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# An Overview of the Application of Blue Light-Emitting Diodes as a Non-Thermic Green Technology for Microbial Inactivation in the Food Sector

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**Abstract**
Blue light is an emerging technology used for the decontamination of food contact surfaces and products. It is based on the activation of photosensitizers by light, determining the release of reactive oxygen species (ROS). ROS causes damage to bacterial cells leading to cell death. Several types of microbes may be treated, such as bacteria, yeasts, moulds and viruses, in planktonic or biofilm form. Blue light technology is affected by several factors: light parameters (i.e., irradiance, dose, wavelength), microbial parameters (i.e., pH, temperature, initial inoculum, grade of biofilm maturation) and surface parameters (i.e., material, roughness, and optical properties). In addition, it may be used alone or coupled with other technologies. The use of blue light shows several advantages, such as safety for food operators, and a lower release of chemicals in the environment. Moreover, it seems unlikely for bacteria to develop resistance to the blue light application.

**Keywords** Blue light technology · Photodynamic inactivation (PDI) · Microbial decontamination · Food industry · Reactive oxygen species (ROS) · Photoactive materials

## Introduction

The World Health Organization (WHO) estimates that every year, about 600 million, or almost 1 in every 10 people, fall ill after consuming contaminated food. In addition, globally, 33 million people die each year due to unsafe food consumption, which is likely an underestimation [1]. Therefore, it is important to maintain a disinfected environment, especially on food contact surfaces (FCS), to avoid contamination. Nowadays, bacterial resistance to disinfectants and antibiotics is a serious concern that drives researchers towards new decontamination solutions. There are several ways to sanitize the industrial environment, and some of these rely on the use of light. Light technologies are widely used as antimicrobials i.e., UV light, divided into UVA, UVB and UVC (Fig. 1), have been studied to be effective against pathogens and spoilage microbes in water and wastewater, on surfaces, in the air and in food products, not releasing residues [2–6]. Nonetheless, its application for microbial decontamination shows several disadvantages, such as a limited penetration capacity, the necessity to place the target sample close to the light source, and the hazard for the operator since prolongated exposures may favour skin cancer formation, facial erythema and eye problems like keratoconjunctivitis, ground-glass eyeballs, welder's flash and snow blindness [7–10]. The urge to find novel sanification solutions in the food sector, combined with the increased interest in light technologies, drove the studies to consider other parts of the electromagnetic spectra. The visible region (400–700 nm) is divided into four parts (Fig. 1): blue (400–480 nm), green (480–560 nm), yellow/orange (560–610) and red (610–760 nm) [11–14].

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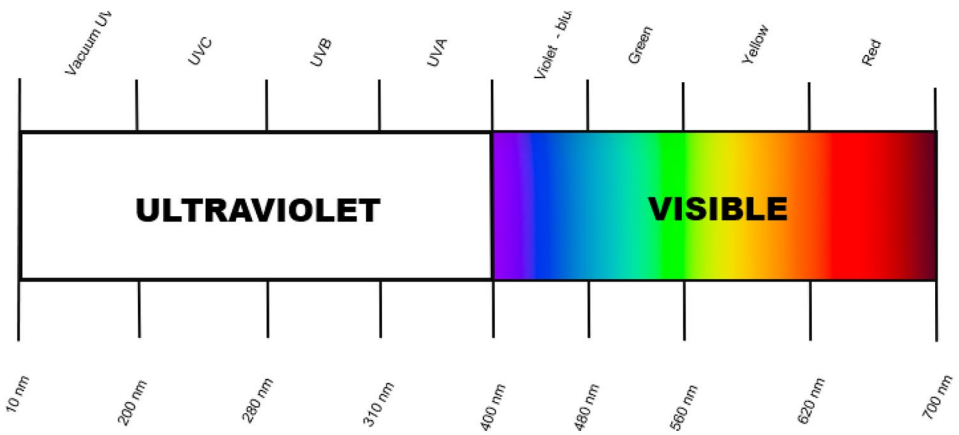


**Fig. 1** Ultraviolet (UV) and visible parts of the light electromagnetic spectra

Studies on blue light started presumably in 2005, according to the articles published on the two scientific literature browsers Scopus (1) and Word of Science (WOS) (3). However, the primary application of blue light was in the medical field and so the first articles were about this topic (Fig. 2a). The employment of blue light in the food sector started later, probably twelve years ago, according to the first articles published by Scopus (1) and WOS (3). Since then, the number of publications has been growing exponentially: in 2021, 15 articles were published on Scopus and 36 on WOS. Differently, UV light started to be used earlier in the medical field, and so the application in the food sector occurs later: the first articles were published at the end of the nineteenth century on WOS but started to be more conspicuous in 2009, as reported in Fig. 2b. (Scopus, 3; WOS, 8).

Among light technologies, blue light seems to be the most promising for microbial inactivation. Thus far, the application of blue light has been employed mainly in the medical field, due to its capability to target microbial cells while sparing host mammalian cells; for this reason, it can be used for treating many localized infections, both superficial and even deep-seated, for instance in the treatment of acne vulgaris, dental infections, otitis media and urogenital infections [15–18]. Other applications concern surface decontamination, and, to a lesser extent, FCS and food products sanitization [19–22]. The application of blue light is a promising technique in this sense since it seems to be unlikely for bacteria to develop resistance (this topic will be discussed in Paragraph 5). Due to the novelty of this technology, most of the studies have been done in culture medium; therefore, the studies applied on surfaces or food products are still limited, and so many features, such as the penetration capacity on different surfaces, have not been studied in detail. Still, it can be assumed that the depth is related to the applied material, its optical properties and its roughness. In the food industry, contamination is a serious concern that must be considered: it has been seen that some pathogens, such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Salmonella* spp., can survive for periods ranging from hours to days on different surfaces [23].

This review article aims to collect the major knowledge about blue LED light as an antimicrobial agent against microorganisms, in particular foodborne microbes i.e., *L. monocytogenes*, *E. coli* and *S. aureus*. In addition, it could also be effective against microbial biofilms, which are complex microbial associations with a higher grade of resistance. For the hazard analysis and critical control point (HACCP), blue light technology may be used as a good manufacture practice (GMP) to sanitize FCS. Furthermore, it could be applied directly to food products without significant modification to the quality but with an increase in shelf-life.

## Light Sources and Parameters

### LED

Traditionally, light technology i.e., UV light, was emitted through low-pressure and medium-pressure mercury lamps; recently, the development of light-emitting diodes (LED) technology has created a preferable alternative for light treatments. In fact, conventional mercury lamps display several disadvantages, such as mercury toxicity, high energy consumption, the necessity to warm up, overheating and a relatively short life span (< 12,000 h) [24–27]. On the other hand, LED lamps exhibit several advantages from both energy and economical point of view: as Haitz's Law said "every 10 years the amount of light generated by a LED increases by a factor of 20, while the cost per lumen (unit of useful light emitted) falls by a factor of 10" [28]. In addition, their lifetime exceeds 100,000 h, allows the use of specific wavelengths, and does not require a special disposal method after their use [29–32].

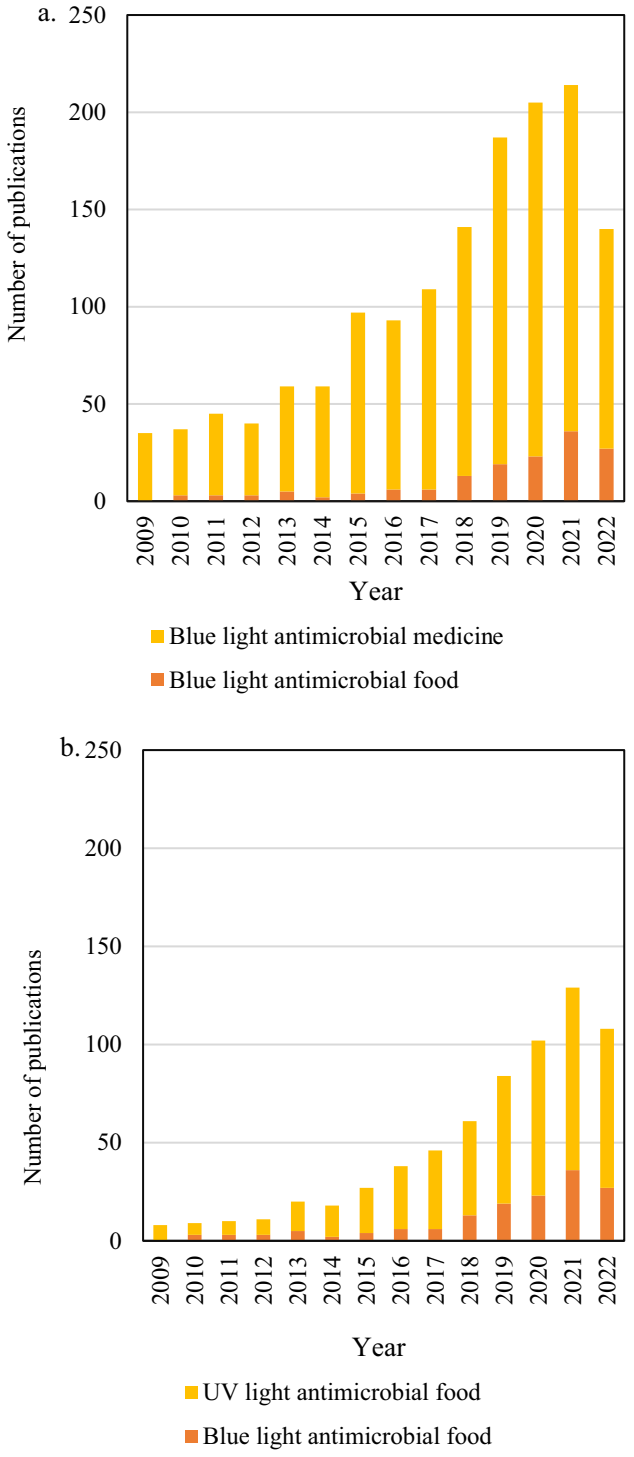


**Fig. 2** Graphics representing the combination of publications present on two literature research databases, Scopus (www.scopus.com) and Web of Science (WOS, www.webofscience.com). **a** Compares the publications regarding blue light in the food and medicine sectors ; **b** compares the number of publications about blue light and publications concerning UV light

LEDs are solid-state semiconductors diodes made of a p–n junction which emits light at a specific wavelength through electroluminescence phenomena [11]. The doping of the semiconductor material forms a p–n junction (Fig. 3) generating an excess of electrons on the negative side (n side) and an excess of holes on the positive side (p side). The n-type semiconductor is created by providing extra free electrons in the substrate by adding an element of group V, like P, into a substrate formed by an element of group IV (silicon), while a p-type semiconductor can be formed by doping a group III element, usually B, into a group IV element substrate to provide extra holes. The additional holes and electrons recombine together forming a non-conductive depletion region. For that reason, LED works only under forward current, with current flowing only from the p-side to the n-side [21, 33]. Based on the energy gap of the semiconductor, which depends on its doping, different wavelengths are produced [11]: the higher the material's band gap, the higher the emitted photon's energy, and the shorter emitted light wavelength [34]. Furthermore, the light wavelength depends on the material used to produce the semiconductor, for instance, aluminium gallium nitride (AlGaN) and aluminium nitride (AlN) are frequently used for UV light, while for visible light gallium nitride (GaN) and indium gallium nitride (InGaN) are more suited [35].

