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Visible-Light–Based Photoinactivation of Listeria monocytogenes: Time-Dependent Response and Operational Implications

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Abstract

Foodborne pathogens remain a major public health concern throughout the farm-to-fork chain. Although thermal decontamination methods are effective, they may compromise product quality in certain foods and increase energy demand, creating the need for alternative technologies that operate with minimal heat load. In this context, 405 nm visible light delivered by violet–blue LEDs has emerged as a promising non-chemical approach for microbial inactivation. Exposure at 405 nm can stimulate endogenous chromophores (e.g., porphyrins), promoting the generation of reactive oxygen species and inducing oxidative damage to critical cellular components. The present study investigates the time-dependent photoinactivation response of Listeria monocytogenes under 405 nm LED illumination and discusses its potential applicability for food safety and hygiene practicesExperimental results revealed that a 120-minute exposure to 405 nm LED light achieved a maximum statistically significant reduction of 0.39 $\log_{10}$ CFU/mL ($p < 0.001$) in the Listeria monocytogenes population compared to the non-illuminated control group.

Keywords: Photoinactivation; LED disinfection; Listeria monocytogenes; reactive oxygen species; food safety.

1. Introduction

Foodborne diseases remain a persistent global burden, affecting public health, food industry economics, and consumer trust [1]. Among foodborne pathogens, *Listeria monocytogenes* is of particular concern because it can cause listeriosis [2], a relatively rare but severe illness with a high case-fatality rate, especially in pregnant women, newborns, older adults, and immunocompromised individuals. The World Health Organization highlights listeriosis as one of the most serious foodborne diseases due to its severity and mortality despite comparatively low incidence [3]. In addition, *L. monocytogenes* is widely distributed in the environment (e.g., soil, water, vegetation, animals) and can persist in food-processing settings, where it may contaminate ready-to-eat foods and survive under refrigeration conditions [4]. These traits complicate control strategies and underscore the need for robust, practical interventions across processing and handling steps.

Traditional microbial control in food systems relies heavily on thermal processing and chemical sanitizers [5]. While effective, these methods are not always ideal for all products and contexts. Heat-based treatments can negatively impact sensory and nutritional quality, particularly for fresh, minimally

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processed, or heat-sensitive foods. Chemical disinfectants may leave residues, require rinsing, or show reduced performance in the presence of organic matter [6]; they can also contribute to material corrosion and, in some cases, raise occupational and environmental concerns. Consequently, non-thermal and residue-free approaches—especially those compatible with continuous or in-line implementation—have gained increasing attention in food safety research and industrial practice.

In this context, visible-light disinfection using violet–blue wavelengths has emerged as a promising alternative or adjunct technology. In particular, 405 nm light delivered by light-emitting diode (LED) arrays has been repeatedly shown to inactivate a broad range of microorganisms without the addition of exogenous photosensitizers [7]. Early foundational work demonstrated that 405 nm LED exposure can inactivate medically relevant bacterial pathogens and provided experimental evidence supporting its antimicrobial potential in practical settings [8,9,10]. The underlying concept aligns with photodynamic inactivation: endogenous microbial chromophores most notably porphyrins and related molecules absorb 405 nm photons and transfer energy to molecular oxygen, leading to reactive oxygen species (ROS) formation. ROS, in turn, can damage proteins, lipids, and nucleic acids, disrupting membrane integrity and essential metabolic functions, ultimately causing loss of culturability and viability [11,12]. Although the exact sensitivity varies across organisms and conditions, the core mechanism is generally consistent across many reported studies.

Evidence specifically targeting *L. monocytogenes* supports the susceptibility of this pathogen to high-intensity visible light within the 400–450 nm range, with 405 nm as a principal wavelength of interest. One experimental study investigating *L. monocytogenes* reported that exposures in the 400–450 nm region at sufficiently high dose levels resulted in complete inactivation of a multi-log population, whereas longer visible wavelengths (>450 nm) produced limited effects. This wavelength dependence is consistent with porphyrin absorption features and reinforces the rationale for focusing on 405 nm as an operationally meaningful target for LED-based systems [13].

