TEM images of four microbial surrogates before (left row) and after UV (middle row) or UV/Sulfite (right row) treatment. ([microbial surrogates] = 5×10^5 cells mL⁻¹, [sulfite] = 1 mM, UV fluence rate = 2.75 mW·cm⁻², salinity = 12 ‰, T = 298.15 K, pH = 8, inactivation time = 40 min for *F. oxysporum*, *E. coli*, and *H. akashiwo*, 5min for *U. marinum*).

Correspondingly, UV irradiation alone triggered mild elevations in extracellular DNA (eDNA) levels for *F. oxysporum* and *U. maritimum*. The accumulation of eDNA was consistent with active physiological DNA extrusion during cellular senescence as previously reported, rather than catastrophic cellular lysis or membrane structural disruption (Takahashi et al., 2017). In contrast, the UV/sulfite system displayed a distinct bimodal pattern in eDNA abundance, featuring an initial transient rise followed by a gradual decline over prolonged reaction time (Fig. S13). For instance, the eDNA concentration in *F. oxysporum* peaked at 7.92 ng μL^{-1} after 20 min treatment, and subsequently declined to 5.53 ng μL^{-1} at 30 min. This characteristic kinetic pattern demonstrates that RRS mediated damage to external structure of cells first triggered massive leakage of intracellular genomic DNA into the extracellular environment. Subsequently, the RRS drove the reductive degradation of the released DNA. Although e_{aq}^- can not directly induce DNA strand breaks, it can form stable molecular adducts (e.g., dihydrothymine, dihydrocytosine) through efficient binding to DNA bases. Accumulation of such adducts triggers the formation of clustered DNA lesions, which ultimately result in cellular inactivation (Kumar et al., 2019; Chomicz-Mańka et al., 2023). Therefore, the compromised cellular integrity, synchronous leakage of intracellular genetic materials and successive degradation of liberated eDNA collectively verify the pivotal role of RRS (predominantly e_{aq}^- and $\bullet\text{H}$) in driving irreversible lethal cellular injury. The inactivation mechanism further corroborates the substantial inactivation enhancement achieved in the UV/sulfite process, confirming that RRS impose supplementary, non-overlapping lethal damage to microorganisms that extends far beyond the intrinsic inactivation of UV irradiation alone.

The differential susceptibility of microorganisms to UV/sulfite treatment is likely associated with variations in cellular architecture and stress response capacity among microbial taxa. The microbial envelope represents the first barrier determining the accessibility of reductive reactive species, including e_{aq}^- and $\bullet\text{H}$, to intracellular targets. Gram-negative bacteria, such as *E. coli*, possess a relatively thin peptidoglycan layer and a lipopolysaccharide-containing outer membrane; once reactive species penetrate the envelope, intracellular components, particularly nucleic acids and proteins, can be readily damaged. e_{aq}^- can induce nucleobase modification through electron attachment reactions, resulting in DNA lesions that impair genomic integrity (Kumar et al., 2019; Ma et al., 2019). These characteristics may contribute to the higher susceptibility of *E. coli* observed in this study. In contrast, eukaryotic microorganisms generally possess more complex cellular architectures that may reduce reactive species accessibility to intracellular targets. Fungal cells such as *F. oxysporum*, contain rigid cell walls mainly composed of chitin and β -glucans, providing structural protection against external stresses (Bowman and Free, 2006). Similarly, algal and protozoan cells possess larger cell sizes and more complex intracellular organization, which may introduce additional barriers for reactive species transport. In addition, differences in antioxidant capacity and DNA repair systems among microorganisms may influence their ability to recover from molecular damage induced by reactive species (Friedberg, 2003; Mittler, 2017). Therefore, the lower inactivation sensitivity of *F. oxysporum*, *H. akashiwo* and *U. maritimum* compared with *E. coli* may result from the combined effects of structural protection, target accessibility, and cellular stress response mechanisms. Overall, the observed variations in microbial inactivation efficiency likely reflect the balance between reactive species accessibility, cellular protection mechanisms, and intracellular damage accumulation. These findings indicate that UV/sulfite-mediated inactivation is not governed by a single target, but rather involves multi-target damage to cellular components, including membranes, proteins, and genetic materials.

3.3. Reduction damage to the cell wall structures as a lethal pathway

Following the confirmation of irreversible microbial inactivation

accompanied by compromised cellular integrity, we further investigated whether reductive stress directly targets key molecular precursors involved in cell wall biosynthesis. Peptidoglycan is the major constituent of bacterial cell walls. MurNAc and UDP-GlcNAc, two essential building blocks of peptidoglycan, were selected as representative model compounds (Unsleber et al., 2017). These components are critical for the bacterial cell wall and play a pivotal role in maintaining cellular structure and integrity. For instance, in *E. coli*, continuous turnover of the murein sacculus involves extensive degradation and recycling of MurNAc containing fragments, where MurNAc-phosphate and UDP-GlcNAc act as indispensable precursors for cell wall maintenance and mechanical stability (Uehara et al., 2006).

