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Minireview

MINIREVIEW – Food Microbiology

Photoinactivation of bacteria by endogenous photosensitizers and exposure to visible light of different wavelengths – a review on existing data

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One sentence summary: Visible violet and blue light is capable of disinfecting all bacteria investigated so far.
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ABSTRACT

Visible light has strong disinfectant properties, a fact that is not well known in comparison to the antibacterial properties of UV light. This review compiles the published data on bacterial inactivation caused by visible light and endogenous photosensitizers. It evaluates more than 50 published studies containing information on about 40 different bacterial species irradiated within the spectral range from 380 to 780 nm. In the available data a high variability of photoinactivation sensitivity is observed, which may be caused by undefined illumination conditions. Under aerobic conditions almost all bacteria except spores should be reduced by at least three log-levels with a dose of about 500 J cm^{-2} of 405 nm irradiation, including both Gram-positive as well as Gram-negative microorganisms. Irradiation of 470 nm is also appropriate for photoinactivating all bacteria species investigated so far but compared to 405 nm illumination it is less effective by a factor between 2 and 5. The spectral dependence of the observed photoinactivation sensitivities gives reason to the assumption that a so far unknown photosensitizer may be involved at 470 nm photoinactivation.

Keywords: photoinactivation; endogenous photosensitizer; visible light; bactericidal

INTRODUCTION

The first studies on the disinfecting properties of visible light were conducted in the late 19th century by researchers such as Arthur Downes and Thomas Porter Blunt as well as Theodor Geisler (Downes and Blunt 1877, 1878; Geisler 1890). Among the pioneers of the field, Percy F. Frankland and H. Marshall Ward reported the disinfecting properties of light on *Bacillus anthracis* (Frankland and Ward 1894; Ward 1894). Most of the very early studies were conducted with the use of sunlight and it was not always possible to precisely delineate the influence of UV radiation on the disinfecting aspects these pioneers noted. However, employing various filters and prisms, Ward could show that the wavelength played a very important role in the bactericidal response – as depicted in Fig. 1 – and that the bactericidal effect was strongest for the shortest wavelength (UV spectrum). Moreover these studies led to the discovery that inhibition of bacterial growth ceased at the border between green and blue light. While these studies are also the first to demonstrate the antibacterial properties of visible light, in the historical perspective these early pioneers are typically regarded as the discoverers of the disinfecting effect of ultraviolet light (UV light), which is generally known and accepted.

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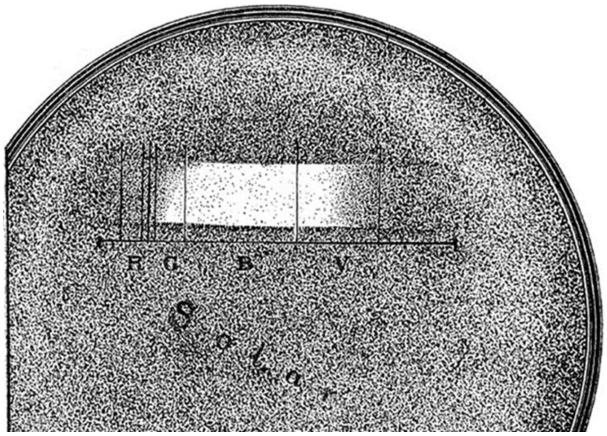

Figure 1. Photograph of an agar plate with *B. anthracis* colonies taken from (Ward 1894). A prism dispersed solar radiation into red (R), green (G), blue (B) and violet (V) light. Bacterial inhibition was observed for the violet, blue and green part of the visible spectrum (Ward 1894).

The UV part of the electromagnetic spectrum is subdivided by its wavelength into three sections: UV-C, 100–280 nm; UV-B, 280–315 nm; and UV-A, 315–380 nm. It is followed by the visible spectral region from 380 to 780 nm. UV-C light is known to be the most effective radiation for the reduction of bacteria, fungi and protozoa, which is caused by the destruction of DNA and RNA (Chevrefils and Caron 2006). However, damage to nucleotides extends to human DNA and represents a considerable disadvantage of using UV light for disinfection.

In contrast to all kinds of UV radiation, visible light is much less harmful (Kleinpenning *et al.* 2010; McDonald *et al.* 2013; Ramakrishnan *et al.* 2014, 2016). It can be applied without higher risks in case of accidental or even intentional illumination of human tissue. An important aspect of the antibacterial effect of visible light is the presence of bacterial photosensitizers. It has gradually been recognized that many bacteria harbor a sufficiently high concentration of endogenous photosensitizers for them to be destroyed from within by intensive irradiation with visible light, especially by violet and blue light of wavelengths 405 and 470 nm, respectively (Ashkenazi *et al.* 2003; Guffey and Wilborn 2006; Maclean *et al.* 2008b). The porphyrins coproporphyrin III, protoporphyrin IX and uroporphyrin III have been identified as the primary endogenous photosensitizers responsible for bacterial photoinactivation (Ashkenazi *et al.* 2003; Feuerstein *et al.* 2005; Maclean *et al.* 2008a,b). Absorption of visible light by these photosensitizers generates reactive oxygen species (ROS) that damage nearby cell structures leading to cell death.

So far this photoinactivation effect has been investigated for about 40 different bacterial species and was even extended to some fungi and viruses. The intention of this review is to compile the existing data on bacterial inactivation for different wavelengths, and to discuss potential reasons for the variations of disinfection success reported in the published data. The observed wavelength dependence of the photoinactivation was also compared to the spectral properties of the assumed involved photosensitizers. Moreover we want to address potential improvements for future experimental setups and to investigate if it is possible to recommend irradiation doses for visible light, similar to the recommendation of 40 mJ cm^{-2} for UV-C irradiation that can be found in international standards (DIN EN 14897 2007).

DISINFECTION WITH VISIBLE LIGHT IN THE MEDICAL AND FOOD SECTOR

The fact that visible light is quite innocuous for human cells represents an enormous advantage with regard to medical applications. The first successful *in vivo* results were published for the treatment of acne vulgaris (Papageorgiou, Katsambas and Chu 2000; Kawada *et al.* 2002; Elman, Slatkine and Harth 2003) and wound infections (Lipovsky *et al.* 2010; Dai *et al.* 2012, 2013; McDonald *et al.* 2013; Zhang *et al.* 2014). Even the successful photoinactivation of *Helicobacter pylori* in the stomachs of human patients has been reported (Ganz *et al.* 2005; Lembo *et al.* 2009) and further medical applications are emerging. Potential future fields of operation are the disinfection of air and surfaces (Murdoch *et al.* 2012; Maclean *et al.* 2014) as well as the disinfection of contact lenses (Hoenes *et al.* 2016; Hoenes, Vogelaar and Hessling 2016).

Another large field of potential applications for photoinactivation by visible light is the conservation and disinfection of food (Luksiene and Brovko 2013; D'Souza *et al.* 2015). So far, reported photoinactivation experiments on real food and obtained without UV radiation are still rare. Among the few published results are the successful disinfection of *Listeria monocytogenes* and *Campylobacter* spp. on hot dogs and chicken surfaces (Haughton *et al.* 2012; Motts *et al.* 2016), respectively, as well as the photoinactivation of *Escherichia coli* in milk (Srimagal, Ramesh and Sahu 2016).

