# Invited Review

# Review of the Comparative Susceptibility of Microbial Species to Photoinactivation Using 380–480 nm Violet-Blue Light

Rachael M. Tomb[1]* ⓘ , Tracy A. White[1], John E. Coia[2], John G. Anderson[1], Scott J. MacGregor[1] and Michelle Maclean[1,3]

[1]The Robertson Trust Laboratory for Electronic Sterilisation Technologies (ROLEST), Department of Electronic & Electrical Engineering, University of Strathclyde, Glasgow, UK
[2]Department of Clinical Microbiology, Glasgow Royal Infirmary, Glasgow, UK
[3]Department of Biomedical Engineering, University of Strathclyde, Glasgow, UK

## ABSTRACT

Antimicrobial violet-blue light is an emerging technology designed for enhanced clinical decontamination and treatment applications, due to its safety, efficacy and ease of use. This systematized review was designed to compile the current knowledge on the antimicrobial efficacy of 380–480 nm light on a range of health care and food-related pathogens including vegetative bacteria, bacterial endospores, fungi and viruses. Data were compiled from 79 studies, with the majority focussing on wavelengths in the region of 405 nm. Analysis indicated that Gram-positive and Gram-negative vegetative bacteria are the most susceptible organisms, while bacterial endospores, viruses and bacteriophage are the least. Evaluation of the dose required for a 1 $\log_{10}$ reduction of key bacteria compared to population, irradiance and wavelength indicated that microbial titer and light intensity had little effect on the dose of 405 nm light required; however, linear analysis indicated organisms exposed to longer wavelengths of violet-blue light may require greater doses for inactivation. Additional research is required to ensure this technology can be used effectively, including: investigating inactivation of multidrug-resistant organisms, fungi, viruses and protozoa; further knowledge about the photodynamic inactivation mechanism of action; the potential for microbial resistance; and the establishment of a standardized exposure methodology.

## INTRODUCTION

Traditionally, visible light inactivation of microorganisms has been associated with photodynamic inactivation (PDI). This inactivation method utilizes exogenous photosensitizers such as methylene blue, rose bengal or cationic porphyrins, which in the presence of oxygen become excited when exposed to different wavelengths of light, including violet-blue (380–500 nm) and red (625–740 nm) (1–5). These excited photosensitizers produce reactive oxygen species (ROS), such as singlet oxygen and hydroxyl radicals, which can damage many structures within microorganisms, including proteins, lipids and nucleic acids (2,6–8). This leads to cellular damage and inactivation of bacteria, yeasts, fungi, viruses and parasites (9).

Research carried out over the last decade has indicated that exogenous porphyrins are not always required for visible light inactivation of microorganisms and that violet-blue light wavelengths possess the potential for antimicrobial capabilities. Early studies by Hamblin *et al.* (10) indicated *Helicobacter pylori* could be inactivated by at least 99% following a dose of 20 J cm$^{-2}$ of violet-blue 405 nm light. The authors believed this was due to the presence of high levels of the intracellular porphyrins coproporphyrin and protoporphyrin IX, which produce ROS upon illumination. These findings were further supported by Guffey and Wilborn (11), who demonstrated a successful 88% and 91% reduction of *Staphylococcus aureus* and *Pseudomonas aeruginosa* following exposure to 405 nm light. Shortly after this, studies by Maclean *et al.* (12,13) demonstrated the oxygen enhancement of the visible light inactivation of *S. aureus*—supporting the theory that inactivation was via a photodynamic process—and that within the violet-blue light region, antimicrobial activity peaked with exposure to wavelengths of around 405 nm ($\pm$10 nm).

Subsequently, many studies have investigated the antimicrobial efficacy of 405 nm light and also the broader violet-blue wavelengths. For example, Haughton *et al.* (2012) inactivated *Campylobacter jejuni* using 395 nm light, while Bumah *et al.* (14,15) demonstrated the antimicrobial efficacy of 470 nm light against *Salmonella enterica* and *S. aureus*. Additionally, a small number of bacterial endospores, fungi and yeasts have been inactivated using violet-blue light (16–23). To date, little is known about viral susceptibility; however, there is now published evidence demonstrating 405 nm light inactivation of a viral surrogate, bacteriophage $\phi$C31, and a mammalian virus, feline calicivirus, without the requirement of additional photosensitizers (24,25).

To allow greater understanding of antimicrobial scope and efficacy of violet-blue light, this systematized review was

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designed to compare the inactivation efficacy of 380–480 nm light on a range of microorganisms including bacteria, fungi and viruses, using data from peer-reviewed research studies. The analysis of the antimicrobial efficacy of violet-blue light includes the following parameters: the effect of population density, the effect of irradiance and comparison of the average doses required for 1 $\log_{10}$ reduction using light between 380 and 480 nm. Performing this analysis will help to gain advanced knowledge and draw conclusions about violet-blue light inactivation of different microbial species, the most effective wavelengths, and additionally indicate further areas of research which require to be investigated.

## METHODOLOGY

### Database search and study inclusion

Two databases, PubMed and Science Direct, were searched for articles from the past thirty years (May 1987–May 2017). Search terms used key words associated with violet-blue light technology (*i.e.* individual wavelengths between 380 and 480 nm, blue light, visible light) and decontamination (inactivation, kill, antimicrobial, decontamination, disinfection, antibacterial, antiviral, antifungal, photoinactivation, photosensitizers). The first 20 pages of each web search were screened (equivalent to 400 papers), or all pages if the search generated less than 20 pages. Additional references added to the screening list included research papers (found in the references section of review papers or in the author's personal collection) as well as 2 PhD theses by Bache (26) and Tomb (27), as these contained inactivation data not currently published elsewhere. Once combined and duplicates excluded, the search yielded 9058 articles (Fig. 1). The titles and abstracts were then screened to identify relevant peer-reviewed papers and those which were not relevant to this review were excluded, for example, studies on PDI, studies inducing endogenous porphyrin production and those which used wavelengths of light outwith 380–480 nm. Following the initial screening process, 153 articles underwent a full-literature review. An additional 74 papers were subsequently removed, including reviews on violet-blue light or research studies which performed experiments outwith the scope of this review, for example, violet-blue light inactivation of microorganisms exposed on food, bacterial biofilms and pathogens on environmental surfaces.

### Data extraction and analysis

The final 79 research articles reviewed (7,10–87) contained inactivation data on clinically relevant and food-associated microorganisms exposed to violet-blue light between 380 and 480 nm. Data from each article were extracted, summarized and tabulated (see Supporting Information). This included information on microorganism (categorized by species and strain number), wavelength of light, irradiance of light source, applied dose and population exposed.

Subsequent analysis of inactivation kinetics was based upon that of Hessling *et al.* (88). $\log_{10}$ reduction values were extracted from text and tables, or if unavailable, extracted from figures. The highest dose reported, for the greatest significant inactivation of each exposed population, was recorded; however, if prominent tailing of results occurred, the previous dose was used in the analysis. Recording data in this way allowed the calculation of the average dose required for 1 $\log_{10}$ reduction (total dose of light/total $\log_{10}$ inactivation) and therefore allowed comparison between studies investigating different: organisms, irradiances of light, wavelengths of violet-blue light and exposed populations.

The data extracted include inactivation at low ($\leq 10°C$) or high ($\geq 37°C$) temperatures. However, as violet-blue light inactivation of bacteria has been shown to be enhanced in low or elevated temperatures (43), the results of these studies were excluded from figures. Similarly, information on inactivation in anaerobic conditions is included in the data set, but excluded from any additional figures on the dose analysis.

