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RESEARCH ARTICLE

Effects of simultaneous and sequential irradiation with pulsed ultraviolet, visible, and near-infrared light on microbial inactivation

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Abstract

The bactericidal efficacy of simultaneous and sequential irradiation with ultraviolet (UV), visible (VIS), and near-infrared (NIR) light was evaluated using an intense pulsed light (IPL) device equipped with optical bandpass filters. *Salmonella enterica* serovar Enteritidis, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* were subjected to three treatment modes: simultaneous UV + VIS + NIR, sequential UV followed by VIS + NIR [UV-(VIS + NIR)], and sequential VIS + NIR followed by UV [(VIS + NIR)-UV]. Simultaneous irradiation achieved ≥ 5 log reductions at lower energy doses than both sequential irradiation modes. Between sequential treatments, (VIS + NIR)-UV exhibited faster microbial reduction than UV-(VIS + NIR), highlighting the importance of irradiation sequence. Notably, (VIS + NIR)-UV induced a photoreactivation mechanism that increased the *S. aureus* population. These results demonstrate that both spectral composition and timing of exposure significantly affect microbial susceptibility to IPL. The findings provide new insights into optimizing wavelength integration and sequencing strategies for effective nonthermal microbial inactivation in food safety applications.

Keywords Microbial inactivation · Ultraviolet light · Visible light · Near-infrared light · Simultaneous treatment · Sequential treatment

Introduction

Food safety is becoming ever more important due to the increasing demand for processed food. Common sterilization methods used in the food industry include heat treatment and gamma irradiation. Thermal treatment is the most widely used technologies due to its reliable sterilization effects in commercial applications and the ease of integrating it into existing processes with simple equipment. However, thermal treatment can cause undesirable subjective as well as quantifiable quality changes. These quality changes include alterations in color and taste, as well as the degradation of heat-sensitive nutrients (Albaali, 2023; Awuah et al., 2007). Irradiation-based technologies, such as gamma irradiation, have been applied as alternative nonthermal sterilization methods because they can effectively inactivate heat-resistant microorganisms while minimizing thermal damage (Jumbe and Suleiman, 2024; Li and Farid, 2016). According to the Joint Expert Committee on Food Irradiation convened by the Food and Agriculture Organization in 1980, the World Health Organization, and the International Atomic Energy Agency, irradiation doses of up to 10 kGy do not pose a toxicological hazard (Joint FAO/IAEA/WHO Study

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Group, WHO TRS No. 890, 1999; Roberts, 2014). Nevertheless, some consumers remain apprehensive about this technology since it involves the use of ionizing radiation. Due to these limitations, there is a growing need for novel sterilization technologies that can ensure food safety while maintaining quality.

Light irradiation is a nonthermal sterilization technology used for the microbial inactivation of foodborne pathogens. This method not only ensures microbial safety but also helps to prevent food deterioration, since it relies on light exposure without heat generation. Irradiation with ultraviolet light (UV) is a well-known and extensively studied technology. The UV spectrum is divided into three main components: ultraviolet-C (UVC, 200–280 nm), ultraviolet-B (UVB, 280–315 nm), and ultraviolet-A (UVA, 315–400 nm). Birmpa et al. (2013) applied UVC irradiation to *Escherichia coli*, *Listeria innocua*, *Salmonella enterica* serovar Enteritidis, and *Staphylococcus aureus* cells inoculated on freshly cut produce. The maximum reduction observed was 1.21–1.75 log CFU/g on lettuce and 1.0–1.4 log CFU/g on strawberries, with no significant color changes observed after exposure for up to 45 min. These findings indicate that conventional UV treatment can preserve product quality but provides only moderate levels of microbial inactivation, thereby motivating the exploration of more efficient light-based disinfection approaches. Intense pulsed light (IPL) is another emerging light-based technology involving irradiation at wavelengths ranging from 200 to 1100 nm, encompassing UV, visible light (VIS, 400–700 nm), and near-infrared light (NIR, 700–1100 nm). IPL has been shown to be effective at decontaminating blueberries while causing no significant changes to their physical properties (e.g., weight loss or firmness) and having only a minimal impact on their biochemical properties (e.g., color, total anthocyanin content, and total antioxidant activity) (Cao et al., 2017). IPL treatment is considered a more-effective and rapid method than UV for microbial inactivation, since the energy is amplified manifold (Dunn, 1996). Cheigh et al. (2013) reported that IPL treatment (180 s, total fluence: 1.6 J/cm^2) achieved a 6 log reduction in *Listeria monocytogenes*, whereas UVC treatment (1000 s, total fluence: 0.08 J/cm^2) resulted in a 4 log reduction, indicating that the higher energy delivered by IPL enabled greater microbial inactivation within a shorter irradiation time than conventional UVC. This difference reflects the distinct temporal energy delivery of pulsed light, which concentrates energy into short, high-intensity pulses, unlike the gradual energy supply of continuous irradiation.