To evaluate their reactivity under reductive conditions, systematic structural analyses were carried out. For MurNAc, orbital calculations yielded a highest occupied molecular orbital (HOMO) energy of -7.0733 eV and a lowest unoccupied molecular orbital (LUMO) energy of -0.9998 eV, corresponding to an energy gap (ΔE) of 6.0734 eV (Fig. S14). These results indicate that MurNAc exhibits favorable electron-accepting capacity under reductive environments. Notably, as presented in Fig. 3a, LUMO electron density was mainly distributed on the ketone carbonyl and carboxyl groups, marking these moieties as preferential electron-accepting sites. Natural population analysis (NPA, Table S3) revealed significant positive charges on the corresponding carbon atoms (C6: 0.4809; C14: 0.7732) and substantial negative charge accumulation on adjacent oxygen atoms (O4: -0.5348; O18: -0.7534; O19: -0.6719). The oxygen atoms with high electron affinity were identified as the primary targets for reductive attack, driving the reduction of carbonyl and carboxyl groups (Fig. 3b). Supported by these electronic characteristics, high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis verified the formation of a reduced MurNAc product ($[\text{M}+\text{H}]^+ = 282.1543$) in the UV/sulfite system (Fig. S15). Free energy calculations further supported the thermodynamic feasibility of the reductive conversion, with a negative Gibbs free energy change ($\Delta G = -20.08 \text{ kcal mol}^{-1}$, Fig. 3c), indicating spontaneous degradation of MurNAc under UV/sulfite treatment (Chomicz-Mańka et al., 2023).

Similarly, UDP-GlcNAc displayed distinct reductive degradation behavior consistent with its molecular structure. Orbital analysis revealed a HOMO energy of -6.9901 eV and a LUMO energy of -1.4049 eV ($\Delta E = 5.5852 \text{ eV}$). Such energy characteristics facilitate electron capture and subsequent reduction reactions. (Fig. S16). The LUMO electron density was primarily concentrated on the uracil moiety (Fig. 3d). NPA revealed obvious charge separation between N13 (-0.5905) and the adjacent C9 atom (0.7833), implying intrinsic instability of the N-C bond (Fig. 3e and Table S4). Upon exposure to RRS generated in the UV/sulfite system, cleavage of the N-C bond occurred, producing a characteristic product ($[\text{M}-\text{H}]^- = 606.0750$) (Fig. S17). Thermodynamic analysis confirmed the spontaneity of this cleavage, with a negative ΔG of $-10.34 \text{ kcal mol}^{-1}$ (Fig. 3f).

The reductive degradation of MurNAc and UDP-GlcNAc indicates direct chemical impairment of peptidoglycan building blocks. These findings are consistent with previous studies demonstrating that damage to peptidoglycan or its biosynthetic precursors directly compromises cell wall integrity and causes lethal structural failure. Destabilized peptidoglycan further induces outer membrane detachment and loss of cellular mechanical resistance, which aligns well with the microbial inactivation phenotypes observed in this study, including morphological damage and eDNA leakage (Jun et al., 2020).

3.4. Reductive degradation of biomacromolecule building blocks

Considering the critical role of genetic material in cellular function and proteins in catalyzing metabolic processes, ribothymidine and two amino acids were selected as models for DNA and proteins, respectively, to investigate their degradation in the UV/sulfite system (Ma et al., 2019). Orbital distribution analysis showed that ribothymidine has a HOMO energy of -6.5653 eV and a LUMO energy of -1.2779 eV, giving

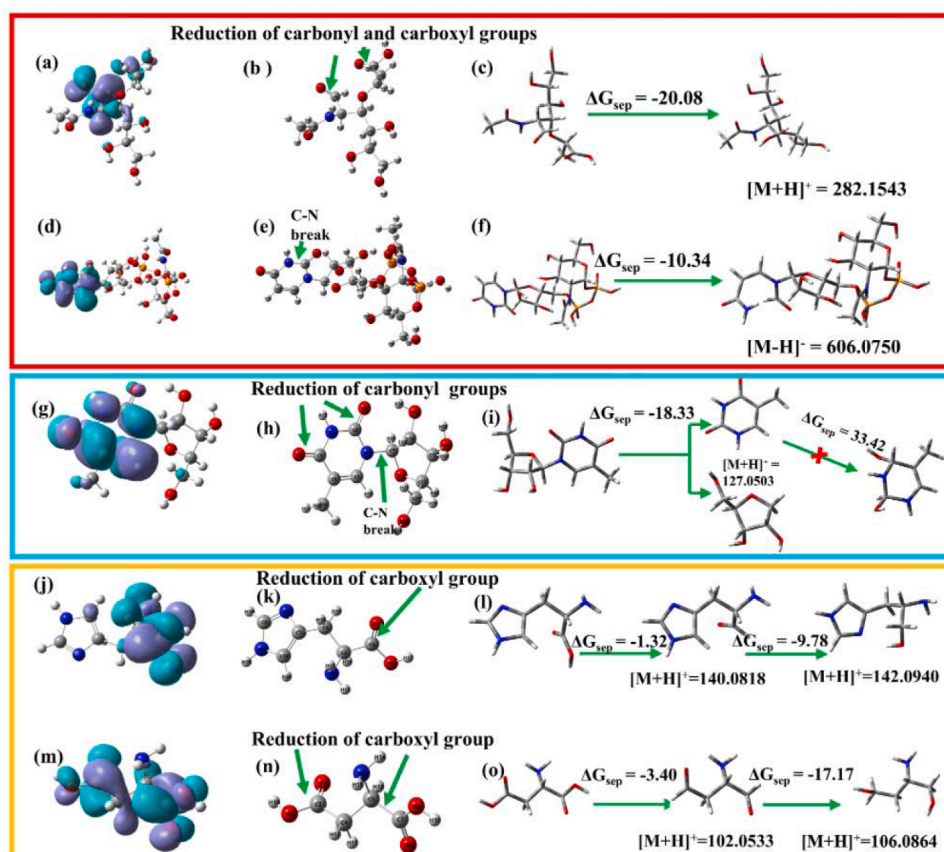


Fig. 3. Reductive degradation of biomacromolecule building blocks (a, d, g, j, m represent the LUMO electron density of MurNAc, UDP-GlcNAc, ribothymidine, histidine and aspartic acid, respectively. b, e, h, k, n represent the reduction site. c, f, i, l, o represent the free energy calculations.).