Statistical analysis of data sets was performed using Minitab statistical software, version 17 (Minitab Ltd, Coventry, UK). One-way ANOVA with *post hoc* Fisher's test was used to analyze dose requirements between microbial groups, with significant differences when $P < 0.05$. Linear regression analysis was used to investigate significant relationships between $x$ & $y$ values, with significant relationships demonstrated when $P < 0.05$.

## RESULTS

Data were retrieved from 79 sources providing information on the inactivation kinetics of a range of microorganisms using violet-blue light between 380 and 480 nm. There were >370 individual entries accumulated, including 57 bacterial strains, 8 yeasts and fungi, 1 bacteriophage and 2 mammalian viruses (see Supporting Information).

As an overall comparison of the data gathered, Fig. 2 represents the dose required for 1 $\log_{10}$ inactivation of the different microbial species, at the various wavelengths investigated. It should be noted that fungal inactivation data were split by morphological group: hyphae, conidia, germinating conidia and yeasts, to demonstrate the varying susceptibility in later analysis. As can be seen in Fig. 2, the majority of light inactivation studies have been carried out using violet-blue light peaking at 405 nm ($n = 51$). Additionally, there is interest in inactivation at longer wavelengths of blue light including 415 nm ($n = 8$), 450 nm ($n = 5$) and 470 nm ($n = 8$). The majority of Gram-positive and Gram-negative vegetative cells required doses <200 J cm$^{-2}$ (246 out of 280 studies). However, Gram-positive endospores, fungal spores, yeast, viruses and bacteriophage generally required >400 J cm$^{-2}$ for a 1 $\log_{10}$ reduction.

Additionally, the data were collated to determine the general susceptibility across the different microbial groups. The average dose for a 1 $\log_{10}$ reduction using light between 380 and 480 nm was calculated for each individual microbial species (where possible), with results demonstrated in Fig. 3. As can be seen in Fig. 3, Gram-negative and Gram-positive vegetative bacteria are most susceptible with mean doses of in the region of 100 J cm$^{-2}$ required for 1 $\log_{10}$ reduction, whereas viruses and bacteriophage were least susceptible with up to 1 kJ cm$^{-2}$ required for a 1 $\log_{10}$ reduction. One-way ANOVA with Fisher's *post hoc* test indicated no significant difference between dose requirements for Gram-positive and Gram-negative vegetative bacteria and yeasts, while these groups were all significantly different to bacterial spores, germinating fungal conidia, viruses and bacteriophage ($P < 0.05$).

Similar to Hessling *et al.* (88), it was possible to perform further analysis on the data collected with regard to individual
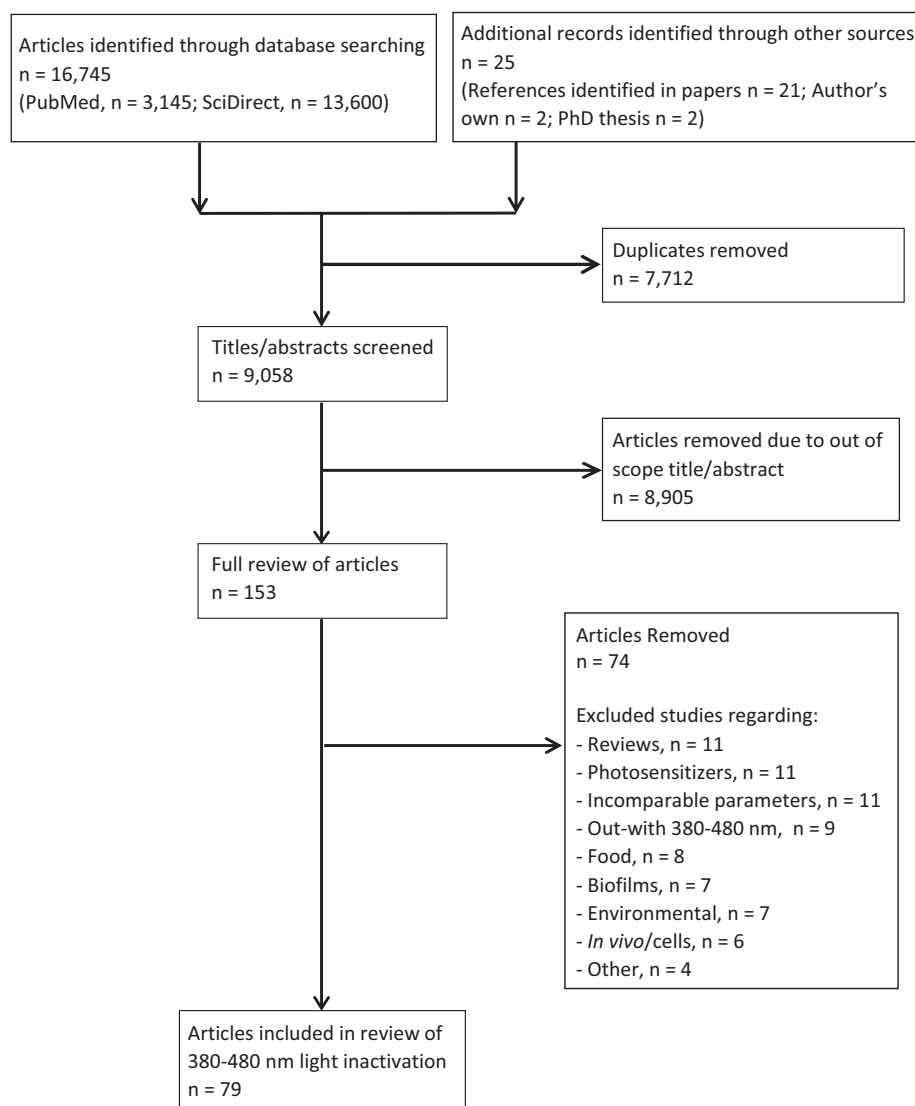
**Figure 1.** Flow chart of the study selection process during the systematized review.

Gram-positive and Gram-negative bacteria. The most common Gram-positive organisms that have been investigated are *S. aureus* (*n* = 30 studies) and *Listeria monocytogenes* (*n* = 8 studies), and the most common Gram-negative bacteria are *Escherichia coli* (*n* = 25 studies) and *P. aeruginosa* (*n* = 14 studies). Using these organisms, comparisons were made between the dose for 1 $\log_{10}$ reduction using 405 nm light and the starting population (Fig. 4) or applied irradiance (Fig. 5). Additionally, the dose for 1 $\log_{10}$ reduction of these organisms was compared between the differing wavelengths (380–480 nm) of violet-blue light (Fig. 6).

Upon comparison of the starting population with the dose of 405 nm light required for 1 $\log_{10}$ reduction, the linear fit indicates that with increasing populations of *S. aureus*, *E. coli* and *L. monocytogenes* higher doses of 405 nm light are required, whereas in the case of *P. aeruginosa,* it appears that as the concentration increases, the dose required decreases (Fig. 4). However, as the Pearson's *r* values for the linear correlation analysis of all the bacteria investigated are between −0.12 and 0.23, and *P* values are >0.05, these indicate that there is no

significant linear relationship, so no true conclusions can be drawn.

Upon comparison of the irradiance of 405 nm light used, the linear fit suggests that for *S. aureus* and *P. aeruginosa,* there is little change in dose required for 1 $\log_{10}$ reduction as irradiance increases (Fig. 5). However, regarding inactivation of *E. coli* and *L. monocytogenes,* this is not the case, with higher irradiances resulting in the dose increasing for *E. coli* inactivation, and the dose decreasing for *L. monocytogenes* inactivation. However, analysis of the Pearson's *r* values for the linear correlation indicates no linear relationship for *E. coli* (0.22) and a weak negative linear relationship (−0.3) for *L. monocytogenes*; therefore, no true significant conclusions can be drawn.