COMPARATIVE EVALUATION OF BACTERIAL DISINFECTION STUDIES

To assess the effects of visible light of different wavelengths on Gram-positive and Gram-negative bacteria, the PubMed database was first searched for different combinations of the key words photoinactivation, endogenous photosensitizer, disinfection, inactivation, bactericidal, visible light, violet light and blue light. In a second step the references in the resulting literature were scanned, as were all papers that cited any discovered articles in PubMed or Google Scholar. Among the identified studies only *in vitro* data were further evaluated in which bacteria in liquid suspensions were irradiated with visible light of one wavelength within the range of 380–780 nm and a specified irradiation dose. Excluded were studies using combinations of different wavelengths or wide spectral ranges, or radiation with UV or IR (infra-red), as were reports on combinations of visible light with additional measures such as extreme temperatures (not within 15–40°C), acidity (not within pH 6–8) or the combination with chemical disinfectants, additional photosensitizers, or nutrient supplements influencing the bacterial porphyrin production.

The data for each strain were included separately in the evaluation and the same was done for each bacterial starting concentration. Most authors have published a series of photoinactivation experiments. In this case usually the value for the highest evaluable irradiation dose was listed. In the case of zero survivors a lower dose was chosen to enable the evaluation that was based on the simplified assumption of a negative exponential dependence between the concentration of surviving bacteria and irradiation dose, usually represented as a straight line in a half-logarithmic diagram. Any observed tailing or shouldering of this dependence was neglected and only the average value for a bacterial concentration reduction of one log-level (change by a factor of 10) was calculated. This method was chosen to ensure that the many different published results with irradiation dose variations of up to three orders of magnitude became

comparable. If figures of disinfection results were published not mentioning exact values in the text, values were extracted from the (magnified) figures. The error associated with this technique is negligible compared to the large variations in the published results.

A compilation of all these results can be found in Table 1, including about 250 data sets for about 40 different bacterial species. Successful photoinactivation data of Gram-positive and Gram-negative bacteria and the necessary irradiation doses for a log reduction in dependence of the wavelength is depicted in Fig. 2.

DATA VARIABILITY AND POSSIBLE EXPERIMENTAL REASONS

A major finding in analyzing the available publications was an unexpected large variability in the calculated irradiation dose necessary for a one log reduction for a specific bacterial species at a certain wavelength (Table 1). The observed differences are up to one order of magnitude. Strain differences among the same species do certainly play a role with regard to this high data variability, but variation is even observed if only a single strain is evaluated such as *Staphylococcus aureus* NCTC 4135. For this strain the irradiation doses for a one log reduction varied between 7.2 and 60.8 J cm^{-2} at 405 nm and the variation even increased when comparing different *S. aureus* isolates. In this case the data were generated by various working groups and setups. However, if only the results of a single working group and a single setup are taken into account minor variability was reported even for different strains, as for example by Halstead *et al.* for *A. baumannii* and *S. aureus* (Halstead *et al.* 2016), a finding which suggests that experimental measurement setups and procedures may be unintended origins of these variations.

INFLUENCE OF ILLUMINATION DURATION, DOSE OR BACTERIAL CONCENTRATION

Comparing the experimental setups of the studies in Table 1, the largest differences are illumination duration (2–975 min), illumination dose (2.1–3000 J cm^{-2}) and bacterial concentration (150–10^9 CFU ml^{-1}). For discussing whether these differences are responsible for the high variability of the log reduction dose, its dependence on these three variables is investigated. Most data exist for 405 nm irradiation of *E. coli* and *S. aureus*. Figure 3a–c depicts exemplarily the dependence of the necessary radiation dose for one log reduction, in relation to illumination duration, actually applied illumination dose and bacterial starting concentration, respectively.

In Fig. 3a we evaluated the dependence of the irradiation dose for a one log-level reduction on the illumination duration for *E. coli* and *S. aureus*. A linear regression delivers an increase of the necessary dose for *E. coli* and a decrease for *S. aureus* with the illumination duration, but the square regression coefficients R^2 of 0.15 and 0.16 for *E. coli* and *S. aureus* imply that the influence of illumination duration is almost negligible and cannot explain the observed variability.

A similar comparison is depicted in Fig. 3b for the dependence of the calculated necessary radiation dose for a one log-level decrease of the bacterial concentration on the actually applied radiation dose. For both bacterial species the linear regression results in an increase of the necessary log reduction dose on the applied dose, but the square regression coefficients R^2 are even smaller than 0.16. Therefore no significant dependence between both parameters could be observed.

Maclean *et al.* (2009) and Bumah *et al.* (2013) investigated the influence of bacterial starting concentrations on the efficacy of 405 nm *S. aureus* inactivation. They observed a reduced disinfection efficacy and a significant decrease of the irradiation intensity in samples with higher bacterial concentrations (included in Fig. 3c). The complete data collection shows an increase of the log reduction dose with rising bacterial concentrations, but once again the square regression coefficients are very low, indicating that the data variability cannot merely be explained by differing bacterial concentrations.

If only the data set of Bumah *et al.* (2013) on photoinactivation of *S. aureus* by 405 nm radiation is investigated, the impression arises that the results with differing irradiation intensities cannot easily be explained by absorption effects. Bumah *et al.* performed measurements with 3×10^6 CFU ml^{-1} and 7×10^6 CFU ml^{-1} and observed an increase by a factor of 3 for the necessary irradiation dose for a one log reduction when increasing the bacterial concentration from 3×10^6 to 7×10^6 CFU ml^{-1}. The illumination intensity was 25 mW cm^{-2} in front of the bacterial suspension and 7 mW cm^{-2} behind it for the 7×10^6 CFU ml^{-1} concentration. By the assumption of a pure absorption effect Lambert–Beer Law would result in an average intensity that is 30% lower in the sample with 7×10^6 CFU ml^{-1} compared to the sample with 3×10^6 CFU ml^{-1}. This difference may even be lower, because bacteria were suspended in a non-absorbing saline solution. The observed attenuation of the illumination may alternatively be explained by scattering. Scattering will lead to multiple reflections of the radiation within the sample, resulting in an average illumination intensity that is possibly even higher in the sample with 7×10^6 CFU ml^{-1}. Why the data of Bumah *et al.* demonstrate an increase by a factor of 3 for the irradiation dose cannot, therefore, be sufficiently explained.

INFLUENCE OF OXYGEN CONCENTRATION

Another factor important for the disinfecting properties of visible light is oxygen. Maclean *et al.* (2008a) reported that the photoinactivation of *S. aureus* is highly dependent on the oxygen concentration in samples with bacterial concentrations of more than 10^5 CFU ml^{-1}. At higher bacterial concentrations oxygen may be consumed faster than it is resupplied by diffusion through the sample surface. This would lead to a lower bacterial photoinactivation sensitivity. It may also explain the outlier in Fig. 3c where at a bacterial concentration of 10^9 CFU ml^{-1} a relatively low log-level reduction dose was observed. In that specific setup a magnetic stirrer was employed, which results in an improved oxygen distribution in liquid samples.

Unfortunately direct measurements of oxygen concentrations have not been reported in any published paper, though oxygen may have a large influence. Riedel *et al.* observed oxygen consumption rates between 3.3×10^{-10} μmol O_2 CFU^{-1} day^{-1} and 2.4×10^{-7} μmol O_2 CFU^{-1} day^{-1} for *E. coli* under nutrient-limited conditions (Riedel *et al.* 2013). By these values a bacterial population of 10^8 CFU ml^{-1}, as employed by some authors in Table 1, would need between 17 min and 9 days to consume 0.284 μmol O_2 ml^{-1} (dissolved oxygen per milliliter at 20°C), if the resupply by diffusion is neglected. In most photoinactivation papers bacterial solutions were diluted in PBS or NaCl solutions which results in nutrient-limited conditions and therefore probably very low oxygen consumption rates. Nevertheless this oxygen consumption may still influence the dissolved oxygen

Table 1. Photoinactivation results reported for different bacteria and wavelengths within the visible spectrum.