Beyond laboratory suspensions, subsequent research has explored real food matrices and food-contact surfaces, where optical shielding, scattering, and absorption can influence dose delivery. For instance, 405 nm LED treatment has been evaluated on ready-to-eat fresh salmon surfaces contaminated with *L. monocytogenes* and *Salmonella* spp. under refrigerated and mild temperature conditions. That work illustrates both the feasibility and the complexity of applying visible-light inactivation on foods, because product geometry, surface properties, and storage temperature can affect microbial responses and practical treatment windows. More recent investigations have broadened the scope to multiple pathogens in thin liquid films and on common materials encountered in food processing and food service environments, providing further evidence that 405 nm LED exposure can reduce microbial contamination under conditions relevant to industrial hygiene [14]. Together, these findings motivate continued research into dose–response behavior, optimization of treatment parameters, and integration strategies for real-world deployment.

From an engineering and process-control perspective, 405 nm LED disinfection offers several operational advantages. LEDs are energy-efficient, compact, scalable, and capable of delivering stable narrow-band emission with low maintenance requirements [9]. Arrays can be configured for uniform illumination over defined areas and can be integrated into enclosures, conveyors, cabinets, or surface-treatment modules. Importantly, visible-light approaches are typically positioned as complementary controls rather than complete replacements for standard sanitation programs. Evidence from healthcare settings, where 405 nm environmental disinfection has been evaluated as an adjunct to routine cleaning, similarly emphasizes optimization and integration rather than substitution. This framing is relevant to food facilities as well: visible-light can potentially reduce background contamination during production downtime or in specific zones, while standard cleaning and sanitation remain essential.

Nevertheless, several scientific and practical considerations must be addressed when developing and evaluating 405 nm LED interventions for food safety. First, microbial inactivation depends strongly on delivered dose (irradiance × time), geometry, and medium optical properties. Turbidity, pigments, and surface roughness can reduce effective photon penetration, leading to lower observed reductions compared with clear media. Second, temperature can interact with photoinactivation outcomes. While 405 nm LED systems are non-thermal in the sense that microbial lethality is not primarily driven by heat, LED arrays can generate some thermal load

depending on power and heat-sinking performance; meanwhile, microbial physiology and repair processes also depend on ambient temperature. Therefore, well-designed experiments should monitor temperature and incorporate appropriate controls to isolate photochemical effects.

Third, stress adaptation and sublethal injury deserve attention, particularly for resilient pathogens such as *L. monocytogenes*. Sublethal treatments may alter subsequent resistance to environmental stresses (e.g., oxidative stress, bile salts, osmotic pressure, cold storage), which can have implications for food safety risk if injured cells recover under favorable conditions. A study investigating 405 nm LED effects on *L. monocytogenes* reported changes in resistance patterns and evaluated membrane integrity and stress responses, highlighting that photoinactivation research should include not only immediate reductions but also potential downstream physiological consequences. Such findings support the value of combining plate counts with complementary indicators (e.g., membrane integrity assays, oxidative stress markers) in advanced studies, especially when aiming for process validation.

Fourth, safety and exposure limits must be considered for both product quality and human operators. Although 405 nm is within the visible spectrum and is used in various lighting contexts, high irradiance exposures can induce ROS-related effects in biological systems [16]. Research examining cytotoxic responses to 405 nm exposure has demonstrated associations with ROS production and oxidative stress markers, underscoring the importance of responsible design, shielding, and adherence to photobiological safety guidance when implementing high-intensity visible-light systems [17]. In food contexts, it is also necessary to assess potential impacts on product appearance (color changes), lipid oxidation, and sensory attributes—particularly for high-fat or pigment-rich foods—so that microbial safety gains do not come at the expense of marketability.

Given these considerations, additional experimental work remains important to clarify time- and dose-dependent inactivation behavior, compare results under controlled laboratory conditions versus real matrices, and define parameter ranges that achieve meaningful reductions with feasible processing times. Within this framework, the present study focuses on the inactivation of *Listeria monocytogenes* under 405 nm LED illumination, emphasizing time-dependent response patterns under controlled conditions and providing a basis for subsequent dose modeling and process optimization. By quantifying reductions relative to appropriate controls and discussing mechanistic and operational implications, this work aims to contribute practical evidence supporting the development of visible-light interventions as part of integrated food safety and hygiene strategies.

