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The potential of violet, blue, green and red light for the inactivation of *P. fluorescens* as planktonic cells, individual cells on a surface and biofilms

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Highlights:

- Violet light (400 nm) slightly inactivates planktonic *P. fluorescens* cells
- Violet light causes reduction of individual cells on a surface at optimal conditions
- Blue light (470 nm) inactivates individual salt-stressed cells on a surface
- Green (522 nm) and red light (644 nm) do not affect any contamination type
- Hydrated biofilms are resistant to visible light inactivation

Abstract: Microbial contaminations are among the major health concerns worldwide. New innovative non-thermal technologies, such as photodynamic inactivation (PDI), are emerging as mild antimicrobial treatments. This research focussed on the effects of four different colours of visible light-emitting diodes (violet, blue, green and red, at 400, 470, 522 and 644 nm) on three different types of *P. fluorescens*

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contaminations: planktonic cells, individual cells on a surface and biofilms. The results demonstrated that only violet light inactivates planktonic *P. fluorescens* in a suspension (0.5-log reduction). Individual cells on a surface could be inactivated (i) effectively by violet light (3.7-log reduction in 30 min) when the cells were at optimal conditions and (ii) below the detection limit by both violet and blue light when salt stress was added to the environment. Finally, for the hydrated biofilms on polystyrene surfaces, no inactivation was achieved with any of the light sources. This study suggests that PDI is a promising technology for decontamination and preservation applications in the food industry where continuous lighting can be easily applied. It is considered more suitable as a technology to prevent microbial proliferation by intensive implementation than as a method to disinfect developed microbial populations.

Keywords: Photodynamic inactivation; *Pseudomonas fluorescens*; light-emitting diodes; planktonic cells; individual cells on a surface; biofilms.

1. Introduction

Microbial contaminations within the food chain, the healthcare sector and the domestic environment are among the major health concerns worldwide (Suetens et al., 2018; WHO, 2015). These contaminations occur when undesired microbes, such as different types of bacteria, yeasts, fungi or even viruses, end up in or on products, materials or organisms. Specifically, bacteria naturally present in the environment pose a serious risk for public health. Although hygiene protocols are compulsory to be followed within the food industry, they fail too often during the production and processing stages (e.g., cutting or packaging). Due to several reasons that include inappropriate handling of the food, use of unsafe water or difficulties in proper cleaning of equipment, these bacteria can reach the consumers in undesirable quantities (Chatterjee and Abraham, 2018). Also, contaminations in hospitals, specifically with multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus*, lead to a crucial public health problem (Enwemeka et al., 2008; Rutala et al., 2018). Equipment, tools and surfaces that have not been properly sterilised are often the cause of cross contamination and infections (Rutala et al., 2018). Finally, the population Journal homepage: <https://www.sciencedirect.com/journal/food-and-bioproducts-processing>

83 worldwide is also constantly exposed to the risk of contamination in the domestic environment. Kitchen
84 surfaces, utensils and sponges, which are reused often to clean several types of surfaces, are typically
85 responsible for bacterial cross contamination and the consequential infections (Biranjia-Hurdoyal and
86 Latouche, 2016).

87 Bacterial species generally live and grow either as planktonic cells that are dispersed in the liquid phase or as
88 sessile cells on surfaces (Ghate et al., 2019). In this latter case, they can be present on the surface as individual
89 cells or as biofilms. Biofilms are bacterial communities that are attached to a surface and embedded in a self-
90 produced matrix, namely extracellular polymeric substance (EPS). These characteristics cause biofilms to be
91 much more tolerant to antibacterial treatments and thus are considered difficult to inactivate (Costerton et al.,
92 1999; Flemming and Wingender, 2010). The surfaces where the individual cells or biofilms occur can be widely
93 different. They can be present on (i) solid flat surfaces such as plastic, stainless steel and other hard materials;
94 (ii) more complex structures (meshed or cross-linked) such as textiles, fibres, nitrile gloves, granular surfaces or
95 patterned microstructures; and finally on (iii) living or dead tissues such as skin, wounds, meat, fish, fruits or
96 vegetables, which represent more complex systems in terms of composition, structure and water content.