Bacterium (Gram-positive/-negative)	Wavelength [nm]	Median dose/log-level [J cm^{-2}]	{Dose/log-level [J cm^{-2}]/strain/applied dose [J cm^{-2}]/illumination duration [min]/start concentration [CFU ml^{-1}]} (Reference)
Acinetobacter baumannii (–)	400	16.7	{15.3/ACI 616/108/30/1E6}, {18.4/ACI 618/108/30/1E6}, {16.0/ACI 642/108/30/1E6}, {17.6/ACI 648/108/30/1E6}, {16.5/ACI 659/108/30/1E6}, {17.6/ACI 665/108/30/1E6}, {17.0/ACI 671/108/30/1E6}, {17.4/ACI 672/108/30/1E6}, {16.9/ACI 698/108/30/1E6}, {16.1/ACI AYE/108/30/1E6}, {16.0/ACI C60/108/30/1E6}, {15.9/ACI 19606/108/30/1E6} (Halstead *et al.* 2016)
	405	14.0	{25.7/NCTC 12156/108/180/1.0E5} (Maclean *et al.* 2009), {2.3/LMG 1041/4.5/15/1.0E3}^{2} (Ramakrishnan *et al.* 2014)
	415	17.6	{17.6/US Army clinical isolate/70.2/60/1.0E8} (Zhang *et al.* 2014)
Aggregatibacter actinomycetem. (–)	460	30.0	{30.0/ATCC 43718/150/2/3.0E7} (Cieplik *et al.* 2014)
Bacillus atrophaeus (+)	470	110.2	{67.4/?/454/3.0E4}, {153.1/?/300/63/3.0E4} (De Lucca *et al.* 2012)
Bacillus cereus (+)	405	93.5	{28.4/NCTC 11143/108/45/5.0E4}^{2} (Maclean *et al.* 2013); {158.6/ATCC 14579/540/1.0E6}^{3} (Kumar *et al.* 2015)
	520	–	{–/ATCC 14579/540/1.0E6}^{1,2} (Kumar *et al.* 2015)
Bacillus cereus spores (+)	405	542.9	{605.3/NCTC 11143/1150/480/5.0E4}^{2}, {480.6/NCTC 11143/1730/720/5.0E4}^{2} (Maclean *et al.* 2013)
Bacillus megaterium spores (+)	405	638.9	{638.9/?/1150/480/5.0E4} (Maclean *et al.* 2013)
Bacillus subtilis (+)	408	69.0	{69.0/DSM 402/300/150/1.0E6} (Hoenes *et al.* 2015)
	451	100.0	{100.0/DSM 402/300/150/1.0E6} (Hoenes *et al.* 2015)
Bacillus subtilis spores (+)	405	676.5	{676.5/?/1150/480/5.0E4} (Maclean *et al.* 2013)
Campylobacter coli (–)	395	0.3	{0.3/1140 DF/2.1/5/1.0E7}^{2}, {0.3/1662 DF/2.1/5/1.0E7}^{2}, {0.3/2124 GF/2.1/5/1.0E7}^{2} (Haughton *et al.* 2012)
Campylobacter jejuni (–)	395	0.3	{0.6/323 BC/4.2/10/1.0E7}^{2}, {0.6/1136 DF/4.2/10/1.0E7}^{2}, {0.3/1135 DF/2.1/5/1.0E7}^{2}, {0.3/1146/DF/2.1/5/1.0E7}^{2}, {0.3/1147 DF/2.1/5/1.0E7}^{2}, {0.3/1354 DF/2.1/5/1.0E7}^{2}, {0.3/NCTC 11168/2.1/5/1.0E7}^{2} (Haughton *et al.* 2012)
	405	3.4	{3.4/LMG 8841/18/30/1.0E5} (Murdoch *et al.* 2010)
Clostridium perfringens (+)	405	10.2	{10.2/ATCC 13124/45/75/3.0E4} (Maclean *et al.* 2009)
Chlostridium difficile (+)	405	13.0	{13.0/NCTC 11204/48/80/5.0E4} (Maclean *et al.* 2013)
Chlostridium difficile spores (+)	405	251.1	{425.9/NCTC 11204/1150/480/5.0E4} (Maclean *et al.* 2013), {76.4/NCTC 11204/252/19/4.0E3} (Moorhead *et al.* 2016)
Corynebacterium striatum (+)	405	120.6	{120.6/clinical isolate/63.9/15/1.0E3}^{2} (McDonald *et al.* 2013)
Elizabethkingia meningoseptica (–)	400	8.0	{8.0/EKIN 502/54/15/1.0E6} (Halstead *et al.* 2016)
Enterobacter cloacae complex (–)	400	92.3	{53.3/ENTCL 525/360/100/1.0E6}, {98.0/ENTCL 801/648/180/1.0E6},{92.3/ENTCL 804/576/160/1.0E6} (Halstead *et al.* 2016)
Enterococcus faecalis (+)	385	81.0	{81.0/ ATCC 19433/–/120/1.0E6}^{3} (Lui *et al.* 2016)
	405	96.0	{96.0/NCTC 00775/216/360/5.2E5} (Maclean *et al.* 2009), {43.8/clinical isolate/63.9/15/1.0E3}^{2} (McDonald *et al.* 2013), {130.0/ATCC 19433/–/165/1.0E6}^{3} (Lui *et al.* 2016)
	430	190.0	{190/ATCC 19433/–/360/1.0E6}^{3} (Lui *et al.* 2016)
	455	410.0	{410.0/ATCC 19433/–/360/1.0E6}^{3} (Lui *et al.* 2016)
	525	–	(–/ATCC 19433/–/1.0E6}^{1,3} (Lui *et al.* 2016)
	590	1 900 000.0	{1 900 000.0/ATCC 19433/–/1.0E6}^{3} (Lui *et al.* 2016)
	623	–	{–/ATCC 19433/–/1.0E6}^{1,3} (Lui *et al.* 2016)
	660	–	{–/ATCC 19433/–/1.0E6}^{1,3} (Lui *et al.* 2016)
	740	–	{–/ATCC 19433/–/1.0E6}^{1,3} (Lui *et al.* 2016)
Enterococcus faecium (+)	400	348.4	{348.4/EFM 513/648/180/1.0E6} (Halstead *et al.* 2016)
Escherichia coli (–)	385	61.0	{61.0/ATCC W3110/–/90/1.0E6}^{3} (Lui *et al.* 2016)
	395	25.2	{25.2/DSM 1607/36/19/1.0E8}^{2} (Birmpa *et al.* 2014)
	400	46.3	{22.9/EC 073/108/30/1.0E6}, {69.7/EC 042/108/30/1.0E6} (Halstead *et al.* 2016)
	405	75.6	{86.0/ATCC W3110/–/75/1.0E6}^{3} (Lui *et al.* 2016), {21.1/K12 (DH5α)/132.1/250/–} (Rhodes *et al.* 2016) {28.6/NCTC 9001/18/60/1.0E3}^{2} (Ramakrishnan *et al.* 2014), {58.1/NCTC 9001/180/300/1.0E5} (Maclean *et al.* 2009), {75.6/NCTC 9001/378/90/1.0E5} (McKenzie *et al.* 2014), {91.2/ NCTC 9001/702/180/1.0E7} (McKenzie *et al.* 2016), {122.7/NCTC 12900/554.7/420/1.0E5} (Endarko *et al.* 2012), {54.3/NCTC 12900/288/480/1.5E5} (Murdoch *et al.* 2010), {57.6/NCTC 12900/288/480/1.5E5} (Murdoch *et al.* 2012), {310.0/RDL 933/310/540/1.0E6}^{2} (Kumar *et al.* 2015), {127.8/clinical isolate/127.8/30/1.0E3} (McDonald *et al.* 2013)

Table 1 (*Continued*).