While the precise mechanisms underlying microbial reductions induced by light irradiation have not yet been fully elucidated, they are generally thought to involve photochemical, photophysical, and photothermal mechanisms. Irradiation in the UV region has been shown to exert the strongest microbial inactivation via photochemical mechanisms. UVC is absorbed by the pyrimidine bases of DNA, leading to the breaking of thymine–adenine double bonds and the subsequent formation of thymine–thymine dimers. This DNA deformation inhibits replication and transcription that ultimately results in microbial death. VIS causes damage to microbial membranes via a photophysical mechanism. VIS light can induce oxidative damage via excitation of endogenous photosensitizers, leading to reactive oxygen species (ROS) generation and membrane disruption (Biscaglia and Gobbo, 2018; Komagoe et al., 2011; Spagnul et al., 2017). Microbial inactivation by light irradiation can also be attributed to photothermal effects, which lead to bacterial disruption and structural changes in target microorganisms when sufficient light energy is absorbed and converted into heat (Takeshita et al., 2003; Wekhof, 2000; Wuytack et al., 2003). Ferrario et al. (2014) reported morphological damage in *Saccharomyces cerevisiae* cells treated with IPL, and this damage increased with increasing IPL dose.

In this study, the contributions of radiation covering different wavelength ranges (UV and VIS + NIR) to microbial inactivation under simultaneous and sequential irradiation conditions were investigated, and the effect of irradiation sequence on inactivation efficiency was evaluated. Gram-negative bacteria (*S.* Enteritidis), Gram-positive bacteria (*S. aureus*), and yeast (*S. cerevisiae*) were selected as model microorganisms to represent distinct cell envelope structures and DNA repair capacities, enabling evaluation of wavelength-dependent and sequence-dependent inactivation behaviors across taxonomically and structurally diverse microorganisms. In addition, the comparison between simultaneous and sequential irradiation strategies was designed to determine whether the temporal order of exposure to different wavelength regions influences microbial susceptibility, which is critical for optimizing multi-wavelength IPL-based nonthermal disinfection processes.

Materials and methods

Experimental setup

The bespoke pilot-scale IPL device designed for this study (Fig. 1) consisted of a power supply, treatment chamber, and chilling system. The capacitor in the power supply was charged before being discharged rapidly to the xenon lamp, generating high-energy pulsed light covering a relatively wide range of wavelengths. To prevent overheating, two cooling fans were installed on the sides of the treatment chamber, and cooling water was circulated continuously around the xenon lamp. The sample was positioned 12 cm beneath the lamp in a foil-covered box that blocked ambient light. A bandpass optical filter placed above the