In order to work with light, it is important to have a thorough understanding of some light and processing parameters, such as the following:

## Wavelength

Blue light is the first part of the visible spectra after UV section. Blue light ranges from 400 to 480 nm. Angarano et al. [36] and others authors [37, 38] identify the wavelength from 400 to 450 nm as violet light. Until now, the majority of scientific studies were done with 405 nm blue LED lamps and, to a lesser extent, 450–460 nm. The great employment of 405 nm LED is probably because this wavelength is near the UV part of the spectra, and so there is a higher probability of microbial inactivation. In addition, the specific wavelength at which the light is emitted is a factor that could determine a different microbial inactivation rate. In fact, cell death occurs by reactive oxygen species (ROS), produced by the excitation of photosensitizers. These molecules absorb light at different wavelengths: for instance, at 405 nm wavelength, light is absorbed by protoporphyrin IX and zinc protoporphyrin, while 450 nm is the wavelength at which flavins absorb mostly [39].

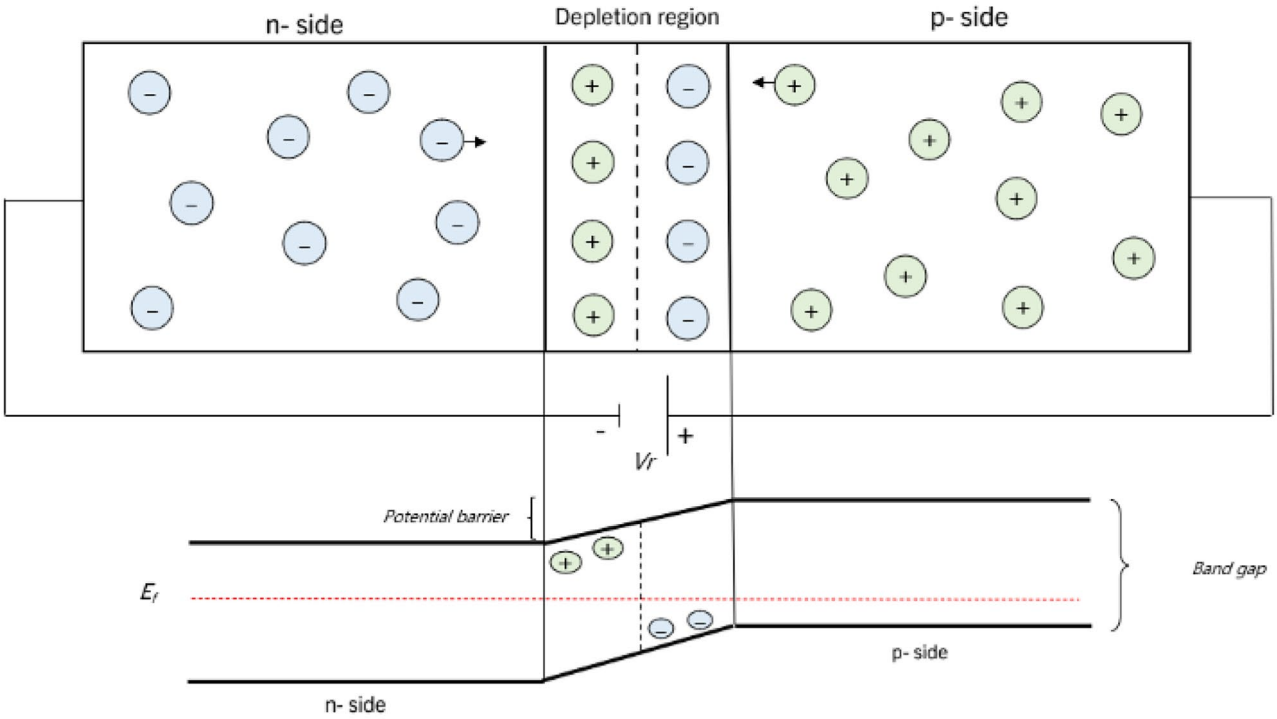


**Fig. 3** Structure of p-n junction of LED. Electrons in the n-side move to the p-side and holes move from p- to n-side. This movement creates a depletion region, where an excess of holes is present on the n-side and an excess of electrons on the p-side. In the depletion region, the Fermi level of the energy is reduced and so the promotion of electrons to an excited state occurs with lower energy

## Irradiance

Irradiance (I) is the radiant power received by a surface per unit area of the sample (W $m^{-2}$): in other words, it measures the rate at which energy is transmitted to the surface, which is affected by the light's spectral composition, as photons of different wavelengths have varying energies. It is fundamental to assess the irradiance values since they represent the energy which reaches the sample. The irradiance of LEDs emitting light at a different wavelength, at a specific distance from the surface, is measured through radiometers, which are tools deploying probes: they consist of an electronic ammeter, to which a light sensor head is connected, which produces a current proportional to the incident irradiance [40]. There are also some chemical methods to determine irradiance value, such as the nitrite-nitrate assay with the addition of Griess reagent, potassium iodide/iodate (KI) actinometry and ferrioxalate actinometry, which are however less used [41–44].

Irradiance is influenced by the light intensity, which depends on the photon fluence (∅), and it is indirectly proportional to the sample light-distance: the higher the distance, the lower the irradiance [21, 45].

## Dose

Energy dose (D), or fluence, is defined as the total radiant energy incident from all directions onto a small sphere divided by the cross-sectional area of that sphere. The energy dose is equal to the product of the irradiance and the exposure time, as reported in Eq. (1):

$$D = I \cdot t \tag{1}$$

where $D$ represents the energy dose of the LED light per unit of area (J $cm^{-2}$), $I$ represents the irradiance of the LED light (W $cm^{-2}$) and $t$ represents the exposure time to LED light (s) [45]. The dose value required for disinfection may vary according to the wide variety of microorganisms and the final effect required for each food product. Also in the same species, there could be differences between strains.

## Microbial Inactivation Calculation

The microbial inactivation produced by blue light may be expressed as reported in Eq. (2):

$$\frac{N_t}{N_0} = e^{-k \cdot I \cdot \Delta t} = e^{-k \cdot D} \quad (2)$$

where $N_t$ is the final microbial population after $\Delta t$, $N_0$ is the initial microbial population, $k$ is the microbe-dependent inactivation constant and $I$ is the irradiance received by the microbes (μW cm$^{-2}$). The equation may also be expressed in terms of dose, since it corresponds to the multiplication of $I$ for $\Delta t$, as reported in Eq. (2) [32].

## Microbial Inactivation

### Mode of Action

The use of visible light wavelength as an antimicrobial requires three main actors: oxygen, a photosensitizer (endogenous or exogenous) and light with an appropriate wavelength able to fit the absorption spectrum of the photosensitizer [46, 47]. After light absorption (Fig. 4), the photosensitizer passes from a low ground energy state to an excited singlet state (lifetime $10^{-9}$ to $10^{-6}$) and then to an excited triplet state ($10^{-3}$ to 10 s); in this form, it can react with the external environment in two ways. The type I reaction entails an electron transfer from the substrate to the photosensitizer forming unstable ions which can produce ROS by reacting again with the substrate. The products include superoxide anions ($O^{2-}$), hydroxyl radicals (•OH) and hydrogen peroxide ($H_2O_2$). Indeed, the type II reaction is based on the formation of singlet oxygen radicals ($^1O_2$) by the energy transfer from the triplet-state photosensitizers to ground-state molecular oxygen [48]. In bacterial cells, both reactions occur during photoactivation,however, one is predominant on the other, based on the levels of intracellular oxygen. In fact, the availability of oxygen is a limiting factor in the production of singlet oxygen [49, 50]. Recently, Hamblin and Abrahamse [51] proposed the 'type III photochemical pathway', they identify three oxygen-independent photosensitizers (psoralens, tetracyclines and inorganic salts), which lead to bacterial death. In the case of psoralens and tetracyclines, cells' death occurs by the binding of the photosensitizer and a molecular structure inside bacterial cells; in the case of inorganic salts, there could be the formation of a PS radical anion and an inorganic radical, which attacks microbial cells. However, many of the mechanisms operating with organic salts depend on the presence of oxygen.

ROS are naturally generated in microbial cells in response to environmental stresses. When an excess of ROS is accumulated, there is oxidative stress [52]. In normal conditions, the ROS are counterbalanced by some antioxidant systems proper of microbial cells, such as superoxide dismutases, catalases, glutathione peroxidases, glutathione reductases, thiol peroxidases, thioredoxins, glutaredoxins and peroxiredoxin [53–58]. In the case of biofilms, also the promotion of the EPS matrix and the adjustment of biofilm heterogeneity are defence mechanisms against oxidative stress [59]. However, if ROS are accumulated faster, the defence mechanisms are not able to detoxify cells, and they could damage cells [60]. The damage could be on different cell components, such as bacterial membrane, DNA, proteins and lipids determining cell death by necrosis or apoptosis (Fig. 5) [61–74].

Makdoumi et al. [75] tested different blue light wavelengths (412 nm and 450 nm) on a methicillin-resistant *S. aureus* (MRSA) suspension at 28.5 J cm$^{-2}$. They showed that the higher wavelength (450 nm) had a higher inactivation efficiency (81%) compared to 412 nm light which was 72%. Zhang et al. [76] evaluated the inactivation of *Candida albicans* suspensions. They saw that when exposing 70.2 J cm$^{-2}$ of 415 nm light, 5.42 log cfu was inactivated on average. Coherently, Gupta et al. [77], using a 405 nm light with 332.1 J cm$^{-2}$ dose value to treat *C. albicans* suspension, obtain a 4.52 log cfu reduction. However, the higher wavelength is not always associated with a higher microbial inactivation. In

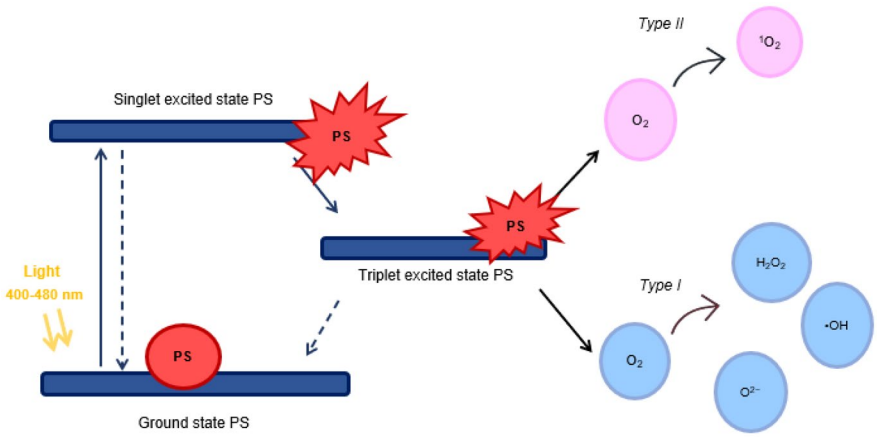


**Fig. 4** Schematic representation of photoactivation. The photosensitizer (PS) is in a ground state. When it absorbs energy from light, it is promoted to a singlet excited state. Here, it can emit all the energy, in the phenomena called fluorescence, or it can pass to a triplet energy state. Now, the excited PS can undergo type I and type II reactions forming different ROS