Bacterium (Gram-positive/-negative)	Wave-length [nm]	Median dose/log-level [J cm^{-2}]	{Dose/log-level [J cm^{-2}]/strain/applied dose [J cm^{-2}]/illumination duration [min]/start concentration [CFU ml^{-1}]} (Reference)
	408	156.7	{156.7/DSM 498/600/300/1.0E6} (Hoenes *et al.* 2015)
	415	60.0	{60.0/ Meir Medical Center isolate 1313/120/20}^{2} (Lipovsky *et al.* 2010)
	425	56.1	{56.1/ATCC 25992/86.4/480/2.0E8}^{2} (Kim *et al.* 2013)
	430	100.0	{100.0/ATCC W3110/–/300/1.0E6}^{3} (Lui *et al.* 2016)
	450	137.6	{137.6/ATCC 25922/117/975/1.5E2} (Keshishyan *et al.* 2015)
	451	500.0	{500.0/DSM 498/600/300/1.0E6} (Hoenes *et al.* 2015)
	455	219.3	{138.6/Meir Med. Center isolate 1313/120/20/?}^{2} (Lipovsky *et al.* 2010), {300.0/ATCC W3110/–/300/1E6}^{3} (Lui *et al.* 2016)
	460	–	{–/ATCC 25992/150/2/3.0E7}^{1} (Cieplik *et al.* 2014)
	461	309.5	{121.8/EDL 933/596.7/450/1.0E6}^{2} (Ghate *et al.* 2013), {497.3/EDL 933/596.7/450/1.0E7}^{2} (Ghate *et al.* 2015b)
	520	38750.0	{38750.0/EDL 933/3100/540/1.0E6}^{2} (Kumar *et al.* 2015)
	521	2859.7	{5400.0/EDL 933/432/450/1.0E7}^{2} (Ghate *et al.* 2015b), {319.4/EDL 933/431.2/450/1.0E6}^{2} (Guffey *et al.* 2013)
	525	785.5	{785.5/ATCC 25992/172.8/480/2.0E8}^{2} (Kim *et al.* 2013)
		–	{–/ATCC W3110/–/–/1.0E6}^{1,3} (Lui *et al.* 2016)
	590	3 100 000.0	{3 100 000.0/ATCC W3110/–/–/1.0E6}^{3} (Lui *et al.* 2016)
	623	32 000 000.0	{32 000 000.0/ATCC W3110/–/–/1.0E6}^{3} (Lui *et al.* 2016)
	625	–	{–/ATCC 25992/172.8/480/2.0E8}^{1} (Kim *et al.* 2013)
	642	–	{–/EDL 933688/450/1.0E6}^{1} (Guffey *et al.* 2013)
	660	1 700 300.0	{3 400 000.0/ATCC W3110/–/–/1.0E6}^{3} (Lui *et al.* 2016), {600.0/ATCC 25992/24/–/1.5E3} (de Sousa *et al.* 2016)
	740	13 000 000.0	{13 000 000.0/ATCC W3110/–/–/1.0E6}^{3} (Lui *et al.* 2016)
Fusobacterium nucleatum (–)	450	55.3	{55.3/ATCC 25586/94/3/5.0E6}^{2} (Feuerstein *et al.* 2005)
ss. Nucleatum	455	17.5	{17.5/ATCC 25586/4.8/1/1.0E8} (Fontana *et al.* 2015)
ss. vincenti	455	9.8	{9.8/ATCC 49256/4.8/1/1.0E8} (Fontana *et al.* 2015)
ss. polymorphum	455	4.0	{4.0/ATCC 10953/4.8/1/1.0E8} (Fontana *et al.* 2015)
	465	361.5	{361.5/ATCC 1594/94/30/5.0E6}^{2} (Feuerstein, Persman and Weiss 2004)
Fusobacterium periodonticum (–)	455	10.3	{10.3/ATCC 33692/4.8/1/1.0E8} (Fontana *et al.* 2015)
Helicobacter pylori (–)	405	5.3	{6.4/ATCC 43504/32/5/?}^{2} (Ganz *et al.* 2005), {4.3/?/20/3/?} (Hamblin *et al.* 2005)
Klebsiella pneumoniae (–)	400	74.8	{73.3/MDR A/504/140/1.0E6}, {76.2/MDR B/504/140/1.0E6} (Halstead *et al.* 2016)
	405	46.2	{46.2/NCTC 9633/180/300/1.0E5} (Maclean *et al.* 2009), {29.7/NCTC 9633/27/90/1.0E3}^{2} (Ramakrishnan *et al.* 2014), {101.4/Clinical Isolate/85.2/20/1.0E3}^{2} (McDonald *et al.* 2013)
Lactobacillus plantarum (+)	405	374.0	{374.0/ATCC 8014/–/420/1.0E6}^{2} (Kumar *et al.* 2016)
	460	1121.0	{1121.0/ATCC 8014/–/420/1.0E6}^{2} (Kumar *et al.* 2016)
	520	–	{–/ATCC 8014/–/420/1.0E6}^{1,2} (Kumar *et al.* 2016)
Leuconostoc mesenteroides (+)	470	257.1	{257.1/?/180/38/?} (De Lucca *et al.* 2012)
Listeria innocua (+)	395	2.3	{2.3/NCTC 11288/2.832/78/1.0E8} (Birmpa *et al.* 2014)
Listeria ivanovii (+)	405	44.9	{44.9/NCTC 11846/184.9/36/1.0E5} (Endarko *et al.* 2012)
Listeria monocytogenes (+)	400	117.4	{117.4/NCTC 11994/123.3/274/1.0E5} (Endarko *et al.* 2012)
	405	45.9	{42.0/NCTC 11994/184.9/360/1.0E5}, {85.0/NCTC 11994/123.3/274/1.0E5}, {49.7/NCTC 11994/184.9/36/1.0E5} (Endarko *et al.* 2012), {21.6/NCTC 11994/108/180/1.5E5} (Murdoch *et al.* 2012), {16.8/LMG 19944/84/20/1.0E5} (McKenzie *et al.* 2014), {175.5/ATCC BAA 679/540/1.0E6}^{3} (Kumar *et al.* 2015)
	410	100.2	{100.2/NCTC 11994/123.3/274/1.0E5} (Endarko *et al.* 2012)
	415	241.8	{241.8/NCTC 11994/123.3/274/1.0E5} (Endarko *et al.* 2012)
	420	493.2	{493.2/NCTC 11994/123.3/274/1.0E5} (Endarko *et al.* 2012)
	430	648.9	{648.9/NCTC 11994/123.3/274/1.0E5} (Endarko *et al.* 2012)
	440	1120.9	{1120.9/NCTC 11994/123.3/274/1.0E5} (Endarko *et al.* 2012)
	450	3082.5	{3082.5/NCTC 11994/123.3/274/1.0E5} (Endarko *et al.* 2012)
	461	263.1	{411.5/ATCC BAA 679/596.7/450/1E7} (Ghate *et al.* 2015b), {114.8/ATCC BAA 679/596.7/450/1E6}^{2} (Ghate *et al.* 2013)
	520	4843.8	{4843.8/ATCC BAA 679/3100/540/1.0E6}^{2} (Kumar *et al.* 2015)
	521	987.0	{1542.9/ATCC BAA 679/432/450/1.0E7} (Ghate *et al.* 2015b), {431.2/ATCC BAA 679/431.2/450/1E6}^{2} (Ghate *et al.* 2013)
	642	–	{–ATCC BAA 679/688/450/1.0E6}^{1,2} (Ghate *et al.* 2013)

Table 1 (*Continued*).