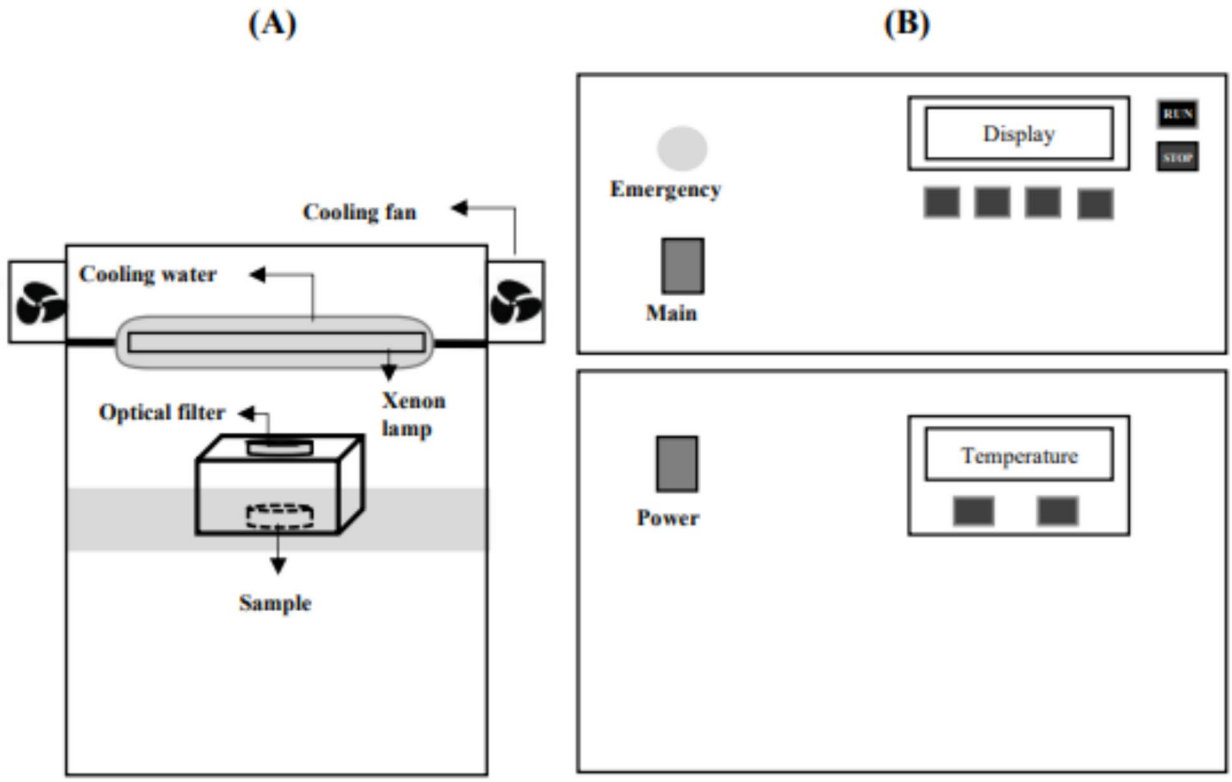


Fig. 1 The IPL device used in this study: **A** treatment chamber and **B** power supply (upper) with chilling system (lower)

box allowed radiation within specific wavelength ranges to reach the sample.

The light emitted by the IPL device covered a broad spectrum ranging from 200 to 1050 nm. The samples were irradiated over specific wavelength ranges using bandpass optical filters (Edmund Optics, Barrington, NJ, USA). The two types of filters used in this study were named the UV filter and the VIS + NIR filter according to the wavelength range they primarily transmitted: the UV region (250–350 nm) and the VIS and NIR regions (350–1050 nm), respectively. The energy fluence and spectra transmitted by each filter were measured using a spectroradiometer (ILT-950 and ILT-960, International Light Technologies, Peabody, MA, USA). Preliminary temperature monitoring was carried out before the main experiments to define treatment conditions that exclude photothermal effects. Under all irradiation conditions, the temperature increase inside the treatment chamber was less than 7 °C, confirming that the IPL treatments were conducted under non-thermal conditions.

Bacterial strains

Gram-negative bacteria *S.* Enteritidis (ATCC 13076), Gram-positive bacteria *S. aureus* (ATCC 12692), and yeast *S. cerevisiae* (IFO 1950) were obtained from the Korean Culture Center of Microorganisms. *S.* Enteritidis and *S. aureus* were cultured on tryptic soy agar prepared form trypticase soy broth (BD, Franklin Lakes, NJ, USA) and agar (Samchun Chemical, Pyeongtaek, South Korea), while *S. cerevisiae* was cultured on yeast mold agar (Difco™ Yeast Mold Broth, BD; Agar, Samchun Chemical). All cultures were stored at 4 ℃ until being used further in the experiments.

A single colony of *S.* Enteritidis and *S. aureus* from each plate was inoculated into 10 mL of tryptic soy broth and incubated at 37 °C for 24 h. For *S. cerevisiae*, yeast mold broth was used as the medium, and incubation was performed at 30 °C for 48 h. Subsequently, 1 mL of the incubated medium was transferred into 100 mL of tryptic soy broth and incubated under the same conditions as in the previous step. For *S. cerevisiae*, yeast mold broth and incubation at 30 °C for 48 h were applied in this step.

Each strain was prepared and treated individually throughout the experiments. Cells were harvested by centrifugation at 4000 rpm for 15 min at room temperature. The supernatant was discarded, and the cells were washed three times with 10 mL of sterilized 0.85% NaCl solution. The final pellets were resuspended in 0.85% NaCl solution to produce an initial population of 10^5 CFU/mL. Finally, 2 mL of the suspension was transferred to a 90 × 15 mm petri dish and used for light irradiation treatments. Sterilized

0.85% NaCl solution was employed as the medium to maintain isotonic conditions while minimizing the influence of nutrients or organic components on microbial survival and light-induced inactivation.

Simultaneous and sequential light irradiation treatments

Simultaneous and sequential treatments were performed to assess the synergistic or inhibitory effects of radiation covering different wavelength regions on microbial inactivation. The experiments were conducted under three distinct conditions. In the first condition, the radiation emitted by the IPL device over the entire wavelength range was utilized without any filter, referred to as UV + VIS + NIR. In the second condition, the UV and VIS + NIR filters were applied sequentially, with the radiation within the UV region administered first, followed by that within the VIS and NIR regions; this protocol was designated as UV-(VIS + NIR). In the third condition, the radiation within the VIS and NIR regions was applied first, followed by that within the UV region, and this was labeled as (VIS + NIR)-UV.

All microorganisms were exposed to radiation until the total energy fluence reached a maximum of 0.62 J/cm^2. In the simultaneous treatment, the energy fluence ratios for UV relative to VIS and NIR were determined to be 12.20% and 87.79%, respectively. These ratios were maintained in the sequential treatments, where radiation within the UV region was applied at 0.08 J/cm^2, and that within the VIS and NIR regions was administered at 0.54 J/cm^2 for the maximum fluence. The xenon lamp was operated under the following conditions: a charging voltage of 2400 V, a frequency of 5 Hz, a charging time of 100 ms, and a pulse width of 0.5 ms, with a maximum treatment time of 300 s.