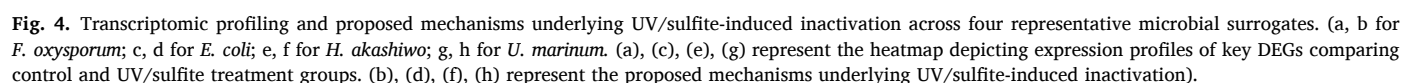
an energy gap (ΔE) of 5.2873 eV, which indicates a propensity for electron acceptance under reductive conditions (Fig. S18). The LUMO electron density is predominantly localized on the pyrimidine ring, particularly around the carbonyl groups and the adjacent unsaturated sites, while the ribose moiety contributes minimally (Fig. 3g). The data suggests that the nucleobase, rather than the sugar scaffold, is preferentially activated under reductive conditions. Consistently, NPA revealed charge separation between N3 and adjacent C7 atom, suggesting intrinsic destabilization of the N-C linkage (Fig. 3h and Table S5). Additionally, the pyrimidine carbonyl region exhibited positive charge on the carbon atoms and negative charge on the oxygen atoms, identifying these sites as preferential targets for RRS attack.

Then, HPLC-MS/MS analysis confirmed that ribothymidine mainly underwent nucleosidic bond cleavage, releasing free thymine ($[M+H]^+ = 127.0503$) (Fig. S19). Reductive activation of the nucleobase destabilized the N-glycosidic bond, thereby promoting bond cleavage and thymine liberation. Although hydrogenation of carbonyl groups and saturation of unsaturated bonds within the pyrimidine ring are possible, no corresponding reduced thymine derivatives were detected experimentally. Free energy calculations provided further mechanistic insight: cleavage of the N-glycosidic bond is thermodynamically favored under UV/sulfite-induced reductive stress ($\Delta G = -18.33$ kcal mol⁻¹), whereas further reduction of the thymine ring is strongly disfavored, as disruption of the π -conjugated pyrimidine-carbonyl framework requires a substantial energy input ($\Delta G = +33.42$ kcal mol⁻¹, Fig. 3i). Consequently, reductive degradation preferentially proceeds through base release rather than extensive nucleobase hydrogenation under the studied aqueous conditions. The above results demonstrate that ribothymidine degradation in the UV/sulfite system was dominated by base-centered transformations, providing a molecular-level explanation for the irreversible loss of genetic information integrity. Similar electron-

driven damage of nucleobases has been reported for 5'-monophosphate of 2'-deoxycytidine, where electron attachment followed by intramolecular proton transfer stabilized nucleobase centered anion radicals under reductive stress, facilitating subsequent bond destabilization (Chomicz-Mańka et al., 2023).

In parallel, the reductive degradation of protein building blocks was examined using histidine and aspartic acid as the representative amino acid models. Orbital distribution analysis revealed that, for both amino acids, the LUMO is primarily localized on the carboxyl functional group, indicating preferential electron acceptance at this site under reductive conditions (Fig. 3j, 3m and Fig. S20). NPA further revealed charge separation within the carboxyl moiety, with the carbonyl carbon atoms carrying a positive charge (C=O) and the adjacent oxygen atoms being electron-rich, rendering the carboxyl group highly susceptible to attack by hydrated electrons and hydrogen radicals (Fig. 3h, n and Table S6-S7). Consistent with these electronic features, HPLC-MS/MS analysis showed that both histidine and aspartic acid underwent reductive transformation initiated by carboxyl reduction. For aspartic acid, reduction of the terminal carboxyl group led to the formation of aldehyde and subsequently alcohol products ($[M+H]^+ = 102.0533$ and 106.0864, respectively). Similarly, histidine underwent carboxyl reduction, generating reduced products with $[M+H]^+$ values of 140.0818 and 142.0940 (Fig. S21). Free energy calculations indicated that stepwise carboxyl reductions are thermodynamically favorable, with carboxyl-to-aldehyde and subsequent alcohol formation exhibiting negative ΔG values, supporting the spontaneous progression of amino acid degradation under reductive conditions (Fig. 3l, o). The results indicate that reductive degradation of amino acids in the UV/sulfite system is dominated by carboxyl-centered reduction, resulting in the loss of acidic functionality and substantial alteration of amino acid structure. In proteins, side-chain carboxyl groups of acidic amino acids

impair protein stability and catalytic function (Bartlett et al., 2002; Miyazawa et al., 2009). Therefore, the observed carboxyl-centered reductive transformations are expected to compromise protein structural integrity and functionality, even in the absence of peptide



backbone cleavage. Such conformational alterations result in the disruption of key cellular processes, as the altered protein conformation may impair enzymatic activity, molecular recognition, and cellular signaling.

The above results demonstrate that the UV/sulfite system drives irreversible microbial inactivation through reductive stress, acting synergistically on three critical targets: cell envelope structure, genetic material, and functional proteins. Specifically, reactive reducing species directly attack the key precursors for cell wall biosynthesis, leading to reductive degradation and structural damage to peptidoglycan building blocks. This results in the loss of cell wall integrity, reduced cellular mechanical stability, and eventual structural collapse. Meanwhile, reactive reducing species target nucleic acid structures and disrupt key chemical bonds in genetic material, causing irreversible DNA damage and the loss of genetic integrity. In addition, reductive stress modifies the essential functional groups of amino acids, altering their chemical structure and charge properties and thereby impairing protein conformation, stability, and catalytic activity, which further disturbs intracellular metabolic processes. These three complementary damage pathways act in concert to cause structural failure, genetic inactivation, and metabolic dysfunction, collectively leading to complete and irreversible microbial inactivation.