2. Materials and method

2.1. Microorganism and culture preparation

Listeria monocytogenes ATCC 19111 (American Type Culture Collection) was used as the test organism. Stock cultures stored in Eppendorf tubes were retrieved and activated prior to experiments. Briefly, the organism was inoculated into sterile nutrient broth (NB Oxoid Ltd, UK) and incubated aerobic. The activated culture was subsequently streaked onto nutrient agar (NA) and incubated at 37 °C for 24h to obtain isolated colonies. Pure cultures were confirmed by repeated streaking until single, well-isolated colonies were obtained. Working stocks were refreshed periodically (e.g., every 15 days) to maintain culture viability.

2.2. *Culture dilution procedure*

Following incubation, 1 mL of the microbial suspension was transferred into 9 mL of NB to prepare a 10^{-1} dilution. Serial ten-fold dilutions were continued in the same manner up to 10^{-6}. For the light exposure experiments, aliquots corresponding to 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions were used.

2.3. *405 nm LED light source and exposure setup*

Photoinactivation experiments were performed using an InGaN/GaN-based 10 W SMD (COB; chip-on-board) LED source with peak emission near 405 nm. A high-intensity illumination module was constructed by arranging nine single LED units in a 3×3 matrix configuration, forming a compact array with dimensions of approximately 29 mm × 20 mm. The LED arrays (model: 1DGL-JC-10W-405; Chanzon Corp) exhibit a spectral bandwidth of approximately 50 nm full width at half maximum (FWHM). The LED beam angle was 120–140°, and the array was positioned centrally to provide uniform illumination across samples.

To minimize heat transfer to the samples, the LED array was mounted onto an aluminum heat-sink plate using a thermal adhesive. The system was powered by a DC supply operating at 700 mA and 9–11 V. The 10 W SMD LED array was operated under

constant current (700 mA) to ensure stable spectral output throughout the exposure periods. While direct irradiance measurements (mW/cm^2) at the sample plane were not recorded, the standardized configuration and electrical parameters were maintained to provide high-intensity violet-blue illumination across all experimental replicates.

2.4. *Light exposure protocol*

Diluted suspensions (10^{-3}–10^{-6}) were transferred into sterile 60 mm Petri dishes and exposed to 405 nm LED illumination for 10, 20, 30, 60, 90, and 120 min.The distance between the LED light source and the surface of the microbial suspension was maintained at 10 cm throughout the exposure periods to ensure consistent irradiance across the sample area. A non-illuminated control group was prepared and handled under identical conditions, except that no light was applied.

2.5. *Microbiological enumeration (CFU counting)*

At each exposure time, 100 µL aliquots were collected from the corresponding Petri dish and plated on nutrient agar (NA). Spread plating was performed using a Drigalski spatula to ensure uniform distribution. Plates were incubated aerobically at 37 °C for 24h, after which colonies were counted. Results were reported as colony-forming units (CFU) per mL**.** All experiments were conducted in triplicate (n = 3), and data were expressed as mean ± standard deviation.

2.6. Statistical analysis

Statistical differences in microbial counts between the 405 nm LED-treated group and the non-illuminated control group were evaluated at each specific time interval using the independent-samples Student's t-test. All experiments were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation. The threshold for statistical significance was set at $p < 0.05$, while values of $p < 0.01$ were considered highly significant. Statistical analyses and data visualization were conducted using data analysis software.

3. Results

In this study, the change in the number of culturable cells following exposure of *Listeria monocytogenes* to 405 nm LED light was evaluated by comparison with a non-illuminated control group, and the outcomes were expressed as $\log_{10}$ reductions. Mean values calculated from three replicates at each time point (n = 3) showed that LED treatment resulted in significantly lower colony counts than the control across all exposure durations ($p < 0.01$ for all points).