Photoreactivation

The above-described experiments demonstrated that subsequent VIS + NIR treatment reduced the microbial inactivation induced by UV treatment, even compared with UV-only treatment. The mechanism underlying this inhibition was investigated by performing an additional experiment in which *S.* Enteritidis cells were exposed to UV at an energy fluence of 0.05 J/cm^2, which did not completely destroy all cells, resulting in a survival count of 1.94 ± 0.33 log CFU/mL (mean ± SD). Following UV treatment, VIS + NIR treatment was applied at energy fluences of 0.10 and 0.60 J/cm^2.

Microbiological analyses

After IPL treatment, the suspensions were serially diluted with 0.85% NaCl solution. Then, 0.1 mL of the diluted suspensions containing *S.* Enteritidis and *S. aureus* were spread onto tryptic soy agar and incubated at 37 °C for 24 h. Similarly, 0.1 mL of the diluted suspensions containing *S. cerevisiae* was spread onto yeast mold agar and incubated at 30 °C for 48 h. After incubation, the number of colonies that formed was counted. The microbial survival value was expressed as log N, where N represents the number of surviving cells, and microbial reduction of cells was calculated as $\log(N_0/N)$, where N_0 is the initial density of the suspension sample.

Statistical analyses

All experiments were performed in triplicate. Statistical analyses consisted of descriptive statistics, and the results are expressed as mean ± standard deviation calculated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Results and discussion

Measurement of energy fluence

The energy distribution within each wavelength range, with and without optical filters, is presented in Fig. 2. In the absence of a filter, the emitted IPL covered a broad spectral range from 200 to 1050 nm, with a fluence per pulse of 11,533.76 μW/cm^2. The fluence contributions from UV, VIS, and NIR were 12.87%, 16.96%, and 70.17%, respectively, indicating that most of the emitted radiation was concentrated in the NIR wavelength region. Applying the UV filter resulted in the transmitted radiation covering wavelengths between 250 and 350 nm, delivering a reduced fluence per pulse of 522.96 μW/cm^2. However, the VIS and NIR regions were not completely blocked, contributing 4.87% and 3.25% of the total fluence, respectively. Similarly, the VIS + NIR filter allowed the transmission of radiation between 350 and 1050 nm, resulting in a fluence per pulse of 9938.85 μW/cm^2. This filter also allowed a small amount of UV radiation to pass through, accounting for 3.19% of the total fluence. The detailed distributions of the UVC, UVB, and UVA fluences are presented in Table 1. Notably, the pulse fluence in the VIS and NIR regions was approximately 19 times higher than that in the UV region, highlighting the predominance of the VIS and NIR wavelength ranges in the total fluence exposure of the microorganisms. This spectral distribution suggests that differences observed between simultaneous and sequential irradiation modes cannot be attributed solely to UV dose, but rather to the interaction between wavelength composition and irradiation sequence.