3.5. Omics-informed cellular responses under reductive stress

To investigate the cellular mechanisms underlying irreversible microbial inactivation, we conducted comparative transcriptomic profiling of the four microbial surrogates. In *F. oxysporum*, UV/sulfite treatment induced 4,471 differentially expressed genes (DEGs), indicating extensive transcriptional reprogramming in response to severe reductive stress. KEGG enrichment analysis revealed that the DEGs were predominantly enriched in pathways associated with ribosome biogenesis and cofactor biosynthesis, alongside pronounced alterations in specific amino acid metabolic pathways, particularly arginine and proline metabolism (Fig. S22). As illustrated in the expression heatmap (Fig. 4a), core genes central to ribosome biogenesis including *RRP7*, *UTP6*, *LSG1*, *RCL1*, and *UTP14* were markedly upregulated. Ribosome biogenesis is a highly energy-intensive and stress-sensitive process in eukaryotes; this widespread upregulation suggests that the protein synthesis machinery is undergoing substantial recombinational and assembly pressure to counteract stress-induced cellular damage (Henras et al., 2008; Woolford & Baserga, 2013).

Concurrently, the cofactor biosynthesis pathway exhibited significant dysregulation. Key genes within this pathway including *ALDH*, *COAB*, and *ilvE* were substantially downregulated, whereas *glyA* was upregulated. Given that cofactors are essential for maintaining cellular redox homeostasis and enabling enzymatic catalysis (Agledal et al., 2010; Brown et al., 2014), the suppression of critical biosynthetic components points to a profound disruption of fundamental energy metabolism and core biochemical networks in the fungus. Moreover, targeted analysis of arginine and proline metabolism uncovered complex, stress-responsive metabolic reprogramming: *GOT1* was upregulated, while *ALDH* was downregulated. This divergent regulation implies an urgent, adaptive reallocation of intracellular carbon and nitrogen resources as the pathogen strives to maintain viability under extreme stress conditions (Divon & Fluhr, 2007; Takagi, 2008). Collectively, these findings demonstrate that UV/sulfite treatment concurrently impairs ribosome assembly, compromises cofactor-dependent metabolic homeostasis, and triggers emergency metabolic rewiring, ultimately culminating in systemic functional collapse in *F. oxysporum* (Fig. 4b).

In *E. coli*, UV/sulfite treatment induced 1,663 DEGs, of which 977 were downregulated. KEGG enrichment analysis revealed that downregulated genes were significantly clustered in chemotaxis, the phosphotransferase system (PTS), and cell cycle pathways (Fig. S23). To validate these transcriptomic shifts quantitatively, the expression

profiles of representative genes within these critical pathways were analyzed (Fig. 4c). As integrated into the mechanistic model (Fig. 4d), within the chemotaxis pathway, key genes including *CheA*, *CheW*, *MotA* and *MotB* were downregulated, indicating impaired environmental sensing and motility (Wadhams and Armitage, 2004). In the PTS pathway, universal components (*PtsI*, *PtsH*) and sugar-specific transporters (*PtsG*, *NagE*, *MalX*) were also downregulated, suggesting restricted uptake and phosphorylation of multiple carbon sources, limiting cellular energy supply. Moreover, genes involved in the cell cycle and division, including the replication-initiation gene *dnaA*, divisome components (*ftsQ*, *ftsA*, *ftsW*), and peptidoglycan precursor synthesis gene *murG* were significantly downregulated (Blaauwen et al., 2017; Egan et al., 2020). These proteins are responsible for replication initiation, divisome assembly, septum formation and peptidoglycan precursor production, their repression directly compromises cell division and envelope integrity. The protein quality control genes including *lon*, *clpX*, and *clpP* were downregulated, indicating reduced clearance of misfolded proteins and amplifying intracellular stress (Gottesman, 1996; Sauer and Baker, 2011). These results suggest that UV/sulfite treatment does not act on a single target but rather coordinately disrupts environmental sensing, nutrient uptake, cell division, and protein homeostasis, systematically compromising bacterial viability.

H. akashiwo exhibited the highest sensitivity to UV/sulfite treatment, with 40,963 DEGs identified, of which 27,321 were downregulated, approximately twice as many as the upregulated genes. KEGG enrichment highlighted significant clustering of downregulated genes in the phosphatidylinositol signaling system, MAPK signaling, endocytosis, autophagy, and ubiquitin-mediated proteolysis pathways (Fig. S24), all of which are critical for plasma membrane and endomembrane integrity and signal transduction (Munnik and Vermeer, 2010; Jahn and Scheller, 2006). To provide quantitative validation for these transcriptomic alterations, expression profiles of representative genes within these critical pathways were analyzed (Fig. 4e). As illustrated in the integrative model derived from these quantitative data (Fig. 4f), within the plant hormone signaling pathway, key components including *TMK1/4*, *AHA1/2*, *SIMKK*, and *MPK3/6* were significantly downregulated across all treatment replicates, indicating compromised membrane receptor function, disrupted proton gradients, and impaired MAPK cascade transmission. Autophagy-related genes spanning *TOR*, *ATG13*, *ATG7* and *ATG5* were also consistently downregulated, suggesting a blockade in autophagosome formation and elongation (Liu and Bassham, 2012). Furthermore, multiple components of the ubiquitin–proteasome system, including E1 (*UBE1*), E2 (*UBE2I*, *UBE2Q*) and several E3 ligases (*NEDD4*, *Parkin*, *CHIP*, *Cul1*) were downregulated, indicating that the sequential steps from ubiquitin activation to substrate recognition were impaired, limiting the clearance of damaged proteins (Hershko and Ciechanover, 1998). As the absence of a rigid cell wall in *H. akashiwo*, the combined inhibition of membrane-associated signaling, autophagy and ubiquitin-mediated proteolysis directly compromises membrane integrity and leads to cytoplasmic leakage, consistent with the observed cellular phenotypes.