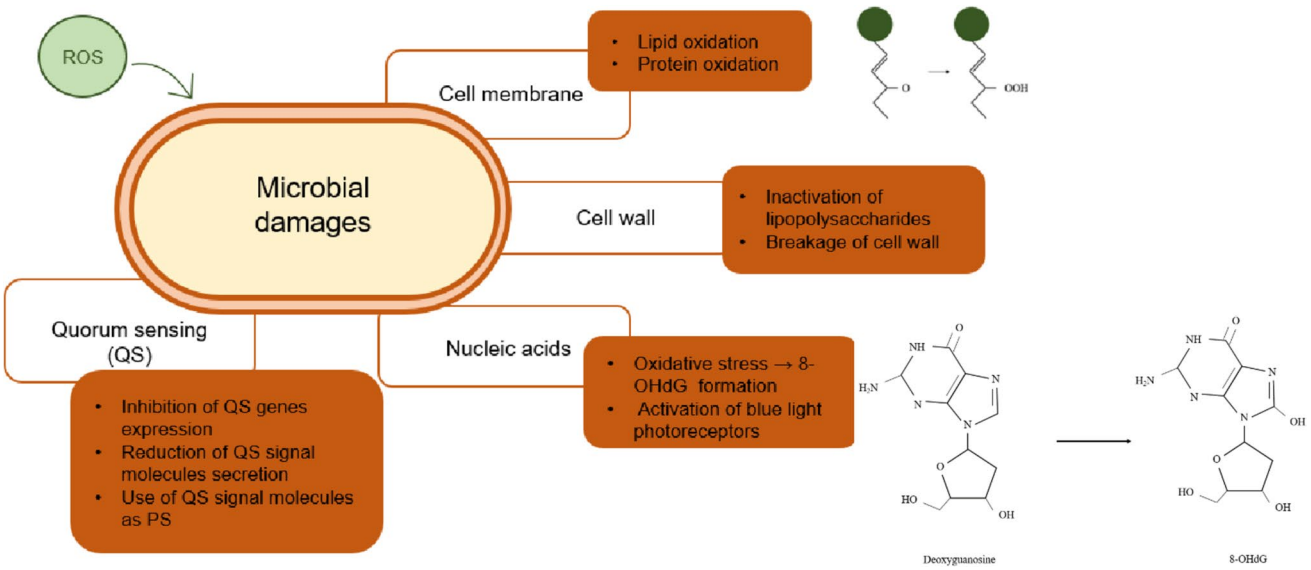


**Fig. 5** Schematic representation of the microbial damages operated by ROS in cell membrane, cell wall, nucleic acids and on the *quorum sensing* system

their study, Roh et al. [78] studied the effect of 405 nm and 465 nm light on several bacterial fish and shellfish pathogens. The findings were that *Photobacterium damselae*, *Aeromonas salmonicida* and *Vibrio harvey* cellular suspensions were more susceptible to 405 nm light compared to 465 nm wavelength. Similarly, it was evaluated that the dose for reducing one log of *S. cerevisiae* suspension was higher at 450 nm light (526 J $cm^{-2}$) compared to the one required at 405 nm (182 J $cm^{-2}$). In total, 22.1 W $m^{-2}$ irradiance, emitted by a 461 nm LED, allowed Ghate et al. [79] to reduce the populations of *E. coli* O157:H7, *Salmonella* Typhimurium, *L. monocytogenes* and *S. aureus* of 4.9, 5.0, 4.3 and 5.2 log CFU/mL after 7.5 h, respectively. Indeed, Endarko et al. [80] irradiated *L. monocytogenes*, *E. coli* and *Shigella sonnei* suspensions with 405 nm light with 85.6 mW $cm^{-2}$ and determined a microbial reduction of 3.72, 4.52 and 3.9 log, respectively. Differently, 18 mW $cm^{-2}$ irradiance of 405 nm light for 7.5 h resulted in a 0.8–2.0 log reduction of *E. coli* O157:H7, *S.* Typhimurium and *S. sonnei* suspensions [81]. With a dose of 306 J $cm^{-2}$, Kumar et al. [69] got a reduction of *S. aureus*, *Bacillus cereus* and *L. monocytogenes* suspensions equal to 4.0, 2.3 and 1.9 log at 405 nm, respectively. Guffey and Wilborn [82], indeed, evaluated the application of different doses with 405 nm and 470 nm light treatments on tryptic soy agar plates. They found that, after 405 nm light irradiation, with a dose of 10 and 15 J $cm^{-2}$, *Pseudomonas aeruginosa* decreased by about 95.1% and 90%, while *S. aureus* under the same doses decreased by 76.1% and 87.9%, respectively. At 470 nm light irradiation, the highest microbial inactivation (96.5%) of *P. aeruginosa* was obtained with a dose of 5 J $cm^{-2}$, while *S. aureus* decreased only with a higher dose, 10 and 15 J $cm^{-2}$. These results underline clearly the differences intra-species after the same treatment conditions. Angarano et al. [36] in their study underlined how the inactivation due to 405 nm light is weaker when the sample-LED distance is longer,2.2 log reduction was observed in solid culture media at 5.0 cm compared to 3.7 log at 2.5 cm. Also, Wu et al. [83] evaluated the inactivation activities of six strains of *E.coli* STEC in a solid culture medium at different distances (2.5, 5, 10, 15 and 20 cm) for 2-h light treatment with 405 nm light. The results have evidenced how the inactivation activity is widely affected by the irradiance, but it is also strictly dependent on the microbial strain. Keyvan et al. [84] treated with 405 nm LED *Salmonella* enteritidis suspensions at 25 °C with 27.7 mW $cm^{-2}$ for 1.5, 3, 7.5 and 24 h. They saw that starting from an initial concentration of 6.48 CFU/mL, after 1.5 h, a decrease in the microbial concentration was obtained. Furthermore, after 7.5 h, its presence was not detectable, meaning that it was sufficient for its eradication. Murdoch et al. [85] found that 405 nm light treatment for 5 min determine a 0.18 log reduction of *Campylobacter jejuni* in liquid media, whereas the microbial reduction increased by increasing the treatment time. Table 1 shows some examples of the inactivation of planktonic cells in different substrates, such as culture media, surfaces and food products. From the data reported, it is evident that the most used wavelength is 405 nm, the one nearest to UV light spectra since the efficacy has been already studied.

## Endogenous Photosensitizers

Microbes have some intracellular photosensitizers, such as porphyrins, flavins, cytochromes and NADH which can be

**Table 1** Effect of the blue light to inactivate planktonic cells in different conditions: in vitro, packaging and food

| 400 nm – 440 nm – 460 nm – 480 nm | Microorganism | Substrate | Dose (J cm$^{-2}$) | Irradiance (mW/cm$^2$) | Microbial inactivation | Temperature (°C) | Reference |
|---|---|---|---|---|---|---|---|
| 415 | *Pseudomonas aeruginosa* ATCC 19660 | *In vitro* Suspension | 48 | 20 | 3.54 log | Not mentioned | (Amin, Bhayana, Hamblin, & Dai, 2016) |
| 400 | *P. aeruginosa* ATCC 15692 | *In vitro* Suspension | 108 | 60 | 5.59 log | Not mentioned | (Halstead et al., 2016) |
| 405 | *P. aeruginosa* ATCC 27853 | *In vitro* Solid media | 15 | Not mentioned | 89.5% 39.3% | Not mentioned | (Guffey & Wilborn, 2006) |
| 470 | | | | | | | |
| 413 | *P. aeruginosa* ATCC 27853 | Milk | 720 | 100 | 5.0 log | Not mentioned | (dos Anjos et al., 2020) |
| 400 | *Staphylococcus aureus* ATCC 29213 | *In vitro* Suspension | 108 | 60 | 6.76 log | Not mentioned | (Halstead et al., 2016) |
| 461 | *S. aureus* ATCC 6538 | *In vitro* Solid media | 596.7 | 22.1 | 4.7 log | 10 | (Ghate et al., 2013) |
| 405 | *S. aureus* ATCC 25923 | *In vitro* Suspension | 15 | Not mentioned | 87.9% 62.0% | Not mentioned | (Guffey & Wilborn, 2006) |
| 470 | | | | | | | |
| 400 | *Stenotrophomonas maltophilia* QEHB | *In vitro* Suspension | 108 | 60 | 7.21 log | Not mentioned | (Halstead et al., 2016) |
| 400 | *Escherichia coli* EPEC CFT_073 | *In vitro* Suspension | 108 | 60 | 4.71 log | Not mentioned | (Halstead et al., 2016) |
| 461 | *E. coli* O157:H7 EDL933 | *In vitro* Solid media | 596.7 | 22.1 | 5.1 log | 10 | (Ghate et al., 2013) |
| 405 | *E. coli* K-12 | *In vitro* Solid media | 67.49 | 9.37 | 100% | Not mentioned | (Barneck et al., 2016) |
| 435 | *E. coli* ATCC 25922 | Cucumbers, tomatoes, lettuce, | 33.8 | 9.4 | 3.0 log | Not mentioned | (Glueck et al., 2017) |
| 461 | *Salmonella typhimurium* ATCC 14028 | *In vitro* Solid media | 596.7 | 22.1 | 4.6 log | 10 | (Ghate et al., 2013) |
| 413 | *S. Typhimurium* ATCC 14028 | Milk | 720 | 100 | 5.0 log | Not mentioned | (dos Anjos et al., 2020) |
| 464 | *S. typhimurium* | Cucumber | 18 | 83.3 | 7.5 × 10$^3$ CFU/mL | Not mentioned | (Guffey et al., 2016) |
| 405 | *Salmonella Enteritidis PT4* | *In vitro* Liquid media | Not mentioned | 27.7 | 5.82 log | 4 | (Keyvan et al., 2022) |
| 405 | *Salmonella spp.* | Fresh-cut papaya | 1.7 x 10$^3$ | 10 | 1.0 - 1.2 log | 4 | (Kim et al., 2017) |
| 405 | *Salmonella* spp. | Cantaloupe rinds | 1210 | 31 | 2.3 log CFU/ cm$^2$ | 4 | (Josewin et al., 2018) |