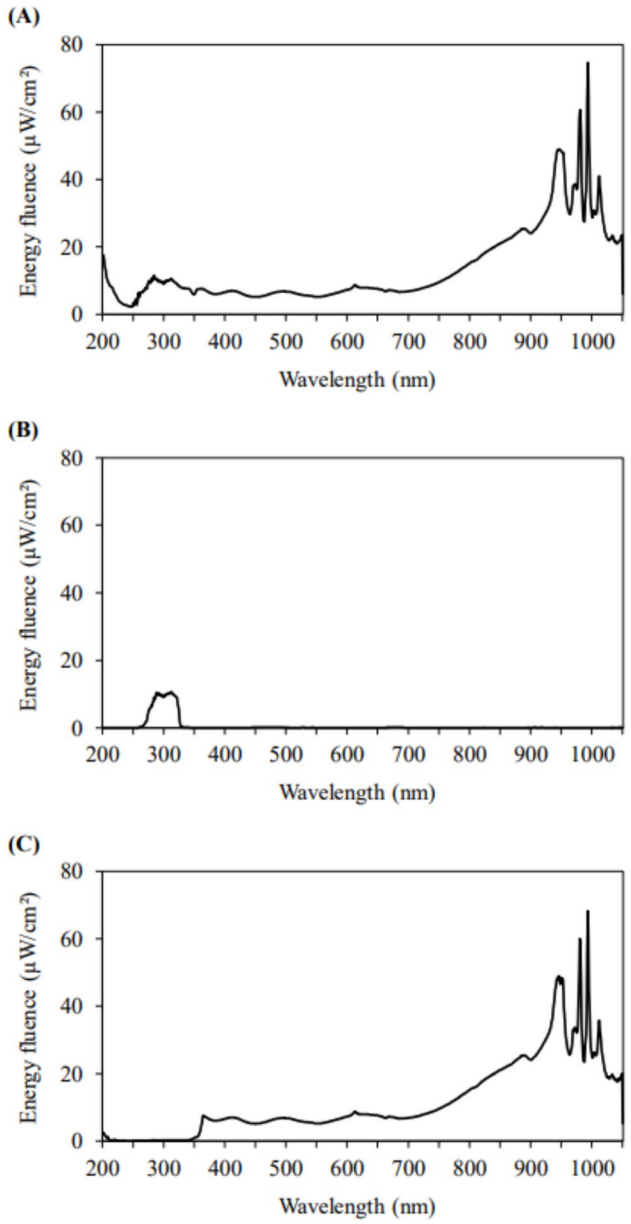


Fig. 2 Energy spectral distribution of a single IPL pulse: **A** without any optical filter, **B** with the UV filter, and **C** with the VIS + NIR filter

Comparison of microbial inactivation according to light wavelength

The microbial reductions obtained from the simultaneous and sequential irradiation treatments with UV, VIS, and NIR are presented in Fig. 3. UV + VIS + NIR treatment resulted in *S.* Enteritidis exhibiting a rapid decrease in survival until a fluence of 0.17 J/cm^2, after which a tailing effect was observed. In contrast, the UV-(VIS + NIR) and (VIS + NIR)-UV treatments resulted in sharp reductions in microbial survival after reaching a fluence of 0.40 J/cm^2. When *S.* Enteritidis first reached undetectable levels (< 1 CFU/mL) under UV + VIS + NIR treatment at a total fluence of 0.29 J/cm^2, the UV-(VIS + NIR) and (VIS + NIR)-UV treatments produced 1.59 and 2.69 log reductions, respectively. UV-(VIS + NIR) treatment at a fluence of 0.62 J/cm^2 resulted in a 4.59 ± 0.94-log reduction, whereas *S.* Enteritidis was completely undetectable under the (VIS + NIR)-UV condition. A similar trend was observed for *S. aureus* under UV + VIS + NIR treatment, while a gradual decrease in survival was noted across all conditions for the UV-(VIS + NIR) and (VIS + NIR)-UV treatments. At the highest applied fluence, *S. aureus* exhibited a 2.64 log reduction for UV-(VIS + NIR) and a 3.69 log reduction for (VIS + NIR)-UV, while UV + VIS + NIR treatment reduced its survival to nondetectable levels from a fluence of 0.29 J/cm^2 onward.

The survival pattern of *S. cerevisiae* differed from the trends observed for the other two microorganisms. Under the UV + VIS + NIR condition, *S. cerevisiae* displayed a continuous decrease in survival as the fluence increased, while minimal variations were observed under the UV-(VIS + NIR) and (VIS + NIR)-UV treatments. Specifically, 1.06 and 1.29 log reductions were recorded for the UV-(VIS + NIR) and (VIS + NIR)-UV treatments, respectively, whereas UV + VIS + NIR treatment resulted in a more-substantial 4.29 log reduction. Among the three tested microorganisms, the inactivation effect was greater for the UV + VIS + NIR simultaneous treatment than for the UV-(VIS + NIR) and (VIS + NIR)-UV sequential treatments. These findings indicate that simultaneous UV + VIS + NIR irradiation achieved greater inactivation than either sequential mode at the same total fluence, whereas sequential treatments did not yield comparable enhancement. To further contextualize these differences in survival patterns,

Table 1 Proportions and fluences of IPL according to the use of the two filters

Filter	UVC (%)	UVB (%)	UVA (%)	VIS (%)	NIR (%)	Energy fluence per pulse (μW/cm^2)
No filter	4.52	3.06	5.29	16.96	70.17	11,533.76
UV filter	10.29	64.52	17.07	4.87	3.25	522.96
VIS + NIR filter	0.25	0.07	2.87	19.70	77.11	9938.85