Reductant exposure induced marked transcriptional changes in *U. marinum*, resulting in 14,345 DEGs, of which 8,517 were upregulated and 5,828 were downregulated. UV/sulfite treatment triggered extensive transcriptional reprogramming, characterized predominantly by a suppressive response to severe reductive stress. KEGG enrichment analysis revealed that DEGs were significantly enriched in core metabolic and signaling pathways, most notably the citrate cycle (TCA cycle), the phosphatidylinositol signaling system, and purine metabolism (Fig. S25). As shown in the expression heatmap, key enzymatic drivers of the TCA cycle, specifically *LSC1* and *ACLY*, were markedly downregulated (Fig. 4g). Given the central role of the TCA cycle in cellular respiration and biosynthesis of macromolecules, suppression of these critical enzymes implies a profound disruption in energy production and carbon flux, precipitating an acute cellular energy crisis (Martínez-Reyes & Chandel, 2020). Concurrently, the phosphatidylinositol signaling

system was substantially impaired, as evidenced by pronounced down-regulation of *FCY1* and *AK91*. This evolutionarily conserved signaling cascade is essential for transducing extracellular stress cues, regulating membrane trafficking and dynamics, and coordinating intracellular adaptive responses; its widespread inhibition suggests a near-complete loss of environmental sensing capacity and structural plasticity in the ciliate (Berridge, 2016). Moreover, key genes associated with purine metabolism including *CALM* and *PIP5K* exhibited significant transcriptional repression. Because purine metabolism underpins both de novo nucleotide biosynthesis and intracellular energy transfer (e.g., via ATP/GTP), this coordinated downregulation reflects a systemic collapse

in genetic information processing, depriving the cell of essential precursors required for DNA repair, RNA synthesis, and other nucleic acid-dependent processes (Fig. 4h) (Pedley and Benkovic, 2017).

It should be noted that the transcriptomic responses obtained from pure cultures may differ from those occurring in complex aquaculture microbial communities. In practical aquaculture waters, microbial interactions, extracellular polymeric substances (EPS) production, biofilm formation, and metabolic exchange may influence reactive species accessibility and microbial stress responses. Therefore, the present transcriptomic results provide species-specific mechanistic insights, while future community-level studies using metagenomic and