Additionally, the wavelength of violet-blue light used was compared with the dose for 1 $\log_{10}$ reduction of the four vegetative bacterial species. As can be seen in Fig. 6, there is very little difference in dose required when exposing *S. aureus* and *P. aeruginosa* to different wavelengths of violet-blue light; however, this is not the case with *L. monocytogenes* and *E. coli*. As the wavelength increases, the dose for 1 $\log_{10}$ reduction
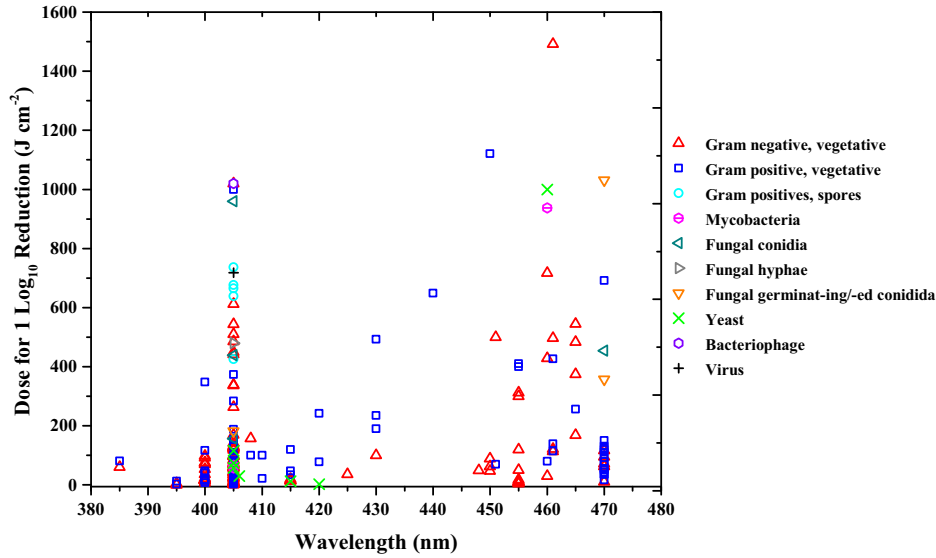
**Figure 2.** Comparison of the dose for a 1 $\log_{10}$ reduction of a range of microorganisms, when exposed to differing wavelengths of violet-blue light.
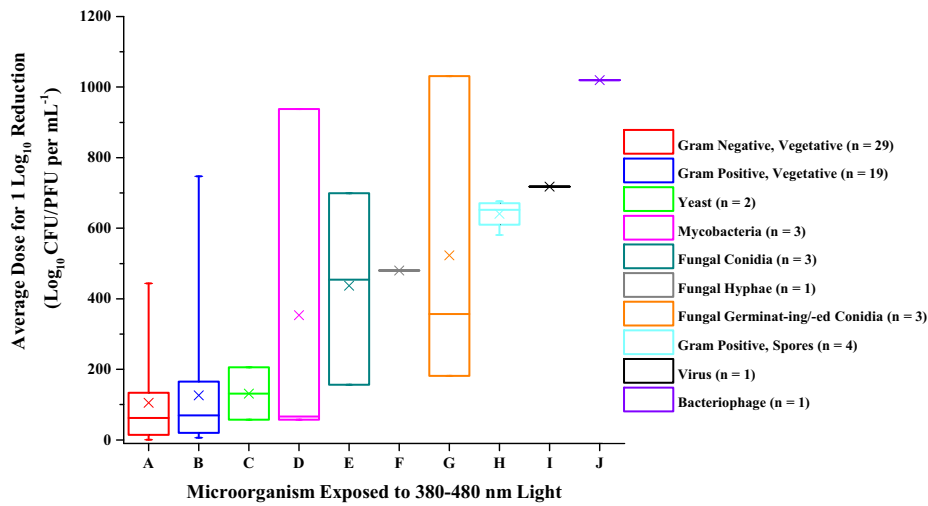


**Figure 3.** Box plot analysis of the average dose for 1 $\log_{10}$ reduction between different microbial groups. Please note that in the case of the Gram-positive and Gram-negative bacteria, the aerobic, anaerobic, facultative aerobic, facultative anaerobic and microaerophilic organisms have been grouped together. Crosses "×" indicate the mean dose for a 1 $\log_{10}$ reduction.

increases, with Pearson's *r* values of 0.39 and 0.73, indicating that there is a moderately strong positive correlation between the wavelength used and dose required for these organisms. This is additionally supported by *P* values of 0.020 and 0.001 for *E. coli* and *L. monocytogenes,* respectively, indicating that there is a statically significant relationship between wavelength and dose for both organisms.

A final analysis was conducted to establish the comparative inactivation efficacy of shorter wavelengths of violet-blue light (~405 nm) to longer wavelengths (450–470 nm) and is demonstrated in Fig. 7. Data were collected from several studies which investigated the inactivation of bacteria, using different wavelengths of violet-blue light, held under similar exposure conditions in each study (11,12,14,31,36,39,45,52,58). Figure 7a demonstrates data for Gram-positive isolates, with the dose required for a 1 $\log_{10}$ reduction increasing for all organisms when wavelength is increased from 405–415 nm to 450–

470 nm. The exception being the dose required for inactivation of *Streptococcus inniae* which decreased from 90.3 to 70.6 J cm$^{-2}$ when wavelength was increased from 405 to 465 nm (31). The data for inactivation of Gram-negative isolates (Fig. 7b) also demonstrate that for all organisms analyzed, higher doses were required for inactivation when longer wavelengths of violet-blue light were utilized. Lower does were required for 1 $\log_{10}$ reduction of *Enterococcus facecalis* and *E. coli* when exposed to 385 nm violet-blue light compared to 405 nm (39); however, this is to be expected as 385 nm lies within the UVA spectrum (320–400 nm).