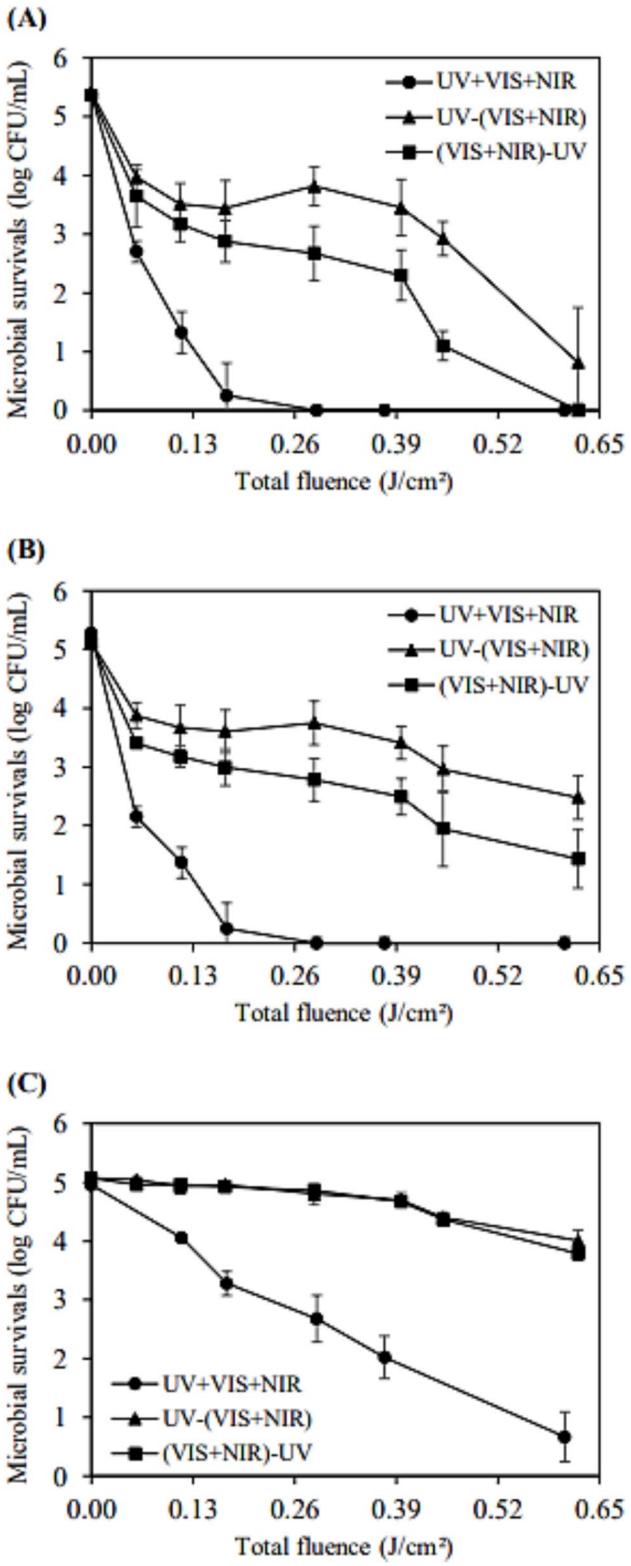


Fig. 3 Survival rates of *S.* Enteritidis (**A**), *S. aureus* (**B**), and *S. cerevisiae* (**C**) under simultaneous and sequential treatments with UV and VIS + NIR. Data are mean and SD values

the relative resistance of the tested microorganisms can be discussed in terms of their structural and physiological characteristics (Kebbi et al., 2020; Wuytack et al., 2003). Gram-positive bacteria such as *S. aureus* possess a thick peptidoglycan layer that may partially shield intracellular targets from UV penetration, whereas Gram-negative bacteria such as *S.* Enteritidis have a thinner peptidoglycan layer but an outer membrane that can influence light interaction and oxidative stress sensitivity (Kebbi et al., 2020; Song et al., 2019a). In contrast, *S. cerevisiae* is a eukaryotic microorganism with a rigid cell wall composed of glucans and mannoproteins and a more complex DNA repair system, which may contribute to its comparatively higher resistance to light-based treatments (Ferrario et al., 2014; Rastogi et al., 2010).

Lu et al. (2021) demonstrated that the combination of 271- and 288-nm UV exerted synergistic effects on the inactivation of *E. coli* O59:H21, *Staphylococcus epidermidis*, and *Salmonella enterica* serovar Typhimurium. Notably, the reduction achieved through simultaneous irradiation at multiple wavelengths exceeded the sum of the reductions observed for individual wavelengths. The maximum synergy ratios, calculated by comparing the log-reduction value for the simultaneous treatment with the sum for the individual treatments, were 0.33, 0.48, and 0.87, respectively. Similarly, Chevremont et al. (2012) found that irradiation at 254, 280, 365, and 405 nm produced 1.7-, 0.8-, 0.5-, and 0.7-log microbial reductions, respectively, for *E. coli* CIP 6224. However, synergistic effects were found when these wavelengths were combined, with 2.3-, 2.0-, and 1.5-log reductions for irradiation at 254 and 365 nm, 280 and 405 nm, and 280 and 365 nm, respectively. Likewise, Kang et al. (2019) reported that simultaneous irradiation with UVC and UVA or visible light resulted in greater microbial inactivation than single-wavelength exposure, indicating that interactions between different spectral regions can enhance disinfection efficiency. In addition, Akgün et al. (2017) demonstrated that combined UV-LED treatments at 280 and 365 nm improved microbial reduction in food matrices compared with single-wavelength irradiation. Chen et al. (2023) further showed that sequential application of different wavelength regions modulated microbial responses depending on the irradiation order, highlighting the importance of temporal arrangement in multi-wavelength strategies. These results collectively indicate that simultaneous treatments can be more effective than single-wavelength treatments, demonstrating synergistic enhancement beyond additive effects in previous studies. This synergy has also been observed in the VIS spectrum. Xiao and Zhang (2025) confirmed that the combination of UVA and blue light (420 nm) enhanced the microbial reductions. The enhanced inactivation observed under

simultaneous irradiation may involve multiple inactivation pathways reported in previous studies, such as protein damage, DNA damage, and ROS-related processes (Matafonova and Batoev, 2022).