Bacterium (Gram-positive/-negative)	Wave-length [nm]	Median dose/log-level [J cm^{-2}]	{Dose/log-level [J cm^{-2}]/strain/applied dose [J cm^{-2}]/illumination duration [min]/start concentration [CFU ml^{-1}]} (Reference)
Listeria seeligeri (+)	405	55.9	{55.9/NCTC 11856/184.9/36/1.0E5} (Endarko *et al.* 2012)
Micrococcus sp. (+)	405	24.9	{24.9/clinical isolate/42.8/10/1.0E3}[2] (McDonald *et al.* 2013)
Mycobacterium smegmatis (+)	405	67.8	{67.8/?/120/200/1.5E5} (Guffey *et al.* 2013)
Mycobacterium terrae (+)	405	57.6	{57.6/LMG 10394/288/480/1.5E5} (Murdoch *et al.* 2012)
Porphyromonas gingivalis (–)	405	40.5	{2.8/ATCC 33277/3.42/5/2.0E8}[4], {78.2/ATCC 33277/98.55/5/2.0E8}[4] (Hope *et al.* 2013)
	425	523.6	{523.6/KCTC 5352/172.8/480/2.0E8}[2] (Kim *et al.* 2013)
	450	47.0	{47.0/ATCC 33277/94/3/5.0E6}[2] (Feuerstein *et al.* 2005)
		–	{–/ATCC 33277/94/3/5.0E6}[2,4] (Feuerstein *et al.* 2005)
	455	48.8	{48.0/ATCC 33277/4.8/1/1.0E8} (Fontana *et al.* 2015)
	465	94.0	{94.0/ATCC 33277/94/30/5.0E6}[2] (Feuerstein, Persman and Weiss 2004)
	525	1270.6	{1270.6/KCTC 5352/172.8/480/2.0E8}[2] (Kim *et al.* 2013)
	625	5958.6	{5958.6/KCTC 5352/172.8/480/2.0E8}[2] (Kim *et al.* 2013)
Prevotella intermedia (–)	455	14.5	{14.5/ATCC 25611/4.8/1/1.0E8} (Fontana *et al.* 2015)
Prevotella melaninogenica (–)	455	7.2	{7.2/ATCC 25845/4.8/1/1.0E8} (Fontana *et al.* 2015)
Prevotella nigrescens (–)	455	9.8	{9.8/ATCC 33563/4.8/1/1.0E8} (Fontana *et al.* 2015)
Propionibacterium acnes (+)	405	13.3	{13.3/different/40/–/–}[2] (Hamblin *et al.* 2005)
		–	{–/ATCC 11827/15/–/1.5E5}[1,4] (Guffey and Wilborn 2006)
	414	45.0	{45.0/ATCC 6919/225/180/1.4E8}[4] (Ashkenazi *et al.* 2003)
	470	–	{–/ATCC 11827/15/–/1.5E5}[1,4] (Guffey and Wilborn 2006)
Proteus vulgaris (–)	405	30.6	{30.6/CN 329/144/240/1.0E5} (Maclean *et al.* 2009)
Pseudomonas aeruginosa (–)	400	17.8	{16.7/PSE 568/108/30/1.0E6}, {19.3/PSE PA01/108/30/1.0E6}, {16.5/PSE 6749/108/30/1.0E6},{18.0/PSE 1054/108/30/1.0E6}, {17.8/PSE 1586/108/30/1.0E6} (Halstead *et al.* 2016)
	405	15.3	{15.3/ATCC 27853/15/30/7.5E4} (Guffey and Wilborn 2006), {42.9/NCTC 9009/180/300/1.0E5} (Maclean *et al.* 2009), {1409.1/ATCC 10145/310/540/1.0E6}[2] (Kumar *et al.* 2015), {15.3/LMG 9009/9/30/1.0E3}[2] (Ramakrishnan *et al.* 2014), {10.5/Clinical isolate/18/4/1.0E3} (McDonald *et al.* 2013)
	415	14.0	{14.4/ATCC 19660/109.9/96/1.0E8} (Dai *et al.* 2013), {13.6/ATCC 19660/48/40/1.0E8} (Amin *et al.* 2016)
	450	142.7	{142.7/ATCC 27853/117/975/1.5E2} (Keshishyan *et al.* 2015)
	470	69.1	{69.1/ATCC 27853/15/–/7.5E4} (Guffey and Wilborn 2006), {25.4/?/10/454/3.0E4} (De Lucca *et al.* 2012),{72.5/?/50/63/3.0E4} (De Lucca *et al.* 2012)
	520	–	{–/ATCC 10145/3100/540/1.0E6}[1,3] (Kumar *et al.* 2015)
	660	64.9	{64.9/ATCC 27853/24/1.5E3} (D'Souza *et al.* 2015)
Serratia marcescens (–)	405	169.6	{169.6/Clinical isolate/191.7/45/1.0E3}[2] (McDonald *et al.* 2013)
Salmonella enterica (–)	405	97.3	{543.8/NCTC 4444/739.6/144/1.0E5} (Endarko *et al.* 2012), {97.3/NCTC 4444/288/480/1.0E5} (Murdoch *et al.* 2010), {82.3/NCTC 4444/288/480/1.5E5} (Murdoch *et al.* 2012)
('Heidelberg')	470	138.4	{138.4/ATCC 8326/110/–/1.0E6} (Bumah, Masson-Meyers and Enwemeka 2015b)
Salmonella Typhimurium (–)	405	516.7	{516.7/ATCC 14028/310/540/1.0E6}[2] (Kumar *et al.* 2015)
	461	917.3	{129.7/ATCC 14028/596.7/450/1.0E6}[2] (Ghate *et al.* 2013), {1704.9/ATCC 14028/596.7/450/1.0E7} (Ghate *et al.* 2015a)
	470	94.8	{94.8/ATCC 14028/110/–/1.0E6} (Bumah, Masson-Meyers and Enwemeka 2015b)
	520	8611.1	{8611.1/ATCC 14028/3100/540/1.0E6}[2] (Kumar *et al.* 2015)
	521	1476.8	{2700.0/ATCC 14028/432/450/1.0E7} (Ghate *et al.* 2015a), {253.6/ATCC 14028/431.2/450/1.0E6}[2] (Ghate *et al.* 2013)
	642	–	{–/ATCC 14028/688/450/1.0E6}[1,2] (Ghate *et al.* 2013)
Shigella sonnei (–)	405	89.5	{142.2/LMG 10473/554.7/11/1.0E5} (Endarko *et al.* 2012), {36.7/NCTC 12984/180/300/1.5E5} (Murdoch *et al.* 2012)
Staphylococcus aureus (+)	400	16.5	{17.5/MRSA 508/108/30/1.0E6}, {7.9/MRSA 520/54/15/1.0E6}, {16.8/MRSA 531/108/30/1.0E6},{40.7/MSSA 10788/288/80/1.0E6}, {16.0/MSSA F77/108/30/1.0E6}, {16.0/MSSA 29213/108/30/1.0E6},{16.1/MSSA 10442/108/30/1E6}, {41.1/MSSA 33807/288/80/1E6}, {47.4/MSSA 4163/288/80/1E6} (Halstead *et al.* 2016), {15.7/NCTC 4135/23.5/120/2.0E5} (Maclean *et al.* 2008b)

Table 1 (*Continued*).