At 10 min, the mean colony count was 28.67 CFU/mL in the illuminated group versus 47.00 CFU/mL in the control group, corresponding to a 0.22 $\log_{10}$ reduction (Student's t-test, p = 0.0001). This level of inactivation was relatively stable during the early stages of exposure, with a 0.22 $\log_{10}$ reduction at 20 min (34.67 vs 57.67 CFU/mL; p = 0.0065), a 0.21 $\log_{10}$ reduction at 30 min (29.67 vs 48.00 CFU/mL; p = 0.0001), and a 0.23 $\log_{10}$ reduction at 60 min (32.33 vs 55.00 CFU/mL; p = 0.0031).

With longer exposure, the inactivation effect became more pronounced. The $\log_{10}$ reduction increased to 0.37 at 90 min (21.00 vs 48.67 CFU/mL; p = 0.0025) and reached its highest value of 0.39 $\log_{10}$ at 120 min (28.33 vs 69.67 CFU/mL; p = 0.0006).

Overall, 405 nm illumination produced a progressive inactivation, achieving up to a 0.39 $\log_{10}$ reduction relative to the control over the 120 min interval. These findings statistically confirm that 405 nm LED light reduces the culturability of *L. monocytogenes* in a clear, time-dependent manner.

Time-dependent changes in the mean colony counts (CFU/mL) of Listeria monocytogenes under 405 nm LED illumination compared to the non-illuminated control. Data points represent the mean of three independent replicates (n = 3), and error bars indicate the standard deviation (± SD). Across all time points, the illuminated group exhibited lower mean colony counts than the control, and this difference became more pronounced with increasing exposure time, particularly at 90 and 120 min (Figure 1).

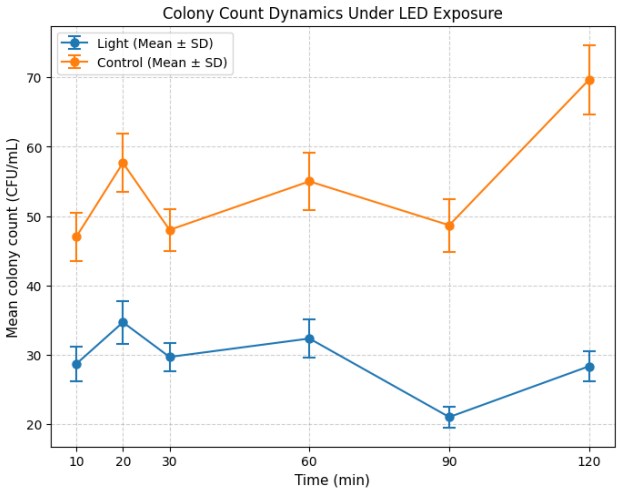

Figure 1. - Colony Count Dynamics Under LED Exposure

Figure 2 shows the Time-dependent log10 reduction (CFU/mL) of *Listeria monocytogenes* following exposure to 405 nm LED illumination. Data points represent the mean of three independent replicates (n = 3), and error bars indicate the standard deviation (± SD). The $\log_{10}$ reduction remained approximately between 0.21 and 0.23 from 10 to 60 min, but increased to 0.37 at 90 min and reached 0.39 at 120 min, indicating a progressively stronger inactivation effect with extended LED exposure

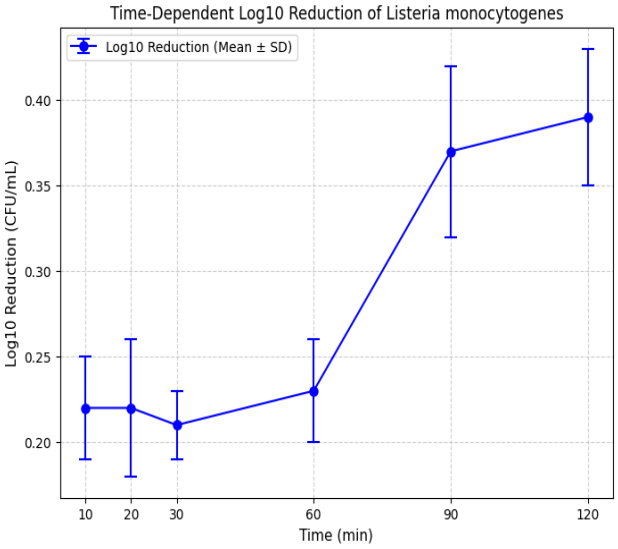

Figure 2. Log_{10} reduction (CFU/mL) of *L. monocytogenes* under 405 nm LED

4. Discussion

The present findings demonstrate that 405 nm LED illumination reduces the culturability of *Listeria monocytogenes* in a time-dependent manner, as evidenced by consistently lower colony counts in the illuminated group compared with the non-illuminated control across all exposure times. The progressive increase in $\log_{10}$ reduction, reaching its highest values at 90 and 120 min, indicates that extended exposure enhances the overall inactivation outcome.