## DISCUSSION

To investigate the antimicrobial scope and efficacy of violet-blue light, a systematized review was carried out to compare the dose required for 1 $\log_{10}$ reduction of a range of microorganisms. This
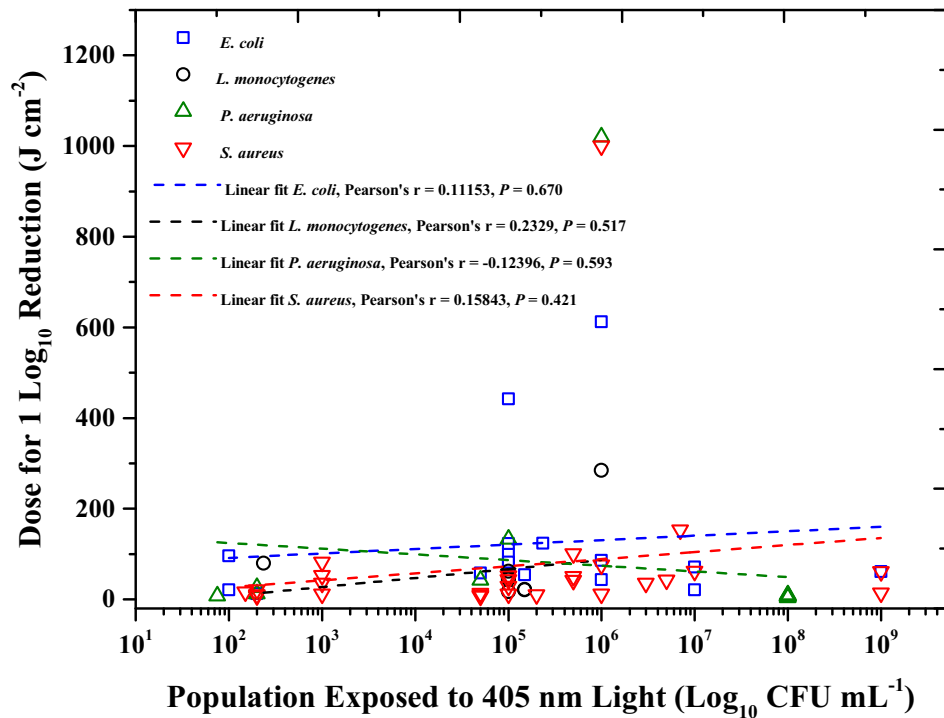
**Figure 4.** Comparisons of the dose for 1 $\log_{10}$ reduction of Gram-positive and Gram-negative bacteria, using 405 nm light, when there are differing population densities. *P* values >0.05 indicate no statistically significant relationship between population density and dose requirement values, using linear regression analysis.
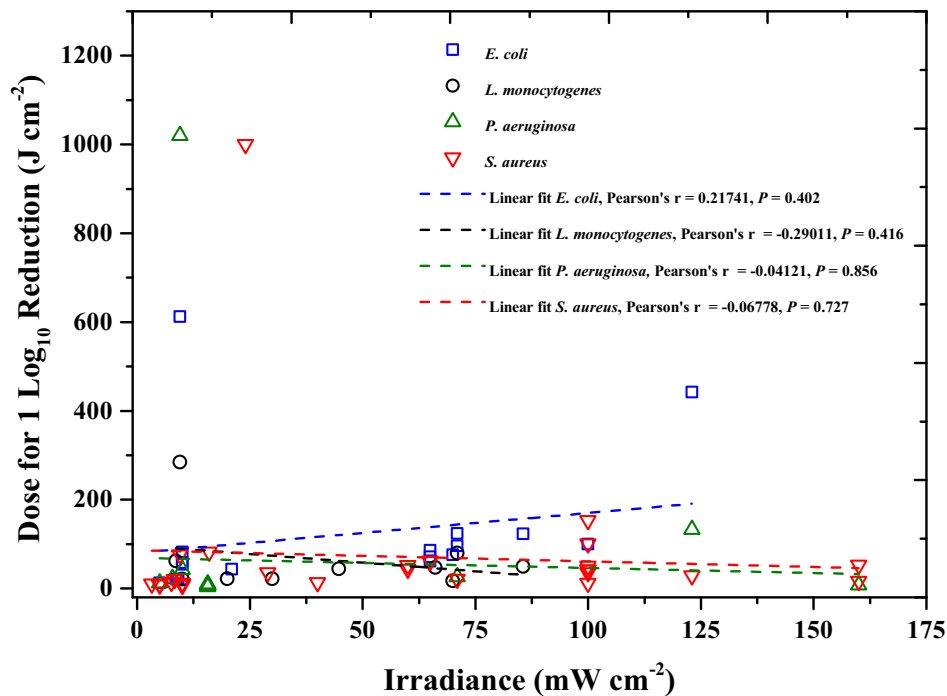


**Figure 5.** Comparisons of the dose for 1 $\log_{10}$ reduction of Gram-positive and Gram-negative bacteria, when exposed to differing irradiances of 405 nm light. *P* values >0.05 indicate no statistically significant relationship between irradiance and dose requirement values, using linear regression analysis.

allowed comparison of the inactivation data generated between research groups, indicated how the efficacy of violet-blue compares between organisms and confirmed areas of antimicrobial violet-blue light research which require further investigation.

The systematized review indicated many differences in the light sources and experimental arrangements used between research groups. Major differences in the experimental arrangement included how microorganisms were exposed, with some

**Figure 6.** Comparisons of the dose for 1 $\log_{10}$ reduction of Gram-positive and Gram-negative bacteria, when exposed to differing wavelengths of violet-blue light. *P* values <0.05 indicate a statistically significant relationship between wavelength of light and dose requirements, using linear regression analysis.
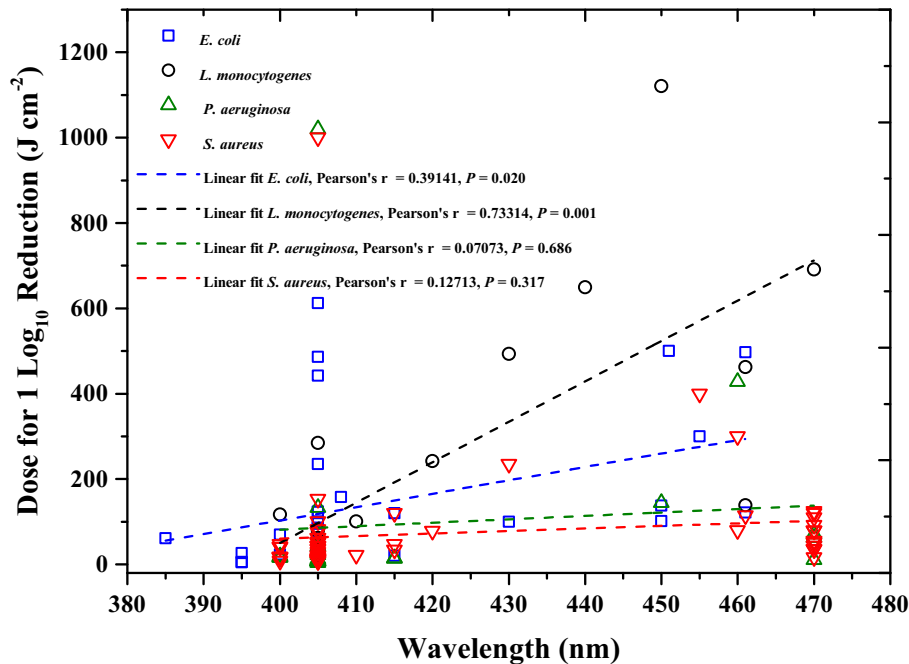
studies exposing organisms on agar plates, while others exposed organisms in suspension (1 $\mu$L-40 mL). Light sources varied with different groups using single LEDs and arrays (1–144 LEDs) to broadband sources with filters, which emitted violet-blue light at irradiances ranging from 1.2 to 520 mW cm$^{-2}$. Exposure times also ranged from 20 s to 48 h, with exposed population densities of between $10^1$ and $10^9$ CFU. Furthermore, as complete data sets were not always available, and at times the experimental arrangement was unclear, it was not possible to use mathematical models of inactivation kinetics such as the Kamau, Gompertz, Weibull or Hom models (33). Therefore, dose for 1 $\log_{10}$ reduction, as demonstrated by Hessling *et al.* (88), was the most appropriate means of comparison.