Bacterium (Gram-positive/-negative)	Wave-length [nm]	Median dose/log-level [J cm^{-2}]	{Dose/log-level [J cm^{-2}]/strain/applied dose [J cm^{-2}]/illumination duration [min]/start concentration [CFU ml^{-1}]} (Reference)
	405	35.9	{16.4/ATCC 25923/15/1.5E5} (Guffey and Wilborn 2006), {60.6/MRSA US-300/60/10/5E6}[2],{58.3/MRSA IS-853/60/10/5.0E6}[2] (Enwemeka *et al.* 2008), (7.2/ NCTC 4135/36/60/1.0E5},{12.0/NCTC 4135/36/60/1.0E3}, {10.3/NCTC 4135/31/52/1.0E5}, {12.0/NCTC 4135/36/60/1.0E7},{13.7/NCTC 4135/41/70/1.0E9}, {9.0/MRSA (clinical isolate 16a, GRI)/45/75/1.0E5} (Maclean *et al.* 2009),{13.6/NCTC 4135/72/30/5.0E4}[2] (Maclean *et al.* 2013), {967.7/ATCC 6538/600/420/1.0E6}[2] (Kumar *et al.* 2016), {118.1/ATCC 35932/–/540/1.0E6}[2] (Kumar *et al.* 2015), {60.8/NCTC 4135/468/120/1.0E7} (McKenzie *et al.* 2016), {9.8/NCTC 4135/23.5/120/2.0E5} (Maclean *et al.* 2008b), (38.7/ATCC BAA-1680/60/10/3.0E6)[2],{35.9/ATCC BAA-1680/55/9/5.0E6}[2], {101.0/ATCC BAA-1680/60/10/7.0E6}[2] (Bumah *et al.* 2015a),{41.8/ATCC BAA-1680/60/10/3.0E6}, {42.9/ATCC BAA-1680/60/10/5.0E6}, {101.7/ATCC BAA-1680/60/10/7.0E6} (Bumah *et al.* 2013), {10.5/NCTC 4135/18/4/1.0E3} (McDonald *et al.* 2013), {27.3/NCTC 4135/9/30/1.0E3}[2] (Ramakrishnan *et al.* 2014), {100.8/ATCC BAA-1680/121/15/5.0E6} (Masson-Meyers *et al.* 2015)
	410	21.4	{21.4/NCTC 4135/23.5/120/2.0E5} (Maclean *et al.* 2008b)
	415	84.1	{47.0/NCTC 4135/23.5/120/2.0E5} (Maclean *et al.* 2008b), {121.2/ATCC 25923/120/20}[2] (Lipovsky *et al.* 2010)
	420	78.3	{78.3/NCTC 4135/23.5/120/2.0E5} (Maclean *et al.* 2008b)
	425	929.0	{929.0/KCTC 1916/172.8/480/2.0E8}[2] (Kim *et al.* 2013)
	430	235.0	{235.0/NCTC 4135/23.5/120/2.0E5} (Maclean *et al.* 2008b)
	450	390.0	{390.0/ATCC 25923/117/975/1.5E2} (Keshishyan *et al.* 2015)
	455	360.4	{360.4/ATCC 25923/120/20/?}[2] (Lipovsky *et al.* 2010)
	460	–	{–/ATCC 6538/1800/420/1.0E6}[1,3] (Kumar *et al.* 2016)
	461	150.7	{150.7/ATCC BAA 679/596.7/450/1.0E6}[2] (Ghate *et al.* 2013)
	470	58.3	{58.3/MRSA US-300/60/33/5.0E6}, {63.8/MRSA IS-853/60/33/5.0E6} (Enwemeka *et al.* 2009),{35.7/ATCC 25923/15/–/1.5E5} (Guffey and Wilborn 2006), {50.0/ATCC BAA-1680/55/–/5.0E6},{107.8/ATCC BAA-1680/55/–/7.0E6} (Bumah, Masson-Meyers and Enwemeka 2015b), {40.9/ATCC BAA-1680/45/25/3.0E6}[2], {50.0/ATCC BAA-1680/60/33/5.0E6}[2], {122.2/ATCC BAA-1680/60/33/7.0E6}[2] (Bumah *et al.* 2015a),{39.2/ATCC BAA-1680/60–/3.0E6}[2] (Bumah *et al.* 2015a), {58.4/ATCC BAA-1680/60/–/5.0E6}[2],{82.2/ATCC BAA-1680/60/–/7.0E6}[2], {96.5/ATCC BAA-1680/60/–/8.0E6}[2], {83.3/ATCC BAA-1680/60/–/1.2E7}[2](Bumah, Masson-Meyers and Enwemeka 2015b), {52.2/ATCC BAA-1680/60/33/5.0E6}, {27.5/ATCC BAA-1680/60/33/3.0E6}, {51.9/ATCC BAA-1680/60/33/5.0E6}, {123.5/ATCC BAA-1680/60/33/7.0E6} (Bumah *et al.* 2013)
	520	1478.6	{127.0/ATCC 6538/596.7/450/1.0E6} (Ghate *et al.* 2013), {2830.2/ATCC 35932/3000/540/1.0E6}[2] (Kumar *et al.* 2015)
		–	{–/ATCC 6538/1800/420/1.0E6}[1,3] (Kumar *et al.* 2016)
	521	287.5	{287.5/ATCC BAA 679/431.2/450/1.0E6} (Ghate *et al.* 2013)
	525	424.6	{424.6/KCTC 1916/172.8/480/2.0E8} (Kim *et al.* 2013)
	625	–	{–/KCTC 1916/172.8/480/2.0E8}[1,3] (Kim *et al.* 2013)
	642	–	{–/ATCC BAA 679/688/450/1.0E6} (Ghate *et al.* 2013)
	660	34.8	{34.8/ATCC 25923/24/1.5E3} (de Sousa *et al.* 2016)
***Staphylococcus epidermis* (+)**	405	18.9	{9.1/NCTC 11964/42/70/1.0E5} (Maclean *et al.* 2009), {46.3/LMG 10474/324/180/1.0E3} (Ramakrishnan *et al.* 2016), {14.8/LMG 10474/9/30/1.0E3}[2] (Ramakrishnan *et al.* 2014), {23.1/NCTC 11964/18/4/1.0E3} (McDonald *et al.* 2013)
***Stenotrophomonas maltophilia* (–)**	400	15.0	{15.0/STEMA 529/108/30/1E6}, {36.4/STEMA 551/108/30/1.0E6}, {14.7/STEMA 558/108/30/1E6} (Halstead *et al.* 2016)
***Streptococcus pyogenes* (+)**	405	10.8	{10.8/NCTC 8198/54/90/1.0E5} (Maclean *et al.* 2009)
***Vibrio parahaemolyticus* (–)**	405	170.0	{170.0/ATCC 17802/420/1.0E6}[2] (Kumar *et al.* 2016)
	460	717.0	{717.0/ATCC 17802/420/1.0E6}[2] (Kumar *et al.* 2016)
	520	–	{–/ATCC 17802/420/1.0E6}[1,3] (Kumar *et al.* 2016)

[1]No photoinactivation observed. [2]Data from figure. [3]Log reduction value from table. [4]Anaerobic condition.