The present study showed that (VIS + NIR)-UV treatment was more effective than UV-(VIS + NIR) treatment in inactivating *S.* Enteritidis and *S. aureus*. To further investigate this phenomenon, the data in Fig. 4 were reanalyzed based on UV energy fluence and subsequently compared with the findings for UV-only treatment (data not shown). This comparison revealed no significant difference between the UV-only and (VIS + NIR)-UV treatments, indicating that pre-exposure to VIS + NIR did not contribute to any additional notable inactivation or inhibition effects before UV irradiation. However, the inactivation effect was smaller for UV-(VIS + NIR) treatment than for UV-only treatment, indicating that subsequent VIS + NIR exposure was associated with reduced net inactivation compared with UV alone. Song et al. (2019a) found that applying UVA treatment before UVC irradiation enhanced the inactivation of *E. coli* and even reduced its reactivation, whereas the inactivation was similar for simultaneous treatment and post-UVC treatment. Xiao et al. (2018) and Nyangaresi et al. (2023) similarly demonstrated the potential of UVA pretreatment. A similar trend was observed between UVA and UVB (Song et al., 2019b), where sequential UVA-then-UVB treatment resulted in greater inactivation than UVB-then-UVA treatment. These findings highlight the importance of the treatment timing. Consequently, the inhibitory effect of VIS and NIR treatment after UV treatment was further confirmed by performing additional experiments in the present study.

Photoreactivation according to energy fluence of VIS and NIR

Among the tested microorganisms, *S.* Enteritidis showed the most distinct photoreactivation-like response after UV followed by VIS + NIR treatment and was therefore selected for further investigation of the photoreactivation mechanism. It is hypothesized that the above-described effects related to treatment order are due to VIS + NIR counteracting microbial inactivation induced by UV treatment. To verify this hypothesis, *S.* Enteritidis cells were treated with VIS + NIR at 0.1 and 0.6 J/cm^2 immediately after UV treatment at 0.05 J/cm^2 (Fig. 4). UV treatment of the *S.* Enteritidis suspension reduced microbial survival to 1.94 log CFU/mL. However, the subsequent VIS + NIR treatments at energy fluences of 0.1 and 0.6 J/cm^2 resulted in microbial survival increasing to 3.38 and 4.07 log CFU/mL, respectively. The increased microbial survival with higher VIS + NIR fluences is probably attributable to the DNA repair mechanism of photoreactivation.

Cells possess multiple mechanisms to repair DNA damage, including photoreactivation, excision repair, and damage tolerance systems (Rastogi et al., 2010). Among these, photoreactivation is specifically triggered by near-UV and