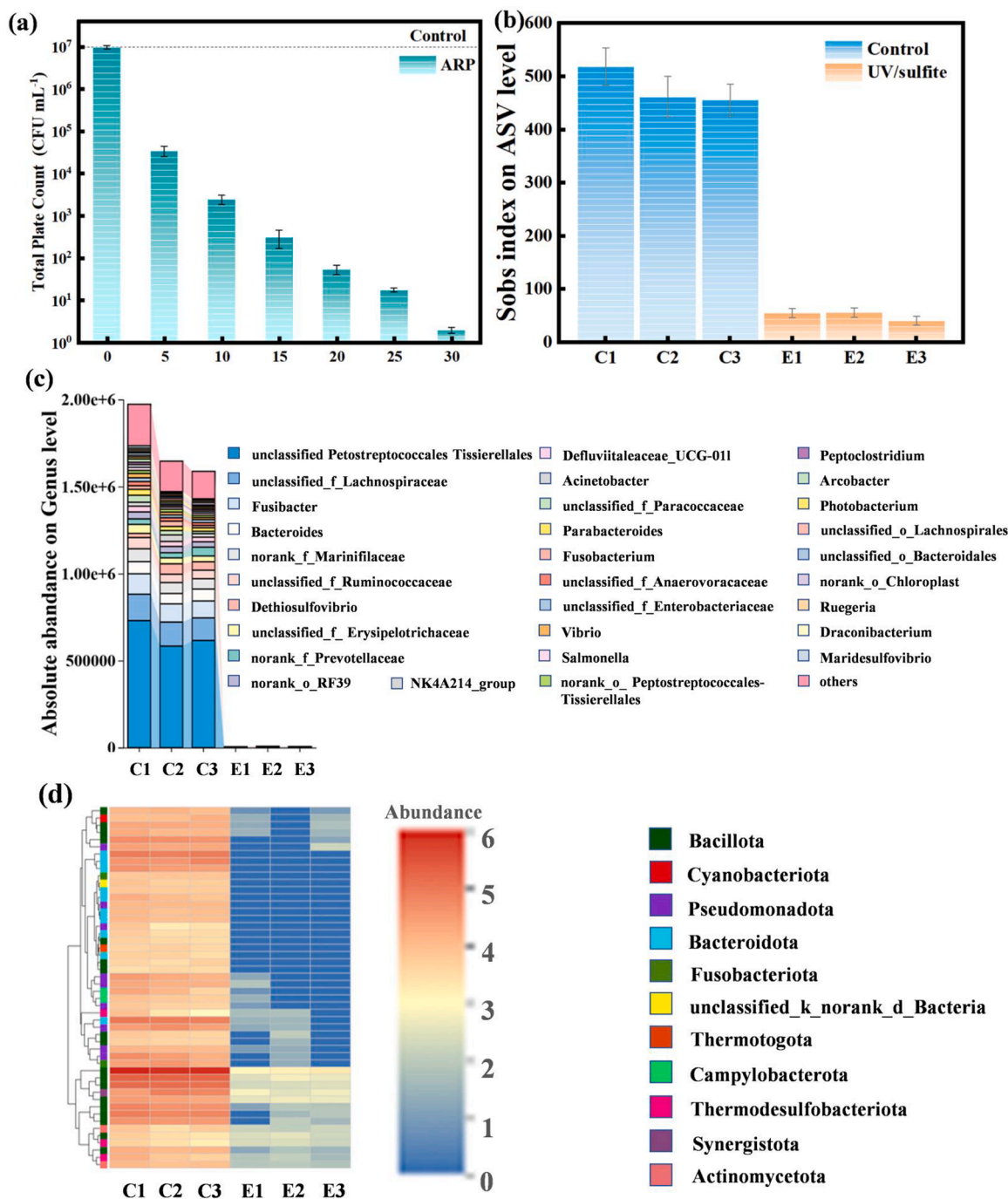


Fig. 5. Disinfection performance under standardized experimental conditions in real marine aquaculture water. (a) Temporal change in total plate counts. (b) Alpha diversity (Sobs index) of microbial communities. (c) Microbial community composition at the genus level. (d) Heatmap of dominant microbial genera. ([sulfite] = 1 mM, UV fluence rate = 2.75 mW cm⁻², salinity = 12 ‰, [Cl⁻] = 6.51 g L⁻¹, [Br⁻] = 22.28 mg L⁻¹, [DOC] = 0.68 mg L⁻¹, T = 298.15 K, pH = 8, treatment time = 60min).

metatranscriptomic approaches are required to evaluate the ecological performance of UV/sulfite disinfection under realistic aquaculture conditions.

3.6. Disinfection performance and DBPs control of the UV/sulfite system in real marine aquaculture water

To evaluate the practical applicability of the UV/sulfite system, its disinfection efficacy and ability to mitigate the formation of DBPs were assessed in real marine aquaculture water. According to the Seawater Quality Standard of China (GB 3097-1997), the total plate count serves as a key parameter for assessing water quality in mariculture environments and provides a crucial indicator of disinfection efficiency. UV-alone treatment was first conducted and presented in Fig. S26. UV irradiation alone achieved approximately 2.75-log reduction of total bacteria within 30 min, indicating that direct UV photolysis contributed to bacterial inactivation. However, UV-induced inactivation is mainly associated with DNA photolesions and such damage can be repaired by microbial photoreactivation or dark-repair pathways. In contrast, as shown in Fig. 5a, UV/sulfite treatment rapidly reduced the total bacterial count from approximately 10^7 CFU mL⁻¹ to near the detection limit within 30 min, corresponding to a reduction of more than 6-log reduction under the same treatment time, and no observable bacterial recovery was detected after treatment. The results suggest that sulfite addition changed the damage pattern from relatively repairable UV-induced DNA photodamage to more extensive multi-target injury. Because direct UV photolysis and sulfite-mediated reductive reactions occur simultaneously and are intrinsically coupled, their absolute contributions cannot be strictly separated in real marine aquaculture water. The enhanced disinfection performance of UV/sulfite is attributed to the combined effects of UV-induced DNA photodamage and RRS-mediated

irreversible biomolecular destruction.

The impact on microbial community structure was further investigated using alpha diversity indices and compositional analyses. Alpha diversity results (Fig. 5b) revealed that the control groups (C1, C2, C3) exhibited relatively high Sobs index values (450–550), reflecting a diverse and stable microbial community. In contrast, the UV/sulfite-treated groups (E1, E2, E3) showed a marked reduction in diversity, with Sobs index values decreasing to 50–100, indicating effective suppression of microbial diversity. Community composition analysis (Fig. 5c) indicated that genera such as *Peptostreptococcales*, *Lachnospiraceae* and *Fusibacter* dominated in the control groups, whereas pathogenic genera including *Vibrio* and *Salmonella* were also present. Following UV/sulfite treatment, the abundance of all microbial groups decreased substantially. Heatmap analysis (Fig. 5d) confirmed these changes, showing that pathogenic genera were nearly eliminated, and the overall microbial load was significantly reduced. Collectively, these findings indicate that the UV/sulfite system is highly effective at suppressing pathogenic microorganisms in marine aquaculture water.

Moreover, an effective disinfection method should not only demonstrate effective microbial inactivation but also control the formation of DBPs, which is crucial for mitigating both environmental and health risks associated with water treatment. As shown in Fig. 6a, the formation of DBPs varied significantly across different UV-based systems. Quantitative analysis revealed that the total DBPs in the UV/sulfite system were approximately $1.61 \mu\text{g L}^{-1}$, significantly lower than those observed in the UV-AOPs, including UV/NaClO ($88.00 \mu\text{g L}^{-1}$), UV/H₂O₂ ($82.50 \mu\text{g L}^{-1}$), UV/PDS ($77.00 \mu\text{g L}^{-1}$), as well as UV/NH₂Cl ($4.75 \mu\text{g L}^{-1}$) and UV/ClO₂ ($4.71 \mu\text{g L}^{-1}$). Further composition analysis indicated that THMs predominantly consisted of TCM, BDCM, and DBCM, with TBM detectable in some systems (Fig. 6b). For HAAs, DCAA and TCAA were the primary species, along with a certain proportion of brominated HAAs (e.