**Table 1** (continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 460 | *Listeria monocytogenes* | Smoked salmon | Not mentioned | 15 | 1.2 log<br>1.1 log | 4<br>12 | (Josewin et al., 2018) |
| 405 | *L. monocytogenes* | Stainless steel coupons contaminated by salmon exudates | 748.8 | 26 | 2.4 log<br>2.4 log<br>1.9 log | 4<br>15<br>25 | (Li et al., 2018) |
| 405 | *L. monocytogenes* | Acrylic coupons contaminated by salmon exudates | 748.8 | 26 | 2.8 log<br>2.4 log<br>2.4 log | 4<br>15<br>25 | (Li et al., 2018) |
| 405 | *Campylobacter jejuni* | Stainless steel coupon contaminated with bacteria chicken exudate | 183.4 | Not mentioned | 4.9 log | 56 | (Gunther et al., 2016) |
| 405 | *Campylobacter coli* | Stainless steel coupon contaminated with bacteria chicken exudate | 180.8 | Not mentioned | 5.1 log | 53.6 | (Gunther et al., 2016) |
| 405 | *Bacillus cereus* ATCC 12826 | Polyolefine, a mixture of polyethylene/polypropylene | Not mentioned | 12 | 4.5 log | Not mentioned | (Luksiene & Paskeviciute, 2012) |
| 402 | *Acinetobacter baumanii* | *In vitro*<br>Liquid media | 84 | 35 | 1.8 log | Not mentioned | (Buchovec et al., 2023) |
| 402 | *Stenotrophomonas maltophilia* | *In vitro*<br>Liquid media | 50.4 | 42 | 1.3 log | Not mentioned | (Buchovec et al., 2022) |

photoexcited by blue light. Porphyrins (i.e., coroporphyrin III, protoporphyrin IX, zinc protoporphyrin and uroporphyrin III) have a peak of absorption around 405 nm which declines at 450 nm. Therefore, at higher wavelengths, other endogenous photosensitizers are involved in photoinactivation, such as FAD or flavins, since they have a peak of absorption around 450 nm, but show a significant absorbance at 470 nm [39, 86]. The employment of endogenous substances to be activated by the light is called antimicrobial blue light (aBL).

The microorganism characteristics affect the effectiveness of blue light treatment. In fact, the susceptibility to photosensitization between gram(+) and gram(−) bacteria is noticeably different, as reported in Fig. 6. This could be explained by the structural differences in the cell wall. In fact, gram(+) microbes have a thick and porous peptidoglycan layer (15–80 nm thickness), which allows photosensitizers to flow into the inner plasma membrane, while gram(−) shows an inner membranes and an outer membrane, and between these two, there is a thin layer of peptidoglycan. The outer membrane shows a highly organized compact structure of gram(−) cells and inhibits porphyrins and porphyrin-like molecules from binding to these bacteria, rendering them less sensitive to some porphyrin-mediated photodynamic inactivation [71, 87, 88]. In particular, gram(−) are resistant to anionic and neutral porphyrins, since after

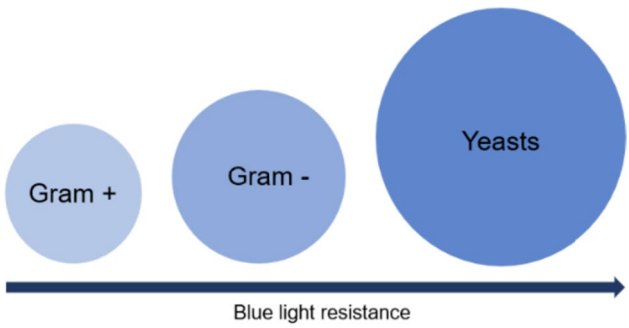


**Fig. 6** Microbial resistance to blue light. As reported in the literature, yeasts are less supsceptible to blue light inactivation due to their complex structure. Between microbes, gram (−) are more resistant than gram (+) for its cell wall structure

illumination, ROS are not produced in sensitive regions but seem to be affected by cationic ones. Related to this, Alves et al. [89] found that a photo-treatment with cationic porphyrins, a light dose of 64.8 J $cm^{-2}$, reduced > 7 log of *E. coli* and *Enterococcus faecalis* bacterial suspension; similarly, in a study of Minnock et al. [90], *E. coli* and *P. aeruginosa* suspensions were effectively photo-inactivated through the presence of a cationic water-soluble zinc pyridinium phthalocyanine (PPC).

Gram(−) species required also longer exposure times than the gram(+) species. For example, a 180-min (108 J $cm^{-2}$) exposure was required to achieve a 4.2 log reduction of *Acinetobacter baumannii* suspension, and 300-min (180 J $cm^{-2}$) exposure was required to achieve a 3.1 log reduction of *E. coli* suspension with 405 nm blue light; while *Staphylococcus*, *Streptococcus* and *Clostridium perfringens* strains were reduced up to 5 log cfu after exposure between 60 and 90 min with 405 nm blue light, with doses of 36, 54 and 45 J $cm^{-2}$, respectively [91].

Table 1 reports some examples of microbial inactivation after blue light irradiation; looking at the data shown in the table, it is clear that the majority of the studies conducted so far are done with a wavelength near the UV spectra since its efficacy has been already confirmed.

### Exogenous Photosensitizers

To increase the antimicrobial effect of blue light, exogenous photosensitizers could be added to the microbial suspension. In this case, the process will be named photodynamic inactivation (PDI). The most used ones, as reported in Fig. 7, are curcumin, porphyrins, riboflavin and hypericin (natural); indeed, the synthetics are for instance Rose Bengal, toluidine blue (TBO), porphyrins and expanded porphyrins, methylene blue and eosin Y [75, 92–99]. The effect is strictly dependent on the microbe to be inactivated, and the type of photosensitizer implemented. Since the use of exogenous photosensitizers has been deeply discussed in other reviews [48, 100, 101], here, some representative examples will be reported.

The application of blue light combined with eosin cumin (5 μM), Rose Bengal (1 μM) and curcumin (5 μM) to *E. faecalis* on polystyrene well produces a reduction from 7.8 ± 0.70 to 2.9 ± 0.30 CFU/mL, 0.5 ± 0.30 CFU/mL and 0.15 ± 0.36 CFU/mL, respectively. As reported, eosin cumin was less active against microbes compared to Rose Bengal and curcumin [102]. Alternatively, it is possible to induce the synthesis of porphyrins inside a bacterial cell by adding their precursor, δ-aminolevulinic acid (ALA), which is a

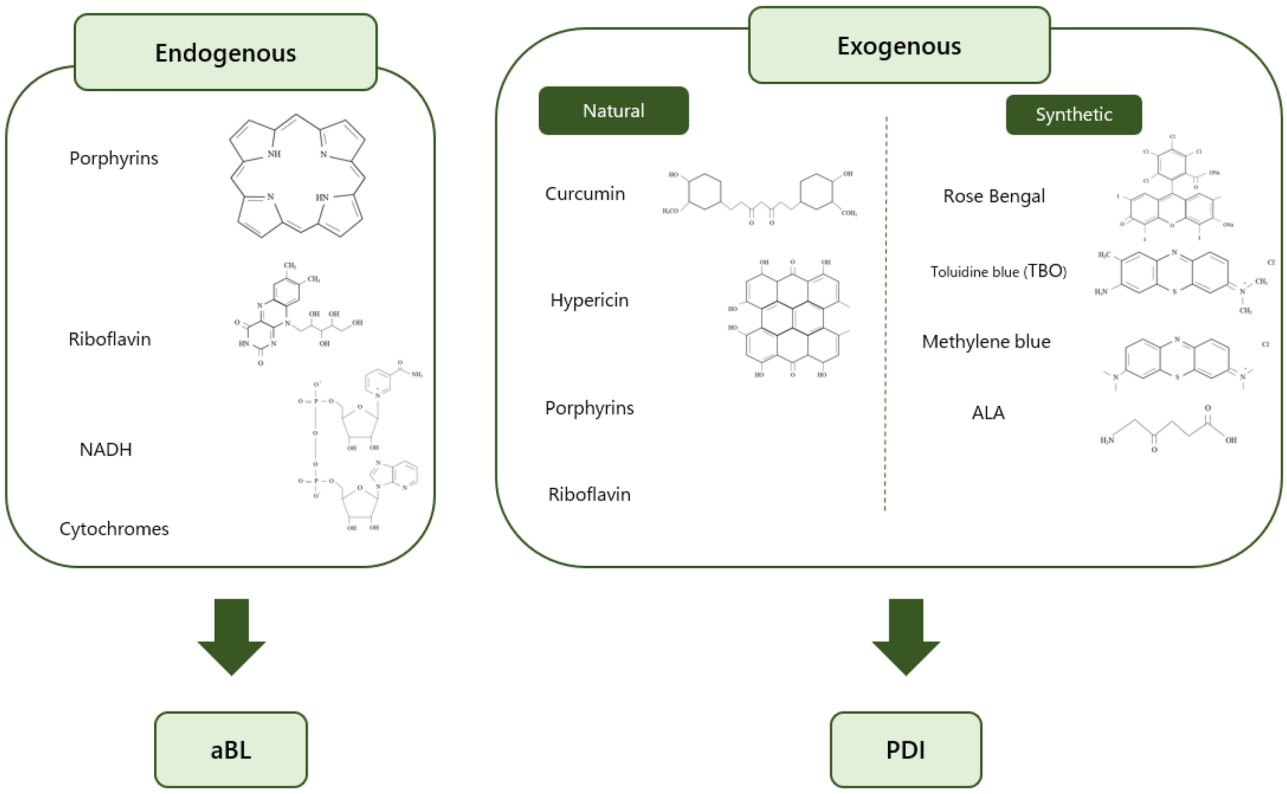


**Fig. 7** Representation of the common endogenous and exogenous photosensitizer (PS) molecules. In the case of the activation of endogenous PS, the process is called antimicrobial blue light (aBL); differently, the addition of exogenous photosensitizers takes the name of photodynamic inactivation (PDI). Furthermore, the exogenous PS could be natural or synthetic

metabolite that occurs naturally in the synthesis of the cellular heme. For instance, Oriel and Nitzan [103] induced the porphyrin synthesis by the addition of ALA and its hydrophobic derivative ALA methyl ester (m-ALA) to inactivate *C. albicans* suspension after visible light treatment. In their study, they found that an initial concentration of 100 mg/mL ALA or m-ALA associated with a light dose of 36 J $cm^{-2}$ caused a reduction of 1.6 or 2.1 orders of magnitude, respectively. Similarly, Buchovec et al. [104] found that, based on the used ALA concentration (7.5–10 mM), *L. monocytogenes* cell suspension decreased from 2.3 to 3.7 log, while the biofilm on packaging material from 1.7 to 3 log, respectively.

The advantage of employing exogenous photosensitizers is to reduce the inactivation time or enhance the inactivation rate. However, in food environments and food products, the added compound should be generally recognized as safe (GRAS), safe for the consumer and not modify the quality properties of the food product (organoleptic and visual characteristics).