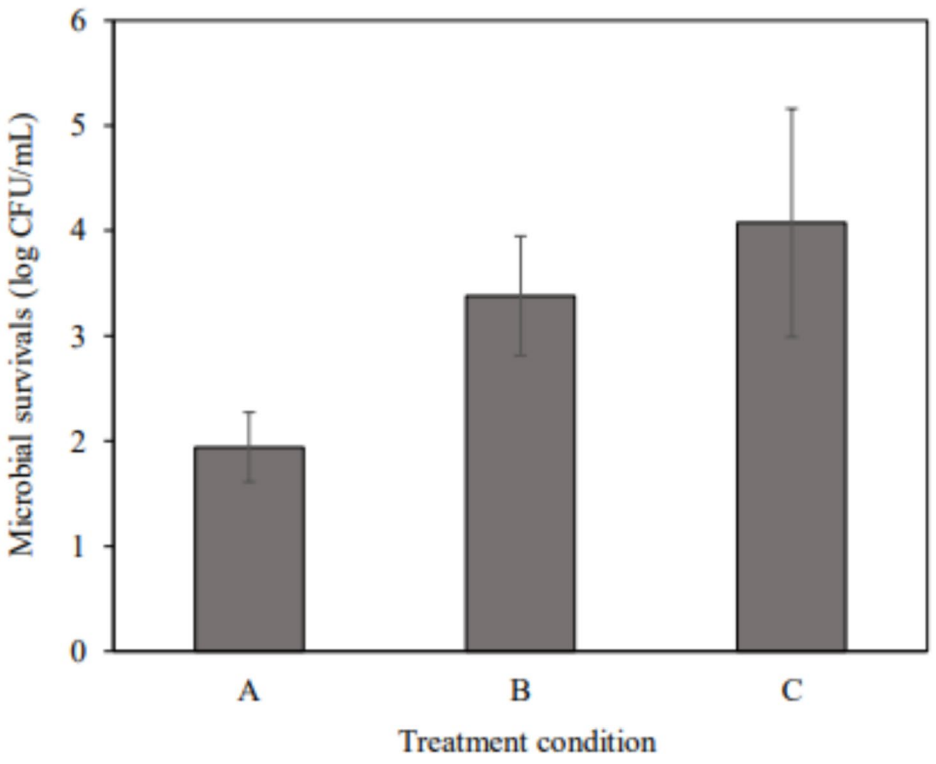


Fig. 4 Photoreactivation of *S.* Enteritidis under different treatment conditions: **a** 0.05 J/cm^2 UV-only treatment, **b** 0.10 J/cm^2 VIS + NIR treatment after 0.05 J/cm^2 UV

blue light, and serves as a mechanism for reversing UV-induced DNA damage (Fitzhenry, 2021). Exposure to UVC in particular leads to the formation of cyclobutane pyrimidine dimers (CPDs), which are covalent bonds between adjacent pyrimidine bases such as thymine–thymine, cytosine–cytosine, and thymine–cytosine bonds (Beggs, 2002). During photoreactivation, the enzyme photolyase binds to CPDs. Photolyase contains two essential cofactors: flavin adenine dinucleotide (FADH) and methylene tetrahydrofolate (MTHF). Upon absorbing blue light at a specific wavelength, MTHF becomes excited and transfers energy to the FADH molecule. The activated flavin then donates a high-energy electron to the CPDs, breaking their cyclobutane ring and restoring the DNA to its original state (Goosen and Moolenaar, 2008). This photoreactivation mechanism provides a plausible explanation for the increase in microbial survival observed after UV-(VIS + NIR) treatment in the present study.

Similar findings have been reported previously. Photoreactivation research has traditionally utilized fluorescent lamps, which emit light within the VIS range. Fitzhenry et al. (2021) demonstrated photoreactivation using fluorescent lamps covering the visible wavelength range (300–700 nm) after pulsed UV treatment delivered at wavelengths below 300 nm (UVC region). Their results showed that at the lowest dose of pulsed UV (486 mJ/cm^2), *S. aureus* exhibited a sharp increase in reactivation within the first 10 to 60 min, after which it stabilized. The extent of reactivation decreased as the UV dose increased, with photoreactivation being negligible at the highest administered dose of 2052 mJ/cm^2. In the case of *E. coli*, photoreactivation increased steadily over 90 min, followed by a tailing effect. However, the degree of reactivation was negligible at UV doses exceeding 1539 mJ/cm^2. These findings suggest that VIS can activate microbial DNA repair mechanisms, although with different microorganisms exhibiting distinct reactivation behaviors.

Furthermore, various factors influence the extent of photoreactivation. Li et al. (2017) investigated the correlation between photoreactivation efficiency and UV energy dose. That study inactivated *E. coli* using 265- and 280-nm LEDs, followed by exposure to light at 300–500 nm. Treatment at 265 nm resulted in 33% and 8.5% photoreactivations for UV doses of 7.27 and 10.91 mJ/cm^2, respectively. A similar trend was noted for the 280-nm treatment, with the 16% photoreactivation for 10.24 mJ/cm^2 decreasing to below 4% for 15.35 mJ/cm^2. These results suggest that increasing the UV energy dose suppresses microbial photoreactivation. Additionally, Salcedo et al. (2007) assessed photoreactivation of coliforms and *Streptococcus faecalis* at various temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, and 30 °C) under fluorescent lamp exposure (310–420 nm), with the findings indicating that higher temperatures were associated with increased photoreactivation. Asymptotic model fitting showed that the survival limit of microorganisms (S_m) increased with the temperature, whereas the kinetic rate constant (k_2) decreased.

In conclusion, this study indicates that microbial responses to broadband IPL are not determined solely by total fluence but are strongly influenced by how different wavelength regions are combined and temporally arranged. The enhanced inactivation observed under simultaneous UV + VIS + NIR exposure suggests that concurrent photochemical, photophysical, and photothermal effects may interact in a complementary manner, whereas sequential application can allow repair-related processes to counteract initial UV-induced damage. In particular, the reduced efficacy observed when VIS + NIR followed UV exposure highlights the practical importance of considering photoreactivation when designing light-based sterilization strategies. From an application perspective, these results emphasize that optimization of IPL treatments should focus not only on energy dose but also on spectral integration and irradiation sequence. Such an approach may contribute to the development of more reliable nonthermal processes while minimizing unnecessary energy input and avoiding conditions that promote microbial recovery.

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