Although the magnitude of reduction varies between time points, the general trend supports the feasibility of violet–blue light as a non-thermal antimicrobial intervention.The maximum inactivation level achieved in this study (0.39 $\log_{10}$ Cfu /Ml reduction after 120 min) aligns with the general time-dependent susceptibility trends reported in the literature, although the magnitude of reduction is lower than in some foundational studies. For instance, Maclean et al. (2009) demonstrated significantly higher log reductions for *L. monocytogenes* and other pathogens using 405 nm LED arrays. These differences in efficacy can primarily be attributed to variations in irradiance levels and the experimental media used. While high-reduction studies often utilize high-intensity systems with precise dose delivery in transparent buffers like PBS, the present study employed a 10 W LED array on cultures in Nutrient Broth. The presence of organic components in the broth may have caused optical shielding or photon scattering, thereby reducing the effective dose reaching the bacterial cells compared to clearer media. Furthermore, the lack of direct irradiance measurements (mW/cm^2) in this setup limits direct numerical benchmarking, yet the statistically significant ($p < 0.001$) response confirms that even under these specific conditions, 405 nm light effectively disrupts the culturability of *L. monocytogenes*.

4.1.Proposed inactivation mechanism: porphyrin excitation and ROS generation

The antimicrobial action of 405 nm light is commonly attributed to an endogenous photodynamic effect [18]. Many microorganisms contain intracellular chromophores—particularly porphyrins and related tetrapyrrole compounds—that absorb photons in the violet–blue region [19]. Upon excitation, these molecules can transfer energy to molecular oxygen, leading to the formation of reactive oxygen species (ROS) such as singlet oxygen and other oxidative radicals [20]. ROS can damage multiple cellular targets simultaneously, including membrane lipids (increasing permeability), proteins (enzyme inactivation), and nucleic acids (oxidative lesions), resulting in loss of viability and/or culturability. Because this mechanism does not rely on exogenous photosensitizers, it offers a practical route for surface or thin-layer decontamination where chemical residues are undesirable.

4.2.Why does the effect become more pronounced at 90–120 minutes?

The stronger relative reduction observed at longer exposure durations likely reflects a combination of cumulative dose effects and time-dependent biological response dynamics. Even when irradiance remains constant, the total delivered dose increases linearly with exposure time. If a substantial fraction

of the population does not reach a lethal oxidative damage threshold at early time points, prolonged illumination can push more cells beyond this threshold, thereby creating a clearer separation between the illuminated and control groups.

In addition, microbial populations are inherently heterogeneous [21]. A subset of cells may exhibit higher tolerance due to physiological state, aggregation, or microenvironmental shielding, which can reduce the effective photon dose reaching individual cells. As exposure time increases, these more tolerant subpopulations may be progressively inactivated, leading to a more pronounced overall reduction.

Another contributing factor may be the delayed manifestation of oxidative injury in culture-based measurements. Reactive oxygen species can induce sublethal damage that compromises a cell's ability to recover and form colonies on agar, causing reductions in CFU/mL that become more apparent as damage accumulates. Under such conditions, some cells may remain metabolically active yet fail to grow on plates, resulting in an apparent increase in inactivation at later time points.

Finally, subtle interactions with environmental conditions may influence the trajectory of photoinactivation over time. Minor variations in temperature, oxygen availability at the sample surface, or mixing/settling behavior can affect ROS generation and the effective delivery of photons to cells. Although the LED configuration was designed to minimize heat transfer, small thermal or oxygen-diffusion effects may still modulate the observed inactivation pattern, particularly during extended exposure periods.