## Yeasts and Fungi

Some publications have been written on the use of photosensitizers and light to kill yeasts and other fungi, but fewer studies have been done on the physiochemical properties of photosensitizers used with them. Compared to bacteria, fungi have complex targets as photosensitizers (glucan, mannan, chitin and lipoprotein in the external wall), but they seem to be less involved in photodynamic treatment. Indeed, some studies have revealed that the common sites of damage in fungi are the plasma membrane and mitochondria, which are the sites where endogenous porphyrins are located. The lower sensitivity is attributed to the presence of a nuclear membrane in the yeasts, which may act as an additional barrier, the greater cell size and the reduced number of targets for $^1O_2$ per unit volume of the cell [38, 64, 105, 106]. Considering exogenous photosensitizers, fungi uptake favours hydrophilicity and the presence of charged groups instead of lipophilicity. After being absorbed, exogenous photosensitizers are distributed to subcellular targets; the effect is oxygen-dependent and is influenced by singlet oxygen formation, its short lifetime ($10^{-6}$ s), its limited diffusivity (movement capability of ca. 0.1 μm), and its reaction with cell targets (e.g., nucleic acids, membrane lipids or cytoplasmic enzymes). In summary, the closer the target is, the highest the likelihood that the cell will be damaged. Since the studies for the food sector are still limited, here some examples in vitro of microbes which could be found mainly in the medical field will be reported. Murdoch et al. [38] exposed *S. cerevisiae* and *C. albicans* suspensions to 405 nm light (dose of 288 and 576 J $cm^{-2}$, respectively) and observed a reduction of 5 log cfu. Fluorescence spectroscopy has revealed emission peaks at 611 nm and 608 nm, respectively, indicating that these species contain predominantly intracellular free porphyrin, like coproporphyrin, due to the similarity in its emission peaks when excited at 405 nm. Hoenes et al. [39] irradiated *S. cerevisiae* suspension with 405 nm and 450 nm with 182 J $cm^{-2}$ and 526 J $cm^{-2}$, respectively, obtaining 1 log cfu reduction. However, the fluorescence spectra analysis revealed that for the 405 nm wavelength, the most important photosensitizers are protoporphyrin IX and zinc protoporphyrin, and not coproporphyrin. Indeed, the analysis showed that no porphyrins are involved in 450 nm photoinactivation; at this wavelength, flavins appear to be the key photosensitizers. Trzaska et al. [107] studied that 405 nm blue light was able to completely inactivate *Fusarium oxysporum*, a parasite of plants (such as bananas, tomatoes and alfalfa), and permanently inhibited its germination. Another study evaluated the inactivation of *Botrytis cinerea* and *Rhizopus stolonifera* in strawberries and tomatoes. *B. cinerea* had a similar behaviour in the two substrates, with an inactivation equal to 33.3% in strawberries and 20.6% in tomatoes. Indeed, *R. stolonifera* was more susceptible in strawberries, with 80.0% of inactivation, and less in tomatoes, achieving a reduction of 29.6% [108]. Temba et al. [109] studied the effect of 420 nm blue light with curcumin to inactivate and activate spores of *Aspergillus flavus* on maize kernels. They obtained a reduction of 2 log CFU $g^{-1}$, but the qualitative analysis was done on maize kernel after the treatment. Curcumin was also used by Huang et al. [110] as exogenous photosensitizer for the inactivation of *B. cinerea* spores, isolated from strawberry fruits. One hundred twenty J $cm^2$ blue light (430 nm) combined with 800 μM of curcumin resulted in no spore germination, meaning that $10^4$ CFU/mL of spores were inactivated by blue light treatment.

## Microbial Biofilms

A biofilm is a well-organized, cooperating, consortium of microorganisms that is firmly associated with a surface and embedded in a matrix of primarily polysaccharide material, produced by the microorganism [111–113]. Its formation is a dynamic multi-step process: irreversible attachment of bacteria to a surface, microbial growth, microcolony formation, mature biofilm and, eventually, detachment and dispersal of biofilms [112–114]. With the advantages of biofilms research, it is clear that they can develop on a plethora of surfaces on which viable microorganisms are present, including plastics, metal, glass, living tissues, natural aquatic systems and food products. Although monomicrobial biofilms have been widely studied, the majority has been found thriving in complex polymicrobial communities, since they show several advantages such as metabolic cooperation, passive resistance, amplified gene pool with more efficient DNA sharing and quorum sensing system [115, 116].

The biofilms' control is a challenge for environments where the microbial communities are problematic, like food industries. In the food sector biofilm eradication is fundamental in order to avoid possible cross-contamination. Several strategies can be employed to eliminate biofilms: conventionally, chemical disinfection has been used but due to antibiotic resistance and environmental and human health, other physical methods have been developed [46, 114, 117, 118]. Blue light can be employed against microbial's biofilm. This technology could be applied to prevent its formation, as a GMP. The produced ROS could damage the biofilm in several ways: for instance, unbalance the microbial community of the biofilm, leading to cell lysis, breaking the matrix structure and interfering with the cell motility and quorum sensing [119–122].

Yang et al. [123] demonstrated that irradiation with 460 nm light, with a dose of 240 J $cm^{-2}$ or 360 J $cm^{-2}$, killed most *S. aureus* MRSA biofilm cells by disrupting their structure, as shown by live/dead staining analysis. Chen et al. [124] used PDI to eradicate *Vibrio parahaemolyticus* biofilm with the use of curcumin. Twenty μM of the photosensitizer plus 60 min of irradiation (455–460 nm) eradicate the biofilm. Ferrer-Espada et al. [125] exposed to 405 nm light biofilms of *Acinetobacter baumannii*, *C. albicans*, *E. coli*, *Enterococcus faecalis*, *S. aureus*, *Neisseria gonorrhoeae*, *P. aeruginosa* and *Proteus mirabilis* on microtiter plates for 30 or 60 min (doses of 108 J $cm^{-2}$ or 216 J $cm^{-2}$, respectively). *E. faecalis*, *E. coli* and *S. aureus* are microbes belonging to both the medical field and the food sector; indeed, it is unlikely to find *P. aeruginosa* in food products. However, errors along the food chain may represent the reason why this microbe is to be found in the food environment. Table 2 reports some examples of blue light application on microbial biofilms.

**Table 2** Conditions of blue light inactivation of microbial biofilm in different substrates

| 400 nm – 440 nm – 460 nm – 480 nm | Microorganism biofilm | Substrate | Dose (J $cm^{-2}$) | Irradiance (mW/$cm^2$) | Microbial inactivation (CFU/mL) | Reference |
|---|---|---|---|---|---|---|
| 405 | *L. monocytogenes* | Stainless steel coupons contaminated by salmon exudates | 748.8 | 26 | 1.5 log | (Li et al., 2018) |
| 405 | *L. monocytogenes* | Acrylic coupons contaminated by salmon exudates | 748.8 | 26 | 1.6 log | (Li et al., 2018) |
| 400 | *Stenotrophomonas maltophilia* QEHB | *In vitro* | 192 | 60 | 92.4% | (Halstead et al., 2016) |
| 400 | *P. aeruginosa* PAO1 | *In vitro* | 192 | 60 | 82.8% | (Halstead et al., 2016) |
| 410 | *Staphylococcus aureus* NCTC 10788 | *In vitro* | 450 | 100 | 6.0 log | (Martegani et al., 2020) |
| 400 | *Klebsiella pneumoniae* (NDM-1 positive) QEHB | *In vitro* | 192 | 60 | 36.3% | (Halstead et al., 2016) |
| 455-460 | *Vibrio parahaemolyticus* | *In vitro* | 13.68 | 380 | 8.0 log | (Chen et al., 2020) |
| 440 | *Acinetobacter baumannii* | *In vitro* | 158.4 | 44 | 1.34 log | (Buchovec et al. 2022) |
| 440 | *A. baumannii* | *In vitro* | 252 | 35 | 1.7 log | (Buchovec et al. 2023) |
| 402 | *A. baumannii* | *In vitro* | 151.2 | 42 | 2.94 log | (Buchovec et al. 2022) |
| 402 | *A. baumannii* | *In vitro* | 189 | 35 | 1.9 log | (Buchovec et al. 2023) |
| 440 | *Stenotrophomonas maltophila* | *In vitro* | 158.4 | 44 | 1.9 log | (Buchovec et al. 2022) |
| 402 | *S. maltophila* | *In vitro* | 151.2 | 42 | 4 log | (Buchovec et al. 2022) |

## Parameters That Influence the Blue Light Microbial Inactivation

There are several parameters which can modify the blue light effect on microbial inactivation, such as initial inoculum, oxygen consumption and the growth phase of the microbes and parameters that are used to evaluate the shelf-life of the food products. These could be characteristics of the matrix, such as pH and water activity ($a_w$), or conservation parameters, like temperature.

### Initial Inoculum, Oxygen Consumption and Growth Phase of the Microbes

The initial inoculum is a factor affecting the efficacy of blue light treatment. In fact, a high optical density of the microbial suspension is the consequence of a higher initial microbial concentration, determining a possible lower microbial inactivation due to the Beer-Lambert law. Furthermore, Vollmerhausen et al. [126] studied the effect of the initial inoculum on the efficacy of 420 nm blue light to inactivate *E.coli*. They saw that the irradiation of $10^3$ cfu $mL^{-1}$ cells reduced *E. coli* concentration under detection level in 6 h; with an inoculum of $10^5$ cfu $mL^{-1}$, irradiation for 8 h showed an initial decrease of the microbial population but cells recovered within 24 h reaching the same population as the dark control. Finally, irradiation of the inoculum of $10^7$ cfu $mL^{-1}$ determined no reduction in cell density compared to the dark control. Together with the higher optical density, they demonstrated that a higher inoculum concentration is associated with greater oxygen consumption, leading to lower bacterial photoinactivation. After 2 h, the dissolved oxygen decreased from a starting concentration of 18.0% and 7.3% for 420 nm, and 8.6% in the dark control [126]. Also, Maclean et al. [50] evaluated the oxygen dependency of *S. aureus*. Irradiating *S. aureus* with 400 nm (450 J $cm^{-1}$), they observed a 3 log cfu difference in samples where extra oxygen was provided during the blue light treatment. In order to be effective, photoactivation needs the presence of oxygen. Usually, the oxygen used is in molecular form ($O_2$) or compounds of $O_2$, dissolved in the cytoplasm of the microorganisms. In the food sector, the presence of oxygen could be compromised by the type of packaging, like vacuum packaging, or by the presence of antioxidants, such as $\beta$-carotene or ascorbic acid, which could quencher the oxygen decreasing the efficacy of blue light [49].

Most of the studies on the effect of blue light against microbes were performed using bacteria in the log phase. However, bacteria in the stationary phase are not the same when they are in log phase. For instance, *E. coli* and *S. aureus* in lag phase have a low population of 'persister' cells, which increases in the stationary phase. These cells show an equal genotype to the active cells, but they differ phenotypically. In fact, through their dormant state, they resist some treatments and are tolerant to some antibiotics. Keshishyan et al. [127] compared the effect of blue light against lag and log phase *E. coli*, *S. aureus* and *P. aeruginosa*. After irradiation with 450 nm (117 J $cm^{-2}$), the microbial inactivation in log phase was lower (14–50%) compared to the lag phase, where a complete inactivation was recorded. A more explicative study, performed by Abana et al. [128], investigated the effect of 455 nm blue light against five *E. coli* strains in log, transition and early stationary phases. From this study, they observed that only *E. coli* DH5α maintained a reduction of 1.5–2.5 CFU/mL along all the phases. However, it was not possible to define a trend in *E. coli* susceptibility to blue light irradiation.