Despite these methodological variations, several comparisons could be made using the data gathered in the systematized review, including comparing the overall susceptibility of microorganisms to violet-blue light with dose for 1 $\log_{10}$ reduction. The data in Fig. 2 compare the dose of 380–480 nm light required between different organisms, with further analysis of these data provided in Fig. 3 comparing the average dose requirements between microbial groups. As can be seen in Fig. 3, Gram-positive and Gram-negative vegetative bacteria appear to require fairly similar doses of violet-blue light for 1 $\log_{10}$ inactivation, with mean doses of 126.5 J cm$^{-2}$ (6–748 J cm$^{-2}$) and 105.6 J cm$^{-2}$ (0.3–444 J cm$^{-2}$) required, respectively. A two-sample *t*-test also revealed the dose requirements for these groups were not significantly different (*P* = 0.655). This differs from previously published data, which indicated that Gram-positive bacteria are more sensitive to violet-blue light inactivation then Gram-negative bacteria (7,38,50). However, the results in this review do mirror those of Hessling *et al.* (88) who also demonstrated no evidence of increased

susceptibility of Gram-positive bacteria when compared to Gram-negative bacteria. It is worth noting, however, that the data for bacterial inactivation include aerobic, anaerobic, facultative aerobic, facultative anaerobic and microaerophilic species. It is possible that grouping these organisms has skewed the data, and this may be particularly pertinent with regard to Gram-negative organisms, as studies have demonstrated high sensitivity of microaerophilic (*Campylobacter*, *Helicobacter*) and anaerobic (*Fusobacterium*) organisms. For example, *C. jejuni* required a dose of 18 J cm$^{-2}$ for >5 $\log_{10}$ reduction (37), *H. pylori* required 10–20 J cm$^{-2}$ for up to 6 $\log_{10}$ reduction (10), and *Fusobacterium nucleatum* required an average dose of 17.8 J cm$^{-2}$ for a 1 $\log_{10}$ reduction (56), whereas facultatively anaerobic organisms such as *Escherichia* and *Salmonella* generally require greater doses of violet-blue for inactivation, with studies demonstrating as much as 2214 J cm$^{-2}$ required for a 5 $\log_{10}$ reduction of *E. coli* (20) and 739.6 J cm$^{-2}$ for a 1.4 $\log_{10}$ reduction of *S. enterica serovar enteritidis* (52). Therefore, future reviews could involve in-depth analysis of these organisms to discover whether sensitivity to violet-blue light is linked with microbial oxygen requirements.

Results in Fig. 3 additionally indicate that yeast cells have similar dose requirements to vegetative bacteria (*P* = 0.874, using one-way ANOVA), with the average dose of 131.6 J cm$^{-2}$ required for 1 $\log_{10}$ reduction. Mycobacteria, fungal conidia, fungal hyphae, germinating/germinated fungal conidia and bacterial endospores all required increasingly greater doses of violet-blue light compared to vegetative bacteria. The mean average doses being 354 J cm$^{-2}$ for mycobacteria, 437 J cm$^{-2}$ for fungal conidia, 480 J cm$^{-2}$ for fungal hyphae, 523 J cm$^{-2}$ for germinating/germinated fungal conidia and 641 J cm$^{-2}$ for bacterial endospores. Viruses appeared to be least susceptible with the highest
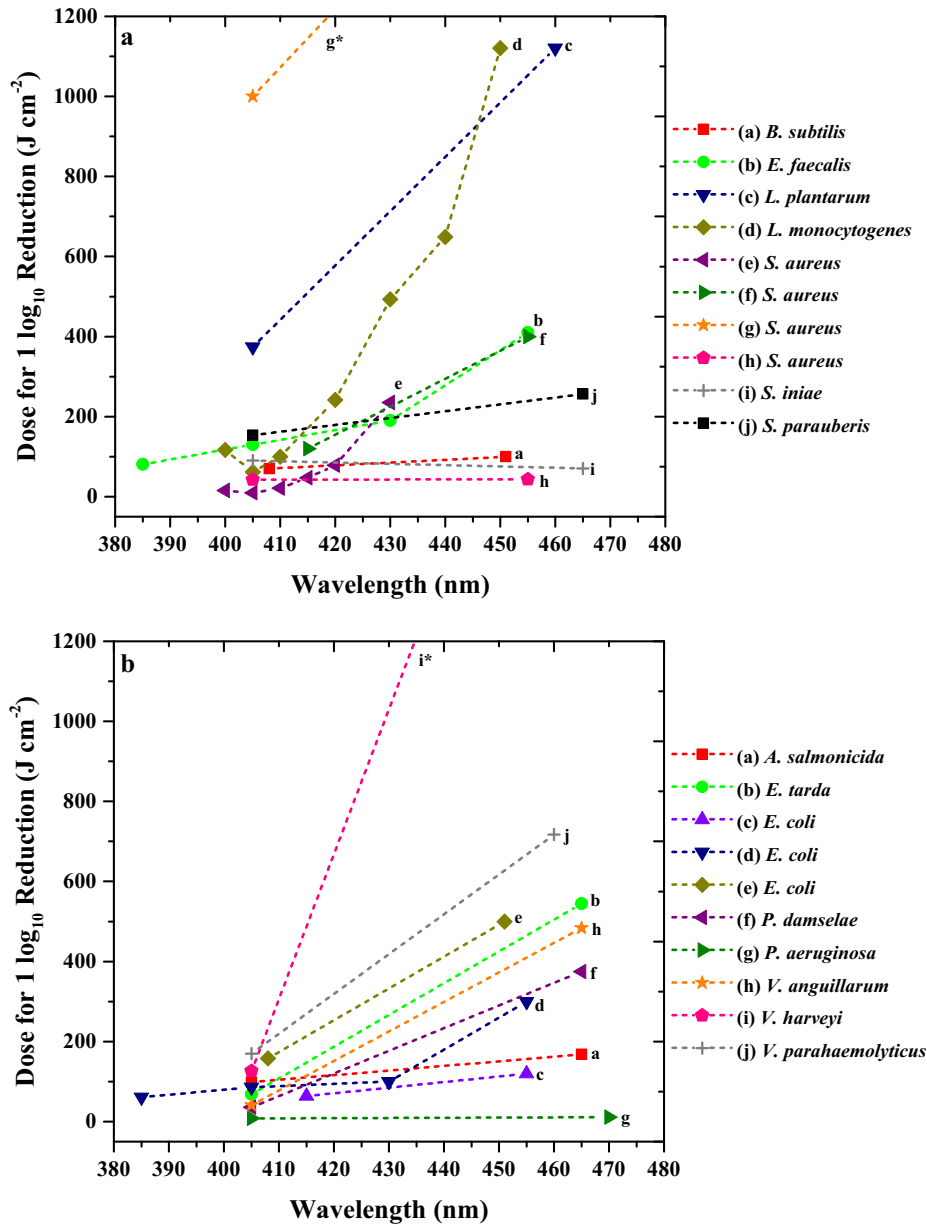
**Figure 7.** Comparison of the efficacy of short *versus* long wavelengths of violet-blue light on specific organisms for 1 $\log_{10}$ reduction: (a) Gram-positive organisms and (b) Gram-negative organisms. "*" indicates doses (>1200 J cm$^{-2}$) which did not result in bacterial inactivation.

mean doses of 718 and 1020 J cm$^{-2}$ required for 1 $\log_{10}$ reduction for viruses and bacteriophage, respectively.