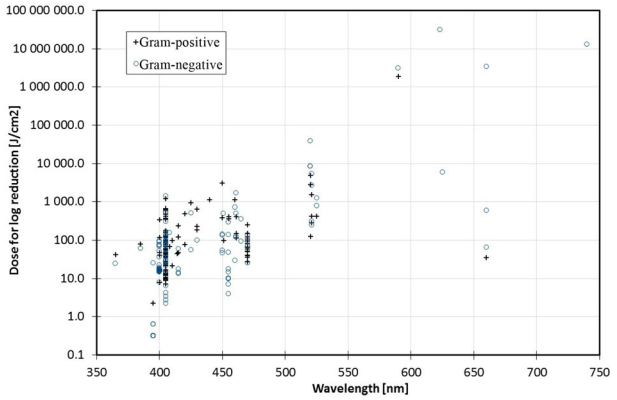

Figure 2. Wavelength-dependent necessary irradiation doses from Table 1 for one log-level reduction of Gram-positive and Gram-negative bacteria.

concentration and therefore interfere with the photoinactivation efficacy, especially if residual metabolic activity of bacteria is present, which is probably true in the cases in which bacteria were diluted in nutrient broth.

IMPORTANCE OF ILLUMINATION SETUP

Another potential origin of variations is the illumination homogeneity. Some authors have tried to guarantee a homogeneous sample illumination, e.g. with an LED array that offered a radiation source area as large as the sample area or even larger. In several setups, however, just a single LED was located very close to the sample. Such a point-like light source results in an inhomogeneous illumination as was illustrated in Murdoch *et al.* (2012) with intensity variations of one order of magnitude over the illuminated area. With this setup the disinfection success in the outer regions of the sample is strongly reduced, an effect which cannot be compensated by a higher irradiation in the middle of the sample. These setups would result in exaggerated necessary irradiation doses for a one log-level reduction.

There is a further potential error source in the illumination setups. In many studies transparent sample vessels, e.g. transparent Petri dishes were used. If these vessels are placed on a reflective metallic or white/gray base, the light is reflected after its first sample transit and it may pass the sample a second time and thereby increases the illumination intensity by almost a factor of two compared to the assumed or previously measured intensity. Thus the calculated necessary irradiation doses would be too low for a one log-level reduction. A similar problem arises when the samples are irradiated directly on an agar plate, where the agar is not black.

SUCCESSFUL PHOTOINACTIVATION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA WITH 405 AND 470 nm IRRADIATION

Despite the high data variability the published studies revealed some interesting conclusions. Under aerobic conditions all bacteria – including spores – investigated so far could be photoinactivated by exposure to 405 nm radiation (Table 1 and Fig. 2). With a dose of about 500 J cm^{-2} of 405 nm radiation almost all bacterial species besides spores should be reduced by three orders of magnitude or more. A similar statement is true for 470 nm light, but in this case higher illumination doses are necessary. Even for some longer wavelengths successful photoinactivation was reported, especially around 525 and 650 nm, but in general the necessary inactivation dose seems to rise exponentially with the wavelength.

Some authors reported Gram-negative bacteria to be more resistant to photoinactivation than Gram-positive (Maclean *et al.* 2009, 2013; Dai *et al.* 2012; Murdoch *et al.* 2012, 2013; Luksiene and Brovko 2013; McDonald *et al.* 2013; Birmpa *et al.* 2014; Aponiene and Luksiene 2015; D'Souza *et al.* 2015) but the data compilation in Fig. 2 shows no clear evidence for this assumption. In fact some of the most sensitive bacteria listed in Table 1 such as *Acinetobacter* spp., *Campylobacter* spp. and *Pseudomonas* spp. are Gram-negative.

INVOLVEMENT OF AN UNKNOWN PHOTOSENSITIZER?

For investigating the wavelength dependence of the observed photoinactivation data and its consistency with the assumed responsible photosensitizers (coproporphyrin III, protoporphyrin IX and uroporphyrin III), a photoinactivation data subset was compiled.

The most complete data for different wavelengths exist on *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*. Evaluating these data the wavelength range was divided in 20 nm intervals, beginning with 380 nm (380–399 nm, 400–419 nm, 420–439 nm, 440–459 nm, 460–479 nm, ...). For each of these bacteria and each interval the median of the existing data from Table 1 was inverted, resulting in the log reduction achieved with one joule per square centimeter. These values can be found in Table 2, together with the photoinactivation sensitivity ratio between the 410 nm interval and the 470 nm interval. The log reductions per J cm^{-2} are depicted in Fig. 4 together with the most important porphyrin absorption spectra.

The observed spectral dependence of the necessary log reduction doses for the selected bacteria in Fig. 4 in the range 400–450 nm is in excellent agreement with the assumed dominant role of porphyrins as ROS-generating photosensitizers: the photoinactivation sensitivity is high around 405 nm and declines towards 450 nm. This coincides with the porphyrin absorption spectra in Fig. 4. In contrast it does not explain the disinfection with 470 nm irradiation, because the observed photoinactivation sensitivity ratios between 405 nm and 470 nm are in a range of 2–5 (Table 2), which is much lower than the absorption ratios of the prominent porphyrins that differ by about two orders of magnitude between 405 and 470 nm (Fig. 4). So the photoinactivation sensitivity at 470 nm cannot be explained just by the previously mentioned porphyrins but may involve a further unknown endogenous photosensitizer. FAD or other flavins are potential candidates that are known for their photoinactivation properties and they show significant absorbance around 470 nm, but they usually have a peak absorption around 440 or 450 nm, which is in contrast to the observed minimum of photoinactivation sensitivity at 450 nm in Fig. 4.

CONCLUSION

Comparing the studies on photoinactivation by visible light published so far some general conclusions can be drawn. The data evaluation showed that considering aerobic conditions every bacterial species investigated so far can generally be photoinactivated by 405 nm and 470 nm irradiation. The necessary irradiation dose is significantly higher for 470 nm illumination.