97 The type of bacterial contamination (i.e., planktonic cells, individual cells on a surface or biofilms) can
98 influence the way that the bacteria react to certain processing technologies that are aimed to inactivate them.
99 These contamination types have shown to cause great differences in the bacterial resistance against
100 antimicrobial treatments that are used in the food industry (Simões et al., 2010; Verran et al., 2002; Wang et al.,
101 2017; Wu et al., 2015). Some examples of technologies used to tackle the planktonic form are thermal
102 inactivation (e.g., pasteurisation) in the food industry for milk and fruit juices, and the use of filtration, UV
103 irradiation or oxidizing agents (chlorination) for water disinfection (Kim et al., 2002; Ranieri et al., 2009). For
104 individual cells on food products, freezing or drying (reducing water activity) procedures are used as hurdle
105 methods for food preservation. Additionally, the use of antibiotics and chemical disinfectants with washing
106 procedures is a common practice applied to wounds, skin burns and abrasion to avoid infections in a medical
107 context or for vegetables and fruits prior to cutting processes in the context of food safety (FDA, 2008). For
108 biofilms, i.e., the most resistant contamination type, harsher chemical disinfectants or mechanical removal
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109 techniques have been suggested for cleaning and decontamination procedures in industry and households.
110 The selection of a decontamination method is determined by the suitability for a specific application (e.g.,
111 compatibility with medical practises, with food products and manufacturing processes, with household usage
112 and consumer habits), the cost and the efficiency. The type of bacterial contamination is expected to be one of
113 the most crucial factors that influence the decontamination efficiency (Haughton et al., 2012; Murodoch et al.,
114 2012; Simões et al., 2010; Verran et al., 2002; Wang et al., 2017; Wu et al., 2015). In the last decades, innovative
115 non-thermal technologies have been suggested for widespread decontamination application: clinical,
116 industrial and commercial. Some technologies that have attracted a lot of attention are cold atmospheric
117 plasma (CAP) (Govaert et al., 2020), cold ice blasting (Stratford et al., 1994), pulsed electric field (PEF) (Bansal
118 et al., 2015), high-intensity light pulse (HILP) (Palgan et al., 2011), nanomaterial usage (Shaban et al., 2019),
119 photocatalysis and smart coatings (Yemmireddy and Hung, 2017), sonication and mild temperatures
120 (Guerrouj et al., 2016), enzymatic solutions (Lequette et al., 2010) and photodynamic inactivation (PDI)
121 (Angarano et al., 2020).

122 PDI is a technology based on the use of visible (VIS) radiation to inactivate different types of bacterial
123 contaminations. The first observation of the PDI phenomenon was done by Downes and Blunt in 1878 and the
124 further exploration of the photochemical phenomenon led to the development of ultra-violet (UV) light as a
125 decontamination technology. However, the potential dangers caused by UV light to humans has sparked a
126 new research trend, which focuses on the VIS region of the light spectrum that does not cause damage to eyes
127 or skin. The narrow emission spectra of LEDs, which can be tuned for the optimization of PDI performance,
128 and their increasingly cheap price are accelerating the research and development of this technology.

129 PDI technology is based on the absorption of light by endogenous or exogenous photosensitizers (PS). The
130 former are naturally present inside the bacterial cells, the latter are added to the cells' environment before the
131 light exposure (Ghate et al., 2019; Josefsen et al., 2008). The PSs lead to the formation of reactive oxygen species
132 that are harmful to cells and cause their death. The last 20 years witnessed an increasing number of studies
133 focusing on *Escherichia*, *Staphylococcus*, *Pseudomonas*, *Listeria*, *Salmonella* and other species that have highlighted
134 the potential of PDI with endogenous PS as an antibacterial technology for food application (Angarano et al.,
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2020; Fysun et al., 2019; Ghate et al., 2019; Haughton et al., 2012; Murdoch et al., 2012). The antibacterial PDI (aPDI), based on endogenous PS, is attracting considerable interest because it demonstrated to be detrimental for pathogenic and spoilage bacteria, so that it can be potentially used to improve food safety and quality. Notwithstanding the abundant research in PDI and endogenous PS, the focus of previous researchers has been mainly on the violet-blue range, which corresponds to the border of the VIS region towards UV. It is important to stress that there is evidence that longer wavelengths (> 450 nm) affect bacteria as well (Ghate et al., 2013; Ghate et al., 2015; Kim et al. 2013; Kumar et al., 2015).

The benefits of the aPDI for food applications rely on (i) the possibility to treat every type of interface of both abiotic and biotic surfaces and (ii) every shape of product, surface and particulate that can be reached by the light, (iii) in combination (synergism) with other treatment (e.g. temperature), (iv) while effectively penetrating transparent materials/media with a (v) non-contact, (vi) non-thermal and (vii) non-destructive treatment. A few of the examples that underline the versatility of its applicability are given. PDI has been applied to fresh products from fruits (Ghate et al., 2017; Kim et al., 2017a) to seafood and meat (e.g., poultry), which have been colonised by bacteria (Ghate et al., 2019; Guffey et al., 2016; Kim et al., 2017a; Roh et al., 2018). With the aim of reducing infections caused by cross contamination, it has been investigated to treat food contact surfaces (contact materials, working benches and areas where food is stored) and equipment that have a more complex structure and geometry (filling hoses, heat exchangers, filling units) (Fysun et al., 2018; Haughton et al., 2012; Subedi et al., 2020). The combination of PDI and incubation temperatures has been studied against different bacterial types (Ghate et al., 2017; Hyun and