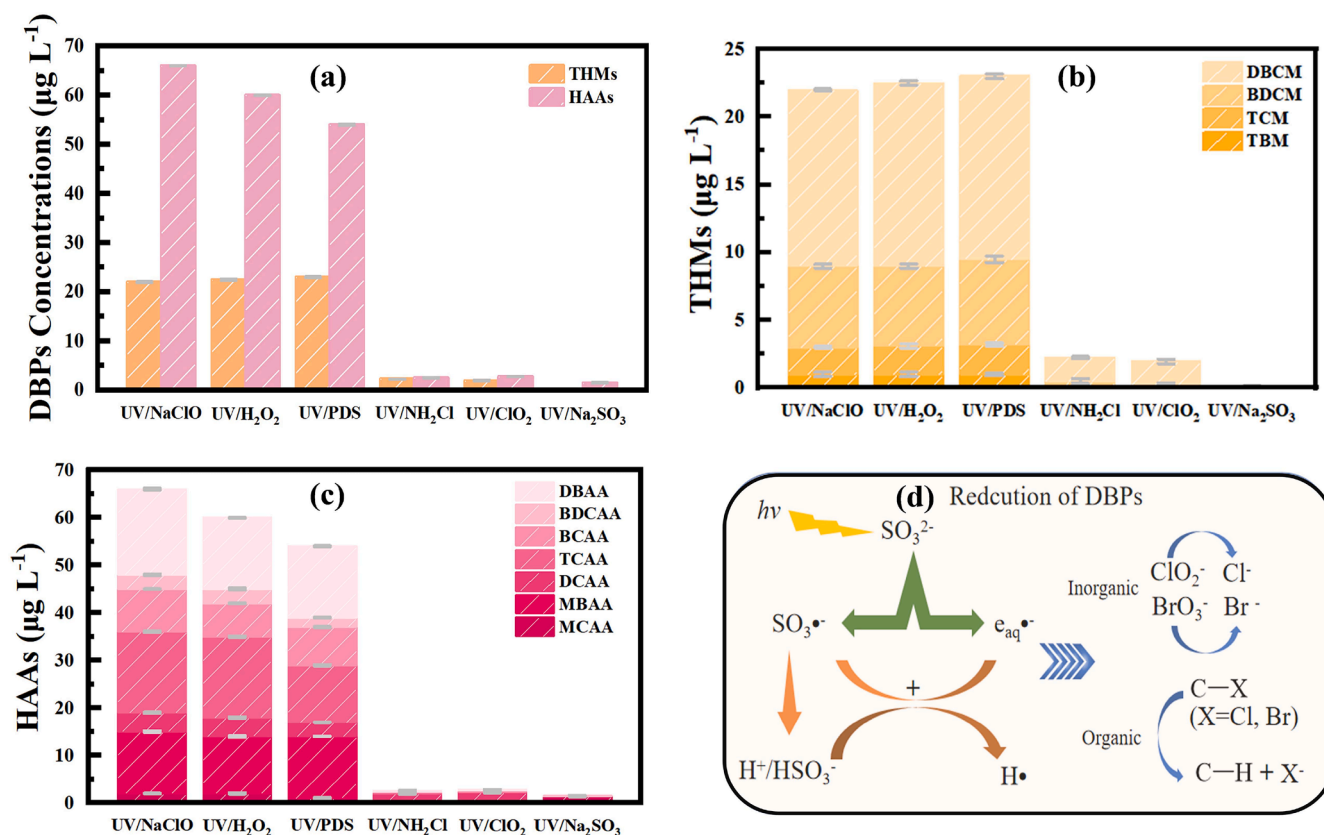


Fig. 6. A preliminary comparison of DBPs formation under standardized experimental conditions in real marine aquaculture water. (a) Total concentrations of DBPs. (b) and (c) Speciation of THMs and HAAs. (d) Schematic illustration of DBPs reduction mechanism in the UV/sulfite system. ([sulfite] = 1 mM, UV fluence rate = 2.75 mW cm^{-2} , salinity = 12 ‰, $[\text{Cl}^-] = 6.51 \text{ g L}^{-1}$, $[\text{Br}^-] = 22.28 \text{ mg L}^{-1}$, $[\text{DOC}] = 0.68 \text{ mg L}^{-1}$, $T = 298.15 \text{ K}$, $\text{pH} = 8$, treatment time = 60min).

g., BDCAA, DBAA) (Fig. 6c). Although H_2O_2 and PDS do not directly introduce free chlorine into the system, seawater inherently contains high concentrations of Cl^- and Br^- . Strong oxidizing reactive species generated in these systems, particularly $\bullet\text{OH}$ in UV/ H_2O_2 and $\text{SO}_4^{\bullet-}/\bullet\text{OH}$ in UV/PDS, can promote the activation of halide ions and the subsequent formation of reactive chlorine/bromine species. These reactive halogen species can further react with organic precursors in seawater and contribute to the formation of halogenated DBPs. These results suggest that oxidative disinfection systems promote halogen substitution and the transformation of organic precursors, leading to higher DBPs formation. In contrast, the UV/sulfite system operates via a different mechanism, generating RRS, primarily e_{aq}^- and $\bullet\text{H}$. These RRS can react with halogenated organic compounds, breaking C-X bonds through dehalogenation or chain-shortening reactions, thereby effectively limiting the formation of toxic halogenated DBPs (Fig. 6d) (Zhang et al., 2022; Gu et al., 2016).