Comparisons were also made between several species of bacteria (two Gram positive and two Gram negative) due to the large number of results collected during the systematized review. The effect of population density and irradiance of 405 nm light on the average dose for 1 $\log_{10}$ reduction was compared between *E. coli, L. monocytogenes, P. aeruginosa* and *S. aureus*. With regard to the population density used, the linear correlation trends indicated a slight increase in the dose requirements upon increasing population, with the exception of *P. aeruginosa* (Fig. 4). This is reflected in a study by Maclean *et al.* (7) who demonstrated that a dose of 36 J cm$^{-2}$ was required for a 3 $\log_{10}$ reduction of $10^3$ and $10^7$ CFU mL$^{-1}$ populations of *S. aureus* compared to a slightly increased dose of 41 J cm$^{-2}$ for

equivalent reduction of a $10^9$ CFU mL$^{-1}$ population. The increase in dose was attributed to attenuation of light passing through a $10^9$ CFU mL$^{-1}$ population of *S. aureus*. Light irradiance reduced from 10 mW cm$^{-2}$ at the sample surface to 5.6 mW cm$^{-2}$ after passing through the sample, which was not seen in samples with a lower population density (7). Additionally, Bumah *et al.* (79) demonstrated that bacterial density does not affect the bactericidal effect of 405 and 470 nm light, but the reduced light penetration of suspending liquids, due to increased bacterial concentration, is likely to limit bactericidal effect. It is also worth noting that the increased doses may also be due to increased oxygen requirements in larger populations, and as oxygen is necessary for inactivation (6,7), this could be a limiting factor reducing inactivation efficacy. However, these results are similar to those of Hessling *et al.* (88) who also found no

significant correlation between the starting population and dose requirements.

Trends in Fig. 5 indicate little change in the dose for 1 $\log_{10}$ reduction with increasing irradiance of 405 nm light (with the exception of *L. monocytogenes*), with no significant correlation seen. In the case of *L. monocytogenes*, as the irradiance increased there was a slight decrease in the dose required. This opposes results demonstrated by Murdoch *et al.* (50) who exposed *L. monocytogenes* to different irradiances (10, 20, 30 mW cm$^{-2}$) of 405 nm light. Following a dose of 108 J cm$^{-2}$ there was a slight decrease in inactivation from 5.18 $\log_{10}$ to 4.9 $\log_{10}$ reduction when irradiance was increased from 10 to 30 mW cm$^{-2}$. However, as the linear relationship was deemed weak in Fig. 5, it is still likely that there is an absorption maxima regardless of the irradiance of light used.

Stronger, significant correlation was observed with the relationship between dose and violet-blue light wavelength. In the case of *S. aureus* and *P. aeruginosa,* there was little change in dose with increasing wavelength; however, stronger positive correlations were seen for *E. coli* and *L. monocytogenes* (Fig. 6). This indicates that some bacterial strains may be more sensitive to shorter wavelengths closer to 405 nm rather than those toward 470 nm. 405 nm light was also demonstrated to be the most effective wavelength for microbial inactivation by Endarko *et al.* (52). Exposure of *L. monocytogenes* to wavelengths between 400 and 450 nm achieved maximum inactivation (1.45 $\log_{10}$ reduction) following exposure to 405 nm light and least inactivation (0.04 $\log_{10}$ reduction), using 450 nm light following a dose of 123.3 J cm$^{-2}$ (52).

This finding was further supported by analysis provided in Fig. 7. As demonstrated, the majority of the data sets (19/20) comparing the efficacy of violet-blue wavelengths found that increased doses were required for bacterial inactivation, or there was no inactivation achieved when using longer wavelengths between 450 and 470 nm. This is particularly pertinent in the case of *Edwardsiella tarda* with the dose for a 1 $\log_{10}$ reduction increasing from 68.4 to 544.5 J cm$^{-2}$ when wavelength increased from 405 to 465 nm (31), *Entercoccus faecalis* with the dose increasing from 130 to 410 J cm$^{-2}$ when wavelength increased from 405 to 455 nm (39), *Lactobacillus planterium* with the dose rising from 374 to 1121 J cm$^{-2}$ when wavelength increased from 405 to 460 nm (58) and *L. monocytogenes* with the dose rising from 61.6 to 1120.9 J cm$^{-2}$ when the wavelength was increased from 405 to 450 nm (52). Studies by Maclean *et al.* (12), Lui *et al.* (39) and Endarko *et al.* (52) further demonstrate the superior efficacy of 405 nm light compared to wavelengths including 400, 420, 415, 420 and 430 nm for inactivation of *E. faecalis*, *E. coli*, *L. monocytogenes* and *S. aureus*. Therefore, although the analysis was made on a small selection of studies ($n = 9$), the general trend does support the current thinking that shorter wavelengths in the region of 405 nm are more effective for bacterial inactivation than longer wavelengths between 450 and 470 nm.

It is also important to note that although the methodology to calculate dose for 1 $\log_{10}$ reduction was adapted from Hessling *et al.* (88), this article has included more papers on bacterial inactivation by violet-blue light than Hessling *et al.* (88) (including those by Barneck *et al.* (48), Decarli *et al.* (60), Fila *et al.* (68), Gillespie *et al.* (74), Gupta *et al.* (20), Hope *et al.* (40), Kawada *et al.* (67), Kim *et al.* (51,72), Kotoku *et al.* (63), O'Donoghue *et al.* (59)) and also included inactivation data on

fungi, yeasts, bacteriophage and viruses. Therefore, this review is a broader representation of the efficacy of violet-blue light between 380 and 480 nm.

Several other interesting comparisons in respect of temperature and anaerobic conditions during exposure can be made using the data from the systematized review (see Supporting Information). Decreased or elevated temperatures during violet-blue light had a varied effect on bacterial inactivation. In the case of 405 nm light exposure in low temperatures ($\leq 10°C$), there was little change in inactivation of *Bacillus cereus*, *Lactobacillus planetarium* and *P. aeruginosa* (33,58); however, there was enhanced inactivation of *E. coli* and *L. monocytogenes* (43). Additionally, inactivation of *S. aureus* was reduced, with approximately 50% less inactivation than that achieved at room temperature (2.1 v 4 $\log_{10}$ reduction) (33,58). When temperature was increased ($\geq 37°C$) during violet-blue light exposure, there was enhanced inactivation of *E. coli, L. monocytogenes and P. aeruginosa* (16,43). This was particularly striking in *L. monocytogenes* with half the dose required (42 v 84 J cm$^{-2}$) for a 5 $\log_{10}$ reduction following 405 nm light exposure at an irradiance of 70 mW cm$^{-2}$ (43). McKenzie *et al.* (43) hypothesized that the enhanced inactivation seen during exposure in these stressed conditions may be a result of structural or metabolic stresses (due to the temperature) which, when combined with 405 nm light, increased microbial susceptibility to ROS and subsequent oxidative damage.

As it is known that oxygen plays an essential role in the photoinactivation of microorganisms using violet-blue light (6,7), it was interesting to compare inactivation of microorganisms exposed in anaerobic environments, when suspended in phosphate-buffered saline or brain–heart infusion broth. Under these conditions, there was little to no inactivation of bacteria, including *S. aureus, E. coli* and *E. faecalis* and significantly reduced inactivation (1–5 $\log_{10}$ less) of fungi and yeasts, including *Aspergillus niger*, *Candida albicans* and *Serratia cerevisiae* (19,40). However, up to 4 $\log_{10}$ inactivation of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens* and *Propionibacterium acnes* could be achieved in anaerobic conditions following violet-blue light exposure (40,62,66). These results further demonstrate that environmental pathogens such as *S. aureus* require oxygen for violet-blue light inactivation (7); however, they also indicate that anaerobic oral bacteria such as *P. gingivalis* may not require oxygen for inactivation during violet-blue light exposure. Hope *et al.* (40) hypothesized that inactivation of these oral bacteria may be due to type I reactions occurring within bacterial cells, producing ion radicals which could cause damage to cellular structures without requiring oxygen as an intermediate. This demonstrates that, in certain oxygen depleted scenarios, violet-blue can still be used for microbial inactivation.