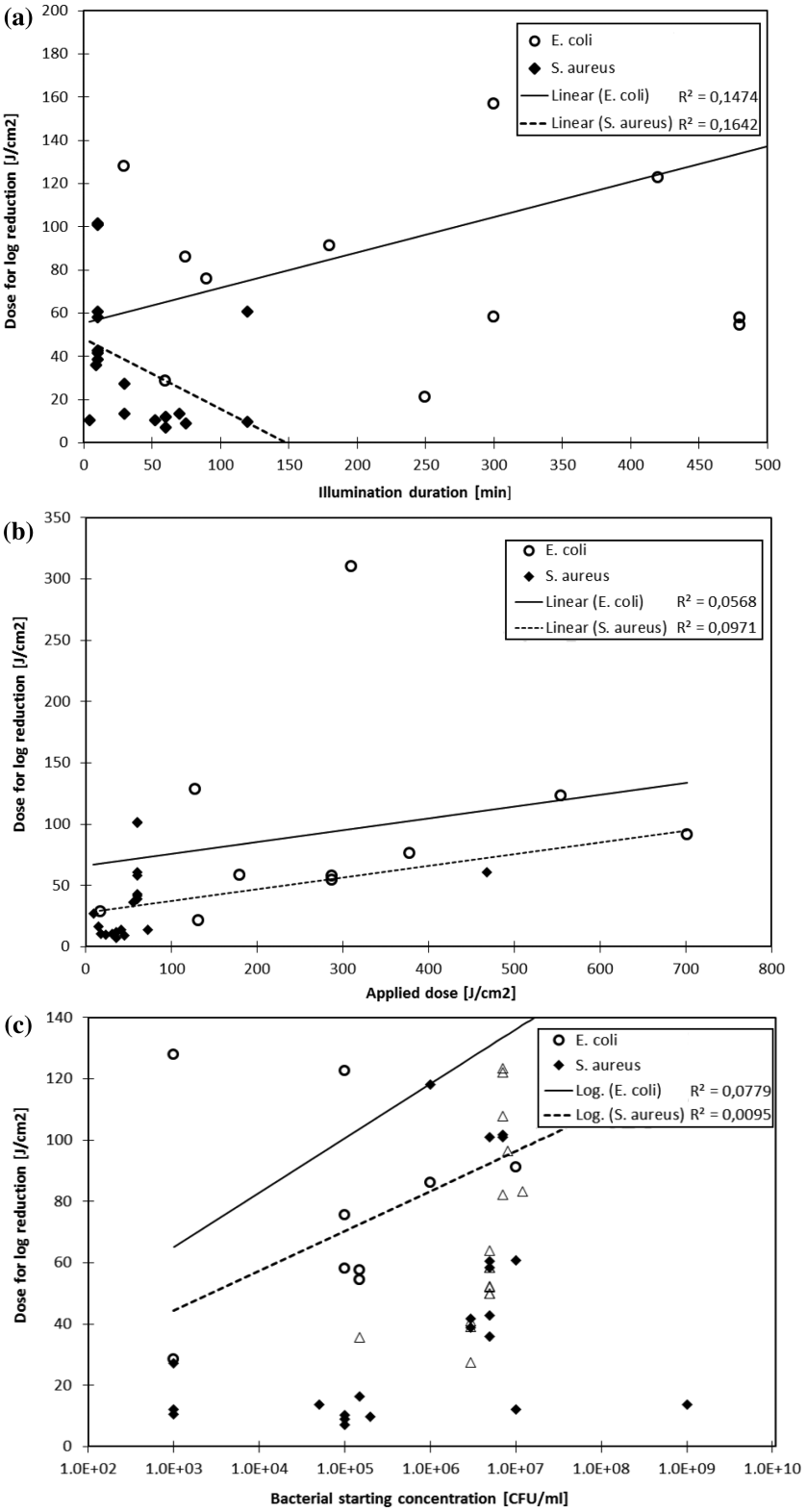

Figure 3. **(a)** Calculated necessary 405 nm illumination doses for a one log-level bacterial concentration reduction for *E. coli* and *S. aureus* for different illumination durations. **(b)** Calculated necessary 405 nm illumination doses for a one log-level bacteria concentration reduction for *E. coli* and *S. aureus* for different applied irradiation doses. **(c)** Calculated necessary 405 nm illumination doses for a one log-level bacteria concentration reduction for *E. coli* and *S. aureus* for different bacterial starting concentrations. (Two extreme outliers, determined under particular undefined illumination conditions, were omitted.)

Table 2. Log reductions achieved with 1 J cm^{-2} for *E. coli*, *S. aureus*, *L. monocytogenes* and *P. aeruginosa* for different wavelengths together with the sensitivity ratio between 410 and 470 nm.

Wavelength [nm]	*E. coli* log reduction per joule [cm^2 J^{-1}]	*L. monocytogenes* log reduction per joule [cm^2 J^{-1}]	*P. aeruginosa* log reduction per joule [cm^2 J^{-1}]	*S. aureus* log reduction per joule [cm^2 J^{-1}]
390	2.3E−02	–	–	–
410	1.4E−02	1.2E−02	6.0E−02	4.1E−02
430	1.3E−02	1.8E−03	–	8.3E−03
450	4.6E−03	4.8E−04	7.0E−03	2.0E−03
470	3.2E−03	3.8E−03	1.4E−02	1.7E−02
530	3.2E−04	6.5E−04	–	2.8E−03
630	3.1E−08	–	–	–
670	5.9E−07	–	1.5E−02	2.9E−02
750	7.7E−08	–	–	–
Ratio @405 nm/@470 nm	4.4	3.1	4.2	2.4

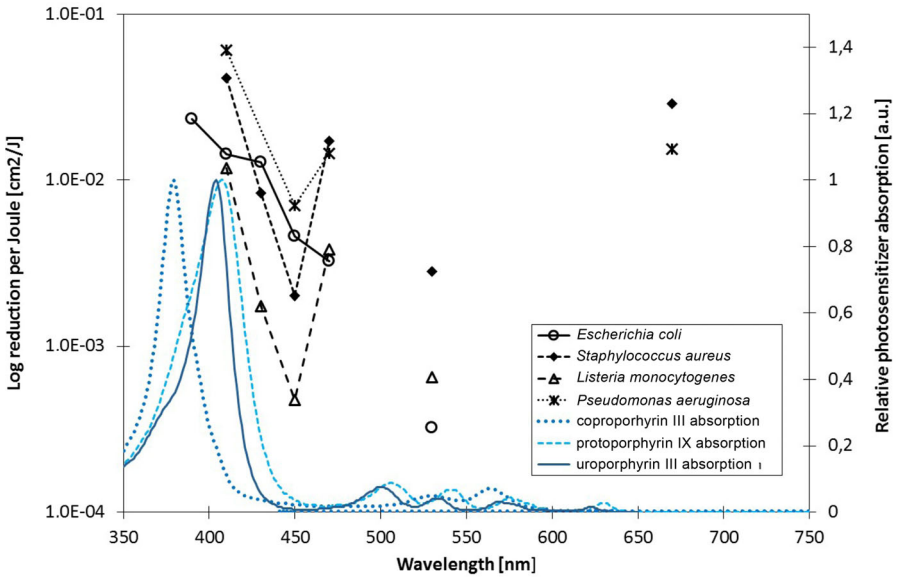

Figure 4. Spectral data of log reduction per joule for *E. coli*, *S. aureus*, *L. monocytogenes* and *P. aeruginosa* and relative absorption spectra of coproporphyrin III, protoporphyrin IX and uroporphyrin III.

Successful photoinactivation has also been reported for longer wavelengths but few data exist and some negative results have then been observed. Differences between different strains of a bacterial species, e.g. strains that are resistant or sensitive to antibiotics, have not been recognized so far, nor are there general differences between Gram-positive and Gram-negative bacteria.

Nevertheless a high data variability was observed in the different publications. The variations in calculated necessary doses for a one log-level reduction most probably originate in experimental setups. The illumination duration for achieving a certain dose does not seem to be an important factor in the analyzed studies, but the illumination homogeneity of some setups is unclear as is the question of whether reflections were always considered. The oxygen concentration within bacterial suspensions is largely unknown but may represent an important influence on photoinactivation results. We therefore recommend a homogeneous illumination without unintended reflections in future setups. Bacterial concentrations should preferably be kept low ($\leq 10^6$ CFU ml^{-1}) and as a precaution the dissolved oxygen concentration should be measured at the beginning and the end of the experiments.

Overall photoinactivation with visible light by endogenous photosensitizers has a high potential for many future applications and should be further investigated.

Conflict of interest

K. Hoenes and M. Hessling filed a German patent application on contact lens disinfection by photoinactivation in 2015. B. Spellerberg declares no conflict of interest.

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