4.3. Limitations of the current study

Several limitations should be considered when interpreting these results and when comparing them with other studies. One limitation of the present study is the absence of precise irradiance measurements (mW/cm^2) and integrated dose calculations (J/cm^2) at the sample surface. While this complicates direct numerical benchmarking with studies using different optical geometries, the reported electrical power (10 W), constant current (700 mA), and standardized exposure times (up to 120 min) provide a robust basis for evaluating the relative photoinactivation kinetics of *L. monocytogenes*. Future investigations should incorporate radiometric sensors to map irradiance at the target plane, enabling the development of precise dose-response models (J/cm^2) to further validate these findings across diverse LED-based decontamination systems.

Second, the study relies on colony counts as the primary outcome. While plate enumeration is a robust indicator of culturability, it does not capture sublethal injury, viable-but-non-culturable (VBNC) states, or the potential for recovery under favorable conditions. Incorporating complementary measurements—such as membrane integrity assays or metabolic activity indicators—would strengthen the interpretation of underlying mechanisms and the assessment of true viability.

Third, the experiments were conducted under controlled laboratory conditions using plated enumeration, and potential matrix or optical shielding effects were not addressed. In real food matrices and on complex food-contact surfaces, absorption and scattering can reduce the effective photon dose delivered to microorganisms, potentially diminishing inactivation efficacy. Therefore, these results should be viewed as foundational and may represent an upper-bound efficacy relative to opaque, pigmented, or highly textured foods.

Finally, the parameter space investigated was limited, with exposure time being the primary variable. Other factors known to influence visible-light inactivation—such as distance to the light source, irradiance intensity, sample thickness, oxygen availability, humidity, and surface material—were not systematically evaluated and should be considered in future studies aimed at process optimization and real-world implementation.

4.4.Implications and recommendations for food safety applications

Despite these limitations, the time-dependent reduction observed in this study supports the potential value of 405 nm LED systems as an adjunct technology for food safety and hygiene. One practical implication is their use for environmental and surface decontamination, where continuous or intermittent illumination in enclosed spaces—such as preparation rooms, packaging areas, storage cabinets, conveyor tunnels, or equipment housings—may help reduce background microbial contamination between routine cleaning cycles.

Importantly, 405 nm LED exposure should be considered a complementary barrier rather than a substitute for validated sanitation programs. When integrated into multi-hurdle control strategies,

visible-light disinfection may provide an additional layer of risk reduction alongside conventional cleaning and chemical disinfection practices, particularly in zones where persistent contamination is challenging to manage.

For effective translation into real-world applications, process design and validation are essential. Future work should prioritize accurate irradiance mapping at the target surface, development of dose–response models (e.g., log-linear or Weibull) for quantitative performance prediction, validation on representative food-contact materials and relevant food matrices, and evaluation of potential product-quality impacts (e.g., color changes or oxidation) when direct exposure to foods is intended.

Finally, safety and compliance considerations must guide implementation. High-intensity visible-light systems should incorporate appropriate shielding, interlocks, and operational procedures to minimize occupational exposure and ensure that the intended dose is delivered consistently to target surfaces under practical conditions.

5. Conclusion

This study demonstrated that exposure to 405 nm LED light reduced the culturability of Listeria monocytogenes in a time-dependent manner, as indicated by consistently lower colony counts in the illuminated group compared with the non-illuminated control. The relative effect became more pronounced at longer exposure durations, with the $\log_{10}$ reduction increasing from 0.22 at 10 min to 0.39 at 120 min, suggesting progressively stronger inactivation with extended treatment.

These results support the potential of 405 nm visible-light irradiation as a non-thermal, residue-free approach that could complement conventional hygiene measures in food safety management. However, to enable reproducible benchmarking and process optimization, future studies should report irradiance and delivered dose, expand evaluation across additional operational parameters (e.g., distance, intensity, surface type, and oxygen availability), and validate performance in representative food matrices and on food-contact materials. Incorporating complementary viability or injury assays would further clarify inactivation mechanisms and recovery potential. Overall, the findings provide a foundational basis for developing LED-based photoinactivation strategies targeting foodborne pathogens.

Conflicts of interest / Competing interests: No conflict of interest..

Ethics approval: No ethic situation.

Availability of data and material: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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