### Temperature

Temperature could be a factor that may influence microbial inactivation operated by blue light. The increased proportion of unsaturated fatty acids in the cell membrane at lower temperatures enhances bacterial inactivation. It is well documented that microorganisms adjust their membrane lipid composition in response to changes in growth temperature to ensure membrane function. The unsaturation of fatty acid chains is the most commonly found change that occurs when the temperature is reduced because it increases the fluidity of the membrane. Unsaturated fatty acids may be more sensitive to oxidation by the ROS generated during photodynamic treatment and therefore, the cell membrane may be more easily damaged by LED illumination at lower temperatures [79, 129, 130]. Hyun and Lee [130] treated pathogens with 460–470 nm LED and a dose of 286.8 J $cm^{-2}$. They evaluated that the reduction in the number of *L. monocytogenes* and *P. fluorescens* cells was higher at 4 °C than at 25 °C. After 4 days of storage at 4 °C, cell suspensions of *L. monocytogenes* and *P. fluorescens* were significantly reduced by 2.72 and 2.01 log CFU/mL, respectively; indeed, storage at 25 °C for the same length of time reductions was equal to 1.50 and 1.32 log CFU/mL, respectively. Similarly, Ghate et al. [79] observed the inactivation of bacterial suspension *E. coli* O157:H7, *S. Typhimurium*, *L. monocytogenes* and *S. aureus* ranging between 4.6–5.2 log CFU/mL at 10 °C and 15 °C after illumination with the 461 nm. Differently, at 20 °C, the illumination was ineffective in reducing the bacterial populations. However, there are some contradictions regarding the role of temperature in bacterial inactivation. Kumar et al. [131] stated that a variation in the temperature from 4 to 10 and 25 °C did not result in any noticeable effect on *Lactobacillus plantarum*, *S. aureus* and *V. parahaemolyticus* suspensions irradiated with 405 nm blue light. In fact, the population of *S. aureus* was reduced up to 0.8, 0.5 and 0.6 log in at 4, 10 and 25 °C, respectively; the *L. plantarum* population decreased up to 3.6 log after 7 h at 25 °C, and *V. parahaemolyticus* reached

below the detection limit at 4 and 10 °C, while at 25 °C, it was reduced to about ca. 3.6 log. Another study [69] evaluated the efficacy of illumination with 405 nm LED (306 J $cm^{-2}$) on *S. aureus* and *S.* Typhimurium at different temperatures, at 25 °C, 4.0 and 0.6 log reductions of *S. aureus* and *S.* Typhimurium, respectively. These values were significantly higher compared to 10 and 4 °C (2.1 and 1.9 log reductions of *S. aureus*, respectively) suggesting that these microbes have a temperature-dependent behaviour: the higher the temperature, the higher the bacterial inactivation. *B. cereus* and *L. monocytogenes* cells did not show an inactivation difference by varying the temperature: all three treatment temperatures (4, 10 and 25 °C) reduced the bacterial population by approximately 2.3 log *B. cereus* and 1.9 log *L. monocytogenes*, in contradiction with what is stated previously. The high inactivation rate of *S. aureus* and *S.* Typhimurium suspensions observed by Kumar et al. [69] may be explained by the fact that at a higher temperature (25 °C), bacterial cells maintain a higher level of metabolic activity as compared to those at refrigeration temperatures. In general, in presence of an antimicrobial agent, the bacterial reduction is higher at room temperature, compared to the refrigeration one, due to the higher metabolic activity of the cells since the value of temperature is similar to optimal growth temperature. Probably, because of this, the rate of bacterial reduction is lower at lower temperatures. However, the results are contradictory, so it could be assumed that the susceptibility of planktonic cells to blue LED light treatment and its temperature-dependence derive from a plethora of factors, including also the microbial strain exposed to treatment [132].

### pH

Also, different pH values may result in different microbial inactivation effects. For instance, Ghate et al. [30] evaluated the decimal reductions (D-value) of 461 nm light on *E. coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* suspensions at pH of 4.5 and 9.5. The log reductions were 5.75 and 2.82 for *E. coli* O157:H7, 5.74 and 2.38 for *S. Typhimurium* and 1.46 and 6.04 for *L. monocytogenes*. Oriel and Nitzan [103] investigated the change in pH values on *C. albicans* suspensions after blue light treatments. The initial pH before incubation was equal to 8.0; before light treatment, this value decreases to 6.5, and after illumination (36 J $cm^{-2}$), it was equal to 5.8. In this study, they state that a pH of around 6.5 was optimal for photosensitization since at lower or higher values the inactivation rate was low.

### $a_w$

$a_w$ is a parameter that influences microbial growth on food products. The effect of different $a_w$ values on blue light decontamination treatment represents a gap in the literature: notwithstanding the importance of this parameter for microbial growth, no studies have considered this so far. Therefore, it could be interesting to investigate how it can affect the light effect together with the parameters previously cited.

## Microbial Resistance to Blue Light

An important question that is yet to be deeply investigated is whether microorganisms can develop some form of resistance against blue light treatment.

Firstly, it is necessary to understand the difference between the terms '*resistance*' and '*tolerance*'. According to the Scientific Committee on Consumer Safety (SCCS), related to non-antibiotic agents, the word 'resistance' is used when a strain is not killed or inhibited by an agent in-use concentration. Furthermore, it is used when the strain survives to a concentration to which most of the strains belonging to same species are sensitive [133]. 'Tolerance', differently, defines the character of a bacterial population that dies very slowly following antimicrobial treatment. Under stressful conditions, cells slow down a lot of their metabolic activity and grow very slowly. At the level of phenotypic behaviour, it has been observed that to kill 99% of a bacterial population of a tolerant specie, it is necessary to substantially prolong the antimicrobial treatment compared to a bacterial population of the same microbial species but sensitive [134]. The application of blue light as an antimicrobial has gained great attention also due to the necessity to develop new approaches to tackle drug resistance [76]. Some authors reported that the blue light technology can eradicate microbes regardless of their antibiotic resistance, and it is rare for bacteria to develop a resistance to these light-based treatments due to the multi-target damages [88, 135–137]. This was confirmed by other authors [61, 138], who found no evidence of tolerance or resistance development of *P. aeruginosa* and *A. baumannii* strain after 10 consecutive cycles of sublethal inactivation with blue light. However, there are contradictory results: Guffey et al. [139] observed that *S. aureus* developed resistance after four cycles of treatment. In the following study, they evaluated that *S. aureus* resistance development to blue light could have been delayed by setting appropriate combinations of dose and wavelength [140]. The problem of developing resistance or tolerance was addressed by Rapacka-Zdonczyk et al. [141], who proposed a possible way of adaptation: the application of sublethal blue light to *S. aureus* determines DNA damages, resulting in activation of RecA gene and SOS response. This increased the release of an enzyme responsible for increased mutation rate, the error-prone DNA polymerase V. In addition, Kim and Yuk [142] assessed how light affects the capability of *Salmonella* cells to repair cellular damage related to the DNA, RNA, protein and cell wall; moreover, they observed the resistance loss to some antibiotics (nalidixic acid, chloramphenicol and ampicillin)

due to the ROS action on different cell targets. However, more studies should be carried out in order to evaluate the possible occurrence of resistance and tolerance for a better understanding of the use of blue light to be applied as a method for microbial reduction.

## Blue Light and Food Contact Surfaces Decontamination

Most of the studies on the blue light efficacy were carried out in vitro, where the effect of the treatments seems to be higher compared to the ones on FCS and food products (Fig. 8) [102, 103, 143, 144]. FCS are surfaces that, during food production, processing and packaging, come into contact with food products [145]. Therefore, their control is essential since a contaminated surface can transmit pathogens to food products [146]. The effect of blue light on FSC in vivo has not been investigated thoroughly; however, some studies in vitro used coupons of different materials, such as stainless steel (SS) or acrylic (AC), to simulate the real conditions [147, 148]. Lang et al. [149] used high-density polyethylene, polypropylene, polished stainless steel and glass coupons as representatives of FCS to test the antimicrobial action of blue light: after 2 min of treatment (901.1 mW/cm$^2$, 405 nm), they observed a reduction > 1 log on all the surfaces, and the best results were the ones on stainless steel (> 4.27 log). The different antimicrobial results may be attributed to the surface characteristics, such as the absorption coefficient, the reflecting properties, the specific heat capacity and the surface colour, porosity, texture and thicknesses [149–151]. Studies that take into account these factors did not investigate the penetration depth of blue light wavelengths in different materials. The penetration depth of visible light ranges from micro to millimetres, depending on the part [49]. Blue light penetration has not been studied in detail, but it was evaluated on human skin, where it is approximately 1 mm [152]. Some more research should be done to evaluate whether specific surface characteristics could enhance the blue light effect. For instance, hydrophobicity or hydrophilicity are aspects that were not taken into consideration in studies published so far.

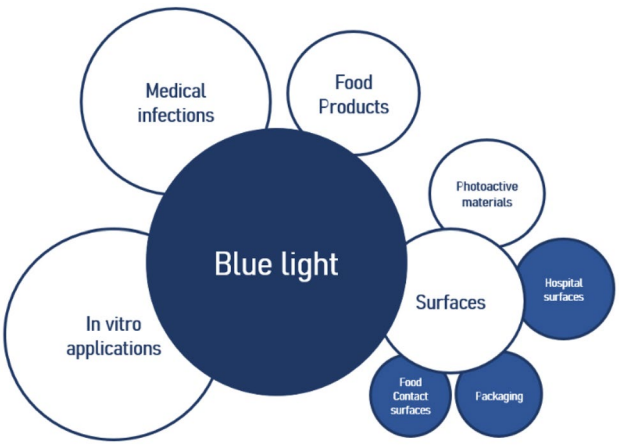


**Fig. 8** Use of blue light. Until now, most of the studies on blue light microbial inactivation are done in vitro. However, some studies have been done to treat medical infections. The research is also moving towards surface disinfection, in particular hospital environments and devices, and to a lesser extent to food contact surfaces (FSC) and food packaging. Also, the activation of materials with blue light is a matter under study. Finally, the exploitation of blue light as an antimicrobial agent for food products is a recent subject