Violet-blue light inactivation of bacteria is thought to occur through excitation of intracellular porphyrin molecules, resulting in ROS production, thus promoting oxidative cellular damage and cell death. This inactivation mechanism has benefits over other antimicrobial treatments such as antibiotics and ultraviolet light, in that the generated ROS cause nonspecific damage (they do not have specific cellular target molecules), making organisms less likely to develop genetic mutations and acquire resistance. Additionally, the nonspecific oxidative damage exerted on exposed microorganisms enables effective inactivation of a wide range of microbial species. However, very few of the papers reviewed attempted to investigate the exact mechanism of

inactivation of violet-blue light. Those which did investigated the production of ROS and its role in inactivation through the use of ROS scavengers (6,83), as well as the presence of photosensitive endogenous porphyrins within microorganism via fluorescence spectrophotometry or high-performance liquid chromatography (10,19,23,33,70).

Additional studies have investigated bacterial damage, with results supporting different hypotheses about the inactivation mechanism. On the one hand, Enwemeka *et al.* (76) hypothesized that damage may occur in the double bond between pyrimidine bases of DNA, causing new bonds to form between incorrect base pairs. As the dose delivered would cause the rate of damage to exceed the rate of repair, cells are likely to die after exposure to violet-blue light and not photorepair (76). A recent study by Kim and Yuk (71) supports this hypothesis, with TEM revealing disorganization of chromosomes and ribosomes following violet-blue light exposure as well as DNA oxidation. The authors hypothesized that violet-blue light inactivation was due to DNA damage and loss of efflux pump activity rather than membrane peroxidation, as no noticeable changes to the cell envelope were witnessed using microscopy (71).

However, a greater amount of evidence has been produced which indicates that inactivation is due to membrane damage. Kim *et al.* (34,51) exposed *B. cereus*, *E. coli*, *L. monocytogenes*, *Salmonella sonnei*, *Salmonella typhimurium* and *S. aureus* to 405 nm light and found no DNA damage. There was no evidence of DNA fragmentation or changes in the DNA ladder profile after performing a comet assay and DNA ladder analysis, respectively (34,51). Additionally, TEM has been used to demonstrate structural damage following 415 nm light exposure of *P. aeruginosa* and *S. aureus*. Membrane degradation, large vacuole formation, release of cytoplasmic material and complete cell disruption were witnessed in *P. aeruginosa*, while there was disruption of cytoplasmic contents, breakage of bacterial cell walls and cell debris seen in *S. aureus* samples (69,77). TEM of *C. albicans* also revealed decomposition of inner organelles, deformed cell walls and unusual vacuole growth following a dose of 35.1 J cm$^{-2}$, and complete loss of cytoplasmic contents due to disrupted cell walls after a dose of 70.2 J cm$^{-2}$ 415 nm light (23). As there are, as yet, no conclusive answers, and it is likely that damage will be a result of a combination of factors due to the nonspecific oxidative damage that the generated ROS can induce within the exposed cells, there is a definite requirement for further work to fully establish the inactivation mechanism of action.

For many of the bacterial species (see Supporting Information), only one strain has been studied; therefore, it would be interesting for future research to investigate a wider range of strains within a bacterial species. Additionally, as antimicrobial violet-blue light is being developed for a range of clinical applications, it is important to expand the number of clinical isolates investigated as there can be large variations in dose requirements. This was demonstrated by Halstead *et al.* (28) who showed variations in the inactivation of isolates from an English hospital exposed to 400 nm light. Variation in dose requirements was particularly notable in clinical isolates of *Stenotrophomonas maltophilia*, with between 2.97 and 7.33 log$_{10}$ reduction achieved following a dose of 108 J cm$^{-2}$ (28). Very few of the organisms tested in the 79 studies reviewed have also been multidrug-resistant (MDR) strains; however, dose requirements do seem to be similar between antibiotic sensitive and antibiotic-resistant organisms. For example, an average dose of 7.14 and 7.85 J cm$^{-2}$ was required for 1 log$_{10}$ reduction of drug-sensitive and MDR *P. aeruginosa* (68). As these pathogens are an increasing problem in the hospital environment with very few treatment options (89), it is very important to continue to establish their susceptibility to violet-blue light. Successful demonstration of the reduction of MDR organisms would certainly support the use of 405 nm light for environmental decontamination in hospitals.

It was also apparent when comparing the data collected during the systematized review that there is a lack of evidence regarding the antimicrobial effect of violet-blue light on fungi, yeasts, protozoa and viruses. Only 10 of the studies reviewed investigated fungi and yeasts with the majority focussing on Gram-positive and Gram-negative bacteria. Although work has been carried out on *C. albicans*, *A. niger* and *Fusobacterium* spp, further work is required to investigate inactivation of additional hospital-acquired pathogens such as *Candida* spp. (*C. glabrata*, *C. parapsilosis*, *C. tropicalis*) *Aspergillus* spp. (*A. fumigates*, *A. flavus*), *Mucorales*, *Fusarium* spp. (*F. moniliforme*, *F. solani* and *F. oxysporum*) and *Scedosporium* spp. (*S. apiospermum* and *S. prolificans*) (90).

Evidence on the potential for violet-blue light inactivation of other microorganisms such as protozoa and viruses is also limited. In all the studies reviewed, only one investigated the inactivation of protozoa (namely *Acanthamoeba polyphaga*, ATCC 30461), with inactivation measured in fluorescence (60). After doses of 300 J cm$^{-2}$ of 460 nm light, there was a 42% reduction in fluorescence of a $10^6$ CFU mL$^{-1}$ population compared with the unexposed control (60). Future work could investigate other protozoa which are harmful to human health such as *Blastocystis hominis*, *Cryptosporidium parvum*, *Entamoeba histolytica* and *Giardia lamblia* (91). Additionally, results by Tomb *et al.* (25) are currently the only published evidence of the virucidal efficacy of 405 nm light against mammalian viruses, demonstrating that very high doses (~700 J cm$^{-2}$) are required for 1 log$_{10}$ inactivation when in minimal media (Dulbecco's phosphate-buffered saline). Similarly, inactivation of a *Streptomyces* bacteriophage, $\varphi$C31, required high doses of 405 nm light when suspended in minimal media (1020 J cm$^{-2}$ calculated for a 1 log$_{10}$ reduction) (24). It is likely that inactivation requires such high doses due to the lack of endogenous porphyrins within viral particles, resulting in inactivation being due to the low-level UVA output (~390 nm) emitted from the LEDs, which could cause protein oxidation (92,93). However, inactivation by 405 nm exposure can be significantly enhanced when viruses are exposed while suspended in organically rich biologically relevant media, with 88–89% less dose required for 1 log$_{10}$ reduction of both FCV and $\varphi$C31 (24,25). Therefore, additional studies are required to support these findings and investigate the most effective wavelength between 380 and 480 nm which has efficacy against nosocomial viruses found in bodily fluids in the environment, such as adenovirus, influenza virus, norovirus and rotavirus (94–97).

It was clear from the systematized review that there is little known about the potential for microorganisms to become tolerant to violet-blue inactivation. Only three of the studies reviewed investigated the potential for tolerance development in bacteria, with differing results (30,70,73). Additionally, a study by Zhang *et al.* (23) investigated the potential for tolerance in *C. albicans* and demonstrated decreased, but not statistically significant, susceptibility to violet-blue light after 10 repeated exposures. It is therefore particularly important to continue research on the

potential for tolerance development, to ensure that evidence is generated from several research groups, using different experimental arrangements, allowing accumulation of data so users can form unbiased opinions. Investigating tolerance will also help to ensure that violet-blue light is effectively used within the clinical environment, and is utilized in a way that in unlikely to result in resistance and provide reassurance to end point users.