As one of the most concerned inorganic DBPs, BrO_3^- was not detected in the UV/sulfite system under all tested conditions. Previous researches have shown that ARPs can effectively reduce BrO_3^- through the synergistic action of e_{aq}^- and $\bullet\text{H}$. e_{aq}^- reduces BrO_3^- to Br^- directly, a thermodynamically favorable process under neutral pH conditions (Xiao et al., 2017b). Meanwhile, $\bullet\text{H}$ contributes to the reduction of BrO_3^- by breaking its Br-O bonds, thereby preventing its accumulation and formation during disinfection (Fig. 6d) (Xiao et al., 2017c). By contrast, BrO_3^- formation is common in several AOPs, where Br^- is oxidized to reactive bromine intermediates (e.g., HOBr/OBr) and subsequently converted to BrO_3^- through reactions involving O_3 , $\bullet\text{OH}$, and other oxidizing radicals (Gunten and Oliveras, 1998; Fang and Shang, 2012; Lee et al., 2020). The UV/sulfite system effectively minimizes organic and inorganic DBPs while maintaining high disinfection efficiency, making it a promising strategy for safe water treatment. Although the UV/sulfite-based ARP demonstrates advantages in minimizing DBPs formation compared with representative AOPs (Table S8), the present DBPs assessment represents a preliminary comparison under standardized conditions. Future studies should conduct DBPs comparisons at equivalent log-inactivation levels.

Besides the formation of DBPs during UV/sulfite treatment, the fate of sulfur-containing residuals derived from sulfite addition should also be considered for practical aquaculture applications. During UV/sulfite treatment, sulfite is activated to generate RRS, the generated sulfur intermediates (e.g., $\text{SO}_3^{\bullet-}$ and other sulfur oxyanion radicals) can be progressively converted into sulfate, which represents the major stable sulfur-containing product under oxygenated aquatic conditions (Text S10) (Seid et al., 2020). Even under a conservative assumption that all added sulfite (1 mM) is completely oxidized to sulfate, the maximum sulfate increase would be only 1 mM ($\sim 96 \text{ mg L}^{-1}$), which represents a minor perturbation relative to the background sulfate levels in marine (2700 mg L^{-1}) and brackish-water systems. Sulfate is an abundant inorganic ion in aquaculture environments and exhibits relatively low biological reactivity at environmentally relevant concentrations (Wang et al., 2016; Cao et al., 2022). Therefore, its formation is unlikely to induce adverse effects on cultured organisms. However, residual sulfite requires further consideration because excessive sulfite may consume DO during oxidation. Thus, aeration before water reuse are recommended to promote sulfite oxidation and ensure the ecological safety of UV/sulfite-treated aquaculture water.

4. Conclusion

This study demonstrates that UV/sulfite-driven ARP is an effective strategy for microbial control in marine aquaculture water. Unlike conventional oxidative disinfection, this system relies on RRS, mainly e_{aq}^- and $\bullet\text{H}$, to induce irreversible microbial inactivation. The enhanced inactivation performance, suppressed post-treatment recovery, and active-species quenching results collectively indicate that reductive pathways provide additional lethal effects beyond direct UV irradiation.

Mechanistic analyses revealed that UV/sulfite treatment damages microorganisms through multiple coordinated pathways. At the cellular level, reductive stress disrupts cell wall/membrane integrity, promotes intracellular leakage, and induces extracellular DNA release and degradation. At the molecular level, model compound experiments, LC-MS/MS analysis, and DFT calculations confirmed that RRS preferentially attack cell wall precursors, nucleic acid-related structures, and amino acid residues, thereby impairing envelope stability, genetic integrity, and protein functionality. Transcriptomic responses further showed that this structural and molecular damage was accompanied by broad dysfunction of ribosome biogenesis, DNA repair, protein homeostasis, membrane transport, and energy metabolism.

Application in real marine aquaculture water further verified the practical potential of the UV/sulfite system. The process effectively reduced bacterial populations and suppressed potentially pathogenic bacteria while maintaining low levels of organic and inorganic DBPs. These findings suggest that UV/sulfite can simultaneously achieve microbial inactivation and DBPs control in complex seawater matrices. Overall, this work provides mechanistic insight into reductive bio-inactivation and supports UV/sulfite as a promising low-risk disinfection approach for marine aquaculture water. However, pilot-scale studies under realistic marine aquaculture conditions are needed to assess the effects of reagent optimization, energy demand, operational stability, and treatment scalability on the overall feasibility of UV/sulfite. Besides, future studies will integrate accumulated kinetic datasets, microbial response signatures, and long-term operational data to develop machine learning-based predictive frameworks for optimizing reductive radical-mediated disinfection processes and predicting microbial inactivation efficiency, DBPs formation risks, and ecological safety. Such data-driven approaches will facilitate the optimization, control, and scale-up of ARPs for practical marine aquaculture water disinfection applications.

CRedit authorship contribution statement

Yuanyuan Zhang: Writing – review & editing, Writing – original draft, Investigation. **Xudong Li:** Investigation. **Fangyu Liang:** Investigation. **Pin Gan:** Methodology. **Pengfei Xue:** Methodology. **Jiayuan Liang:** Writing – review & editing, Writing – original draft. **Kefu Yu:** Writing – review & editing, Supervision.

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 materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2026.127119](https://doi.org/10.1016/j.watres.2026.127119).

Data availability

Data will be made available on request.

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