### Photoactive Material

Over the past few last years, self-disinfecting surfaces have been studied to control pathogens. Some of the most recent strategies include surfaces with anti-adhesive and antimicrobial properties. Antimicrobial surfaces can exert their properties by adding specific additives, which can be stably incorporated into or be slowly released from the material. One class of these antimicrobial surfaces is represented by photoactive materials that perform the disinfection activity through a photodynamic mechanism. They are synthesized by the direct polymerization of the PS agent or are the result of the immobilization of a photosensitizer into a support [153, 154]. For this purpose, several supports are available, for instance, resins, polymers and nanoparticles [93, 155–159]. There is a plethora of photoactive materials that could be activated by visible light [160]; for instance, 5-[4-(1-dodecanoylpyridinium)]-10,15,20-triphenylporphyrin was tested against *S. aureus* MRSA at 416 nm irradiation. Thandu et al. [161] irradiated four adipates added with phorphyrin and expanded phorphyrins to inactivate *S. aureus* (460 nm, 50 W m$^{-2}$). In 60 min, a complete inactivation ($10^8$ CFU/mL) of *S. aureus* was observed with the irradiation of long alkyl chains (dioctyl adipate, $OA_{20}$). In a further study, the authors investigated the addition of different photosensitizers on $OA_{20}$. Four porphyrins (5,10,15,20-tetrakis(4-methylphenyl)-21H,23H-porphine (4MeP, 1); 5,10,15,20-tetra- kis(4-methoxyphenyl)-21H,23H-porphine (4OMeP, 2); 5,10,15,20-tetra-4-pyridinyl-21H,23H-porphine (4PyP, 3); and 5,10,15,20-tetrakis(4-aminophenyl)-21H,23H-porphine ($4NH_2P$, 4)) were studied for their antimicrobial activity against *S. aureus* in solution and immobilized into a PVC/octyl adipate composite. The results show that $4NH_2P$ in solution was the most active porphyrin and 4PyP, the less active; indeed, 5,10,15,20-tetrakis(4-aminophenyl)-21H,23H-porphine 5,10,15,20-tetrakis(4-aminophenyl)-21H,23H-porphine octyl adipate ($4NH_2P5$-$OA_{20}$) and 5,10,15,20-tetra-4-pyridinyl-21H,23H-porphine octyl adipate ($4PyP5$-$OA_{20}$) were the most active composites, determining about 4 log CFU/mL and 3 log CFU/mL reductions. 4PyP,

which shows the worst photosensitizing ability in solution, became an active additive by immobilization into PVC. The author showed that the honeycomb-shaped morphology of the composite surface confers antimicrobial properties. This suggested that the molecular assembly and also the surface organization of the material should be taken into account to design new photoactive materials [156]. Also, some studies investigated the use of poly(vinyl) chloride (PVC), a plastic material used for drinking bottles and food packaging [162], added with curcumin [163] or a gelatin/chitosan film incorporated with curcumin (GEL/CS/Cur). The GEL/CS/Cur was demonstrated to reduce > 4 log (99.99%) of *L. monocytogenes*, *E. coli* and *Shewanella putrefaciens* after 455 nm light irradiation [164]. Lopez et al. [165] developed an antimicrobial coating by the electropolymerization of a porphycene able to inactivate $10^5$ CFU/mL of MRSA and *E. coli* EC7 after being irradiated with 455–800 nm light (30 mW $cm^{-2}$). Another light-activated antimicrobial coating was developed by Gusev et al. [166]. They used an electrodeposited phthalocyanines (Pc)-based photoactive layer with the addition of zinc (tetraamino)phthalocyanine ($ZnPcNH_2$) as photosensitizer. This coating was able to inactivate ca. 3 log (99.8%) of *S. aureus* (400–800 nm light). Lourenc [167] evaluated the water-soluble zinc(II) phthalocyanines (ZnPc) peripherally substituted with 4-dimethylaminopyridine (DMAP) against *E. coli*. 20 mM of ZnPc derivatives irradiated with 150 mW $cm^{-2}$ light (400–800 nm) determined a factor of inactivation of 4 log [168].

π-conjugated photoactive polymeric films (FDP 5) is a self-sterilizing surface obtained through the electrochemical oxidation of the carbazole groups of a biscarbazol triphenylamine end-capped dendrimeric zinc(II) porphyrin (DP 5). The photoactivation of this film determined a complete inactivation of *S. aureus* and > 2 log (> 99%) of *E. coli*, while microbial biofilms formed on it were reduced by > 4 log (> 99.99%) [169]. Two antimicrobial films were formed by electrochemical polymerization of 5,10,15,20-tetra(4-*N*,*N*-diphenylaminophenyl)porphyrin ($H_2P$-film) and its complex with Pd(II) (PdP-film) on indium tin oxide (ITO) electrodes. The photoactivation of these films in aqueous solutions produced an inactivation of ca. 3 log of *E. coli* (99.9%) and ca. 2 log of *C. albicans* (99.7%) [170].

Thandu et al. [171] studied the effect of a magnetic porphyrin nanoconjugate (SPION-TPP) against two Gram(+) bacteria evaluating that 0.5 μM of SPION-TPP was sufficient to kill $10^7$–$10^8$ CFU/mL of *S. aureus* and, in a lower amount, *Streptococcus mutans*. Feese et al. [172] built a crystalline material made of cellulose nanocrystals modified on the surface with a cationic porphyrin (CNC-Por) and demonstrated its capability to photo-kill *Mycobacterium smegmatis* and *S. aureus*. Its decontaminating efficacy after light irradiation was also demonstrated towards *A. baumannii*, multidrug-resistant *A. baumannii* (MDRAB) and MRSA, achieving a reduction of 5–6 log cfu [173]. Sah et al. [174] developed a nano-composite able to photodynamically inactivate microbial cells by combining single-walled carbon nanotubes (SWCNTs) and amine-functionalized porphyrin. A cationic porphyrin-grafted paper was developed by Mbakidi et al. [175] for microbial photoinactivation. They studied that the photosensitizing filter paper kills $10^5$ CFU/mL of *S. aureus* and *E. coli*. Also, Comuzzi et al. [155] studied the photokilling effect of modified PET-ITO electrodes against *S. aureus*. A reduction of 4 log was obtained by using Poly-5-(4-pyridyl)dipyrromethene (Poly-5-py-DP/PCox), and 5-Phenyl-dipyrromethane (5-ph-DP/PCox) at 60 min and 90 min, respectively. The strength of these materials is represented by their regeneration ability. The electropolymerization of ZnPc-EDOT and CuPc-EDOT (made by the electrochemical polymerization of Cu(II) and Zn(II) modified ethylenedioxythiophene phthalocyanines, EDOT Pcs) on a glass surface coated with ITO can inactivate microbes by enhancing the ROS formation after light irradiation. This feature is improved due to the addition of iodide salt (KI). In particular, both of the films added with KI reduced *S. aureus* of ca. 4 log cfu (99.98%); indeed, for *E. coli*, CuPc-EDOT plus KI inactivated only 95%, whereas for ZnPc-EDOT plus KI, it was 99.98% [176].

Reynoso et al. [153] developed a material by the combination of fullerene $C_{60}$ and 3,4-ethylenedioxythiophene (EDOT) that, after being photoactivated, was able to kill > 99.9% of planktonic *S. aureus* and > 99.9% of biofilms formed on it. In another study, a porphyrin-fullerene $C_{60}$ dyad (TCP-$C_6$) was made by attaching a carbazoyl porphyrin derivative to fullerene $C_{60}$. The irradiation of the film was effective in the inactivation of 4 log of *S. aureus* and *E. coli* [177]. *E. coli* and *S. aureus* were reduced by 99% and 98% respectively by a modified fabric, composed of a bottom layer of ε-polylysine, able to kill bacterial membrane, and a second layer of zinc phthalocyanine, a photosensitiser [178].

## Blue Light and Food Products

The application of photoinactivation has recently started to consider the food sector and, in particular, food products. As reported in Fig. 8, the treatment of food products is one of the possible applications of blue light. In Tables 1 and 2, the efficacy of blue light against microbes has been evaluated on some food products. Based on the food commodities that were treated, different quality parameters were analysed.

### Milk and Dairy Products

Several studies have been carried out on milk and dairy products, confirming the antimicrobial effect of blue light without any significant alteration in any of the milk

constituents (e.g., sugars, proteins and lipids) but increasing the shelf life [179–182]. As an example, Srimagal et al. [183], to achieve a reduction of 5 log, as required for a pasteurization process, and the minimum colour change, used values of wavelength, temperature, and treatment time corresponding to 406 nm, 13.8 °C, and 37.8 min, respectively. Furthermore, no significant differences in composition and physic-chemical properties were underlined between light-treated and untreated samples. The shelf life for samples packed in low-density polyethylene (LDPE) pouches was about 9 days for light-treated milk under refrigerated storage, almost twice that of untreated milk samples that had a 5-day shelf life.

### Horticultural Products

The microbial inactivation of blue light was also conducted on horticultural produce, such as cucumber, tomato, lettuce, mangos, pineapple, grapes, cantaloupe rinds, fenugreek seeds, mung beans and mung bean germlings [19, 96, 144, 150, 184, 185]. Studies confirmed that blue light exerts an antimicrobial effect (1.0–5.0 log reductions); the differences in inactivation efficiencies can be attributed to differences in initial microbial populations, any addition of exogenous photosensitizers and the process parameters (wavelength, light dose, temperature, etc.). Also, some studies investigated the effect of the physico-chemical properties of horticultural produce after light treatments. In most of the cases, there were no significant differences in the number of anthocyanins, phenols, flavonoids, surface colour and antioxidant activity and, generally, the shelf life was increased [150, 184, 185]. Aurum and Nguyen [19] evaluated the effects of 465 nm light on grapes with the addition of curcumin. *E.coli* inactivation accounted for 2.4 log cfu/g after 36.3 J $cm^{-2}$ light exposure. The evaluation of physicochemical properties revealed that untreated grapes underwent significant quality degradation indicated by losses of weight, firmness, visual appearance and nutrients. Related to the sensorial quality, photoactivated samples experienced less textural degradation, better maintenance of fruit firmness and reduced weight losses.

### Meat and Fishery Food Products

Few studies have been carried out on meat and fishery food products [31, 147, 148, 186, 187]. Light treatments seem to be less effective on meat and seafood surfaces compared to in vitro treatments: this could be due to the opaque and uneven surface of the products, which creates shadows and to the presence of proteins that attenuated ROS [187, 188]. Kim et al. [188] evaluated the effect of 405 nm treatment on *Salmonella enteritidis* in PBS and on the surface of cooked chicken. While the microbial inactivation in PBS at 0.45 kJ $cm^{-2}$ was 1.3–2.1 log CFU/mL, on the cooked chicken surface, it accounted for only 0.8–0.9 log CFU $m^{-2}$ at a higher dose (3.80 kJ $cm^{-2}$). Also, Gunther et al. [147] confirmed the higher microbial inactivation effect on stainless steel surfaces with respect to chicken skin. In fact, 405 nm treatment (185.8 J $cm^{-2}$) produced a reduction of *Campylobacter coli* of 2.1 log, while a 5.1 log reduction was achieved with treatment on stainless steel. Luo et al. [186] observed that the application of 44 J $cm^{-2}$ light had no negative impacts on fresh beef quality, only very slight changes in colour, total free amino acids and taste. To improve microbial inactivation, other authors investigated the addition of an external photosensitizer: 460 nm light treatment and 100 μL of riboflavin produced a reduction of 1.1–1.2 log CFU/$cm^2$. However, riboflavin can release its yellow-orange colour after LED illumination causing a change in smoked salmon [31]. Gao and Matthews [189] tested the inactivation of *L. monocytogenes* on chicken skin with the addition of water-soluble curcumin obtaining a reduction of 2.9 log, and no significant colour change was observed. The authors suggest that fats and the complex surface of chicken skin may interfere with the results.