As the systematized review only focussed on laboratory inactivation studies, future reviews could compare the use of violet-blue light for: wound decontamination (30,70), dental hygiene (98,99), acne treatments (100,101), prevention of food spoilage and disinfection of food (102–104) and environmental decontamination purposes (105–108). It is also likely that research papers will become more transparent in the future as research councils require data to be deposited in accessible online databases, such as the UK Data Archive. It may therefore be possible to retrieve the majority of the raw data from studies and apply mathematical models to provide more accurate estimates of microbial inactivation using violet-blue light, which would in turn improve the value of any systematic reviews published.

Accessible data sets may also include detailed information on the experimental arrangement, which would allow a more in-depth analysis on the effect of the set-up between studies. This could include comparisons between variables such as the light sources, volume of samples, irradiances and temperatures, which are all likely to have an impact on the inactivation kinetics. For example, data on the inactivation of an organism, with known endogenous photosensitizers, could be compared between studies using a narrowband light source, to that of a monochromatic source, emitting light at the wavelength associated with peak absorption by the organism's porphyrins. This would indicate whether efficacy is improved using a monochromatic light emitting a single wavelength, or whether inactivation is similar when a small range of wavelengths are emitted from the LEDs. Therefore, analysis of experimental arrangements may highlight important aspects for future studies, such as the importance of LED selection, and tailoring the wavelength of light to the organisms exposed.

Additionally, to further support the clinical application of violet-blue light, it would be important to be able to standardize microbial inactivation studies between differing research groups. One way of doing so would be to distribute a standardized test panel of organisms, suspended on a 96-well plate, to research groups working with visible light decontamination technologies. The results could then be collected into a database and analyzed to ensure that all groups involved are achieving similar levels of inactivation. Therefore, outcomes from future studies could be considered more robust and could be directly compared between groups ensuring greater transparency and higher impact. Collated results could then be used when producing standardized procedures for violet-blue light exposure experiments and for clinical treatment applications.

## CONCLUSION

This review on the efficacy of antimicrobial 380–480 nm light is the first to encompass the inactivation of a broad range of microorganisms including Gram-positive and Gram-negative bacteria, fungi, yeasts, bacteriophage and viruses. Data gathered indicated that Gram-negative and Gram-positive vegetative bacteria are the most susceptible organisms, while bacterial endospores, viruses and bacteriophage present the highest dose requirements for inactivation. This systematized review has

additionally allowed the antimicrobial efficacy of violet-blue light to be compared when using different irradiances of light, exposing different starting populations and also between the different wavelengths of light used, as well as highlighting areas of violet-blue light research which need further work. Analysis indicated that population density and irradiance of violet-blue light used are unlikely to have an effect on the average dose requirements for Gram-positive and Gram-negative bacteria, but this requires further investigation, with higher populations and peak absorption maxima potentially influencing dose requirements of other organisms not included in the analysis. Interestingly though, results demonstrated that higher doses are required for inactivation when longer wavelengths of violet-blue light are used, particularly in the case of *E. coli* and *L. monocytogenes*. This finding supports the use of light in the lower region of 405 nm light for inactivation of microorganisms. However, the review demonstrated the lack of published data on inactivation of MDR isolates, fungi, viruses and protozoa, as well as highlighting a requirement for standardized method for efficacy testing and the need for further evidence on the mechanism of inactivation and potential for bacterial tolerance.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.1.** Details inactivation of Gram positive and Gram negative bacteria, Gram positive endospores and mycobacteria.

**Table S1.2.** Details inactivation of yeasts and fungal conidia, germinating/germinated conidia and hyphae.

**Table S1.3.** Details inactivation of bacteriophage and viruses.

This data, underpinning the review, is also openly available from the University of Strathclyde KnowledgeBase, https://doi.org/10.15129/8d4383bd-c3b0-4ec3-b4bb-4b2885546b8d

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## AUTHOR BIOGRAPHIES

**Rachael M. Tomb** obtained a BSc(Hons) in Biomedical Science from the University of Strathclyde in 2013. Following on from this, she was awarded competitive funding for a Doctoral Fellowship from the Scottish Infection Research Network and Chief Scientist Office. Her PhD project focused on the antiviral efficacy of 405 nm light and potential of bacterial tolerance development. Rachael graduated with a PhD in 2017, and continues to work in this exciting area of photobiology within The Robertson Trust Laboratory for Electronic Sterilisation Technologies, at the University of Strathclyde.

**Tracy A. White** received a BSc(Hons) in Marine Science from the Scottish Association for Marine Science in 2006. Following graduation she worked within the Environmental Research Insitute, before moving to Aquapharm Biodiscovery Ltd to develop anti-infectives. Tracy was then invited to undertake a PhD on Pharmaceutical Microbiology at Queens University, Belfast in 2009. Following PhD completion, Tracy worked as a Development Scientist for Quotient Biodiagnostics before joining the University of Strathclyde in 2015 as a Research Associate in the The Robertson Trust Laboratory for Electronic Sterilisation Technologies.

**John E. Coia** trained in Medical Microbiology, having obtained degrees in Medicine and Molecular Biology from the University of Glasgow. He is a Fellow of the Royal College of Pathologists and the Royal College of Physicians (Edinburgh). He is a Consultant Microbiologist with NHS Greater Glasgow & Clyde and Honorary Professor in the School of Medicine, University of Glasgow, and Visiting Professor in The Robertson Trust Laboratory for Electronic Sterilisation Technologies (University of Strathclyde). He is Director of the Scottish *Salmonella, Shigella & C. difficile* and Scottish MRSA Reference Services, and Medical Director of the Scottish Microbiology Reference Laboratories, Glasgow.

**John G. Anderson** was born in Glasgow, UK, in 1942. He received a BSc degree in Applied Microbiology and a PhD degree in fungal physiology from the University of Strathclyde, Glasgow, in 1968 and 1971, respectively. He was with the Department of Bioscience and Biotechnology, University of Strathclyde, from 1971, where he became a Professor of Microbiology, and the Head of Department. He is currently an Emeritus Professor with the University of Strathclyde. His current research interests include various aspects of food, biomedical and environmental microbiology with particular focus on the development of optical and electrical antimicrobial technologies.

**Scott J. MacGregor** received his BSc and PhD degrees from the University of Strathclyde, Glasgow, U.K., in 1982 and 1986, respectively. He is currently with the University of Strathclyde, where he became a Pulsed-Power Research Fellow in 1986, a Lecturer in pulsed-power technology in 1989, a Senior Lecturer in 1994, and a Reader and a Professor of high-voltage engineering in 1999 and 2001, respectively. He has been the Vice Principal of the University of Strathclyde since 2014. His current research interests include the development of electronic and optical methods for antimicrobial decontamination and sterilization applications.

**Michelle Maclean** is a Senior Lecturer at the University of Strathclyde. Born on the Isle of Lewis, Scotland, in 1980, she received her BSc(Hons) degree in Microbiology and Immunology in 2002, and her PhD in 2006 (University of Strathclyde, UK). Based at The Robertson Trust Laboratory for Electronic Sterilisation Technologies at Strathclyde, her research is concerned with microbial infection control, with particular focus on the fundamental antimicrobial effects of violet-blue light and its development for infection control applications. Since her PhD, she has held positions as post-doctoral Research Fellow (2006-2013), and Strathclyde Chancellors Fellow (2014-2017) at the University of Strathclyde.