## Blue Light and Synergy with Other Applications

### Compounds

#### Natural

The application of blue light can be improved by the contribution of exogenous photosensitizers. The addition of compounds such as curcumin or ALA determined an increased microbial inactivation due to the higher production of ROS.

Essential oils are products of the secondary metabolism of aromatic plants. Some of them have strong antibacterial and antifungal effects. Hydrophobicity is the main characteristic of essential oil components since it allows them to move through bacterial cell membrane's lipids, disorganizing cell structures and making them more permeable. This determines a loss of chemiosmotic control which cause cells' death [190, 191]. The combination of essential oils and blue light could be a way to increase microbial inactivation, compared to the use of these compounds alone. However, the effect depends on the type and concentration of essential oils, the light wavelength and the microorganism species [192]. Clove (*Eugenia caryophyllata*) and thyme (*Thymus vulgaris*) essential oils, in a concentration of 5% in combination with 470 nm light treatment, were able to decrease *Staphylococcus epidermidis*, *P. aeruginosa* and *C. albicans*.

The antiseptic qualities of plant-derived compounds from herbs and spices and their extracts have been recognized

since antiquity. The antimicrobial activity of plant extracts may be due to phenolic compounds or other hydrophobic components present in essential oils. Polyphenols are secondary metabolites ubiquitously distributed in all higher plants. Among them, flavan-3-ols, flavonols and tannins exert great antimicrobial activity against a wide range of microbes and can suppress microbial virulence factors (e.g., inhibition of biofilm formation, reduction of host ligands adhesion and neutralization of bacterial toxins) [12, 191, 193, 194]. Polyphenols can exert a synergistic effect with blue light treatment for microbial inactivation. Nakamura et al. [195] evaluated the effect of caffeic acid, gallic acid, chlorogenic acid, epigallocatechin, epigallocatechin gallate and proanthocyanidin (1 mg/mL in polyphenol aqueous solution) exposed to 400 nm light in liquid medium against gram(+) bacteria (*E. faecalis, S. aureus and Streptococcus mutans*) and gram(−) bacteria (*Aggregatibacter actinomycetemcomitans, E. coli, and P. aeruginosa*). It was confirmed that the bactericidal activity of polyphenols can be increased by photoirradiation. In particular, caffeic acid and chlorogenic acid reduced all bacterial species studied up to 4 log CFU/mL when irradiated for 10 min.

### Chemicals

Chlorinated disinfectants are widely used for surface decontamination in sanitisation protocols in the food industry. Moorhead et al. [196] studied the effect of blue light combined with chlorinated disinfectants on *Clostridium difficile*, both in vegetative and spore form. The results were encouraging because *Cl. difficile* was inactivated by 405 nm light; the spores were tenfold more resistant compared with the vegetative form. Even more promising results were achieved by the combined use of the chlorinated disinfectants and 405 nm light obtaining a synergic microbial inactivation effect. The spore inactivation was enhanced dramatically by the treatment with blue light and reduced the concentration of the disinfectants. Other authors investigated the synergic effect between blue light and hydrogen peroxide ($H_2O_2$) to increase the phototoxic activity against *S. mutans* Feuerstein et al. [197]. Compared with the control sample, the $H_2O_2$ treatment (0.3 mM) by 30% while the combination of the two methods yielded 96% growth inhibition. Also, other authors studied the combination of $H_2O_2$ (33.3 mM) and chlorin e6 (Ce6, 50 μM) under blue light irradiation (450 nm). The antimicrobial activity improved strongly, especially in the multi-species biofilm eradication, reaching a reduction of 6.6 log [198].

Silver nanoparticles (AgNPs) are considered a new class of antimicrobials due to their action against gram(+) and gram(−) bacteria. AgNP activity would depend not only on their concentration and size but also on their shape [199]. Pal et al. [200] demonstrated that triangular-shaped nanoparticles are qualitatively more effective against *E. coli* probably because they give a greater positive charge to the nanoparticles, which together with the active facets on a triangular-shaped particle can ensure a greater activity. The use of AgNPs in combination with blue light could enhance the effect of light alone: this was confirmed for both *S. aureus* MRSA [201, 202] and *P. aeruginosa* solutions [199, 203].

The action of photosensitizers could be markedly increased by the addition of non-toxic inorganic salt solutions by accelerating type I or type II reactions [204]. The photodynamic inactivation was enhanced using potassium iodide solution (10 Mm) to potentiate methylene blue (10 μM) with irradiation of 50 s and an irradiance of 100 mW/cm$^2$ to reduce *S. aureus* and *E. fecalis* [205, 206].

New polymers such as carbon dots (CDots) have been recently discovered for their antibacterial activity when activated by blue light [207]. Nonetheless, the application of these kinds of materials is strictly related to their safe use. However, the methods to include these polymers inside materials should be examined.

### Technologies

Some studies have also investigated the synergistic effect of ultrasound (US), combined with blue light to inactivate microbes. The combination of US and blue light treatment increased cell viability loss, DNA damage, and, in the medical field, clonogenicity inhibition of mammary cancer cells [208]. A recent study investigated the in vitro effect of S. aureus biofilms with 450 nm light and 1 MHz US, achieving 3.48 log inactivation [209].

Magnetic fields could also be implemented with blue light treatments to enhance the antimicrobial effect. In fact, the application of a magnetic field stresses the bacterial cells, which respond by the activation of genes ALA dehydratase (ALAD), which promotes the synthesis of porphyrins [210]. Astuti et al. [211] investigated the antimicrobial photodynamic effect on *E. coli* activated by magnetic fields 1.8 mT using different wavelengths (469 nm, 541 nm and 626 nm). After a radiant exposure of 18.81 J cm$^{-2}$, *E. coli* on solid culture medium were reduced up to 80%.

## Safety of Blue Light

A study conducted by Kleinpenning et al. [212] tested the effect of blue 420 nm light (100 J cm$^{-2}$ per day) on eight healthy volunteers by illuminating them over 5 consecutive days at 20 J cm$^{-2}$. From skin biopsies, the results were the following: no DNA damage, no inflammatory processes of the cells and no sunburn before and after the test, although they observed transient melanogenesis and vacuolization of

keratinocytes even though these alterations did not lead to cell apoptosis.

Notwithstanding the safety of skin application, blue light could be detrimental to the eyes. In an in vitro study, the application for 17 h of 420 and 430 nm at 1.13, 1.16 mW $cm^{-2}$ on human corneal and conjunctival epithelial cells was evaluated. The results show that a significant decrease in cellular viability, changes in cellular morphology, ROS accumulation and an alteration of mRNA expression implicated in the inflammatory response were observed [213]. Damages due to the blue light were observed on the retina caused by oxidative stress and cataract formation due to accumulating ROS [214].

The Italian Legislation (Legislative Decree 81/2008, Chapter V, Art. 213 -218 and Annex XXXVII), in accordance with Directive EU 2006/25, has set the limit values for exposure to blue light radiations: $L_B = 10^6 \cdot t^{-1}$ W/$m^2$sr and $L_B = 100$ W/$m^2$sr for $\alpha \geq 11$ mrad ($L_B$ is the effective radiance and α the angular subtense) [215, 216]. The International Commission for Non-Ionizing Radiation Protection ICNIRP [217] has stated that the maximal exposure duration to blue light should be 100 J/$cm^2$·sr for about 167 min per day [101]. Other guidelines were provided by the Castleman and Ziem [218], that recommend daily exposure of personnel to blue light with these limits: for 10,000 s (2.8 h) or more exposure, the maximum intensity of the light source is ≤ 0.01 W/$cm^2$.sr; for light intensity above 0.01 W/$cm^2$.sr, the maximum dose of light is 100 J/$cm^2$.sr. (i.e. light dosage (J/$cm^2$·sr) = light intensity (W/$cm^2$·sr) × time of exposure (s)) and for a light source subtending an angle less than 0.011 rad, the maximum light intensity is $10-4$ W/$cm^2$ for exposure over 100 s. It is therefore of fundamental importance to use a tested protective filter to cover the eyes completely, such as the use of specific glasses that guarantee adequate protection for the workers [219]. It is fundamental to develop safety standards to promote the application of blue light technologies.

## Conclusions and Future Perspective

Blue light has great potential to be used against pathogen microbes present in the environment and food sector. The natural presence of photosensitizers inside bacterial cells represents a great potential to exploit this technology, which could also be improved through the addition of exogenous molecules. Furthermore, the safety aspects together with the unlikely possibility to develop microbial resistance represent an advantage for its application.

While it is a very promising non-thermal technology, there is still a lack of in-depth studies on its effects due to its novelty. Since it seems promising for microbial inactivation, studies have focused mainly on the medical field and to a lesser extent on the food sector. As reported in this review article, for example, there are few studies on the blue visible region penetration through the layers of surfaces, food or water. This research would allow us to better understand the mode of microbial inactivation and the chemical modifications inside different food matrices, being able to adapt the blue light to better conditions of dose and treatment time. Among the various studies, scientists should focus also on the sensorial properties of blue light–treated food products to consider the acceptance for consumers in terms of colour, texture and perception of shelf life.

It is also necessary to amplify the spectrum of microbes treated with blue light. Most of the published studies regarding blue light to reduce the microbial load on food and in the environment are related to aerobic pathogens. Only a few articles investigate the effect of anaerobic microorganisms, and they are often related to the medical field [220, 221]. More knowledge on the antimicrobial efficacy of blue light on anaerobic species would provide important insights for applying the technology also to vacuum-packed products. Furthermore, it would be important to understand the effects of blue light on the spores of *Clostridium* spp.

Some recent articles have tested the use of blue light in some food products with promising results [151, 184, 222], however, products treated with blue light must be studied to avoid damage to the product due to oxidative reactions or alterations of taste and colour.

New polymers such as carbon dots (CDots) have been recently discovered as having antibacterial activity when activated by blue light [207]. However, the application of these kinds of materials is strictly related to their safe use. Nonetheless, the methods to include these polymers inside materials should be examined.

In addition, studies should be directed towards the discovery of new exogenous molecules that should be investigated for their specific properties and different methods of release to improve photodynamic activity. A lot of recent works have involved the study of the properties of nanomaterials, such as silver (Ag), silver oxide ($Ag_2O$), titanium dioxide ($TiO_2$) and zinc oxide (ZnO). These compounds exploit the photocatalysis process to produce ROS with antimicrobial activity, and they can be enhanced by blue light [199, 223]. Also for these substances, it would be interesting to know the physical–chemical effects in the environment and the food as the possibility of releasing toxic compounds, food changes or how they can be included in the materials.

The application of photoactive materials as food packaging should be exploited to treat packaged food to minimize the possible microbial cross-contamination.

Another important aspect of this technology is sustainability, the long-term LED duration and low heat emission. Blue light can be defined as an eco-friendly technology because it does not leave residuals after treatments.

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## Declarations

**Competing Interests** The authors declare nocompeting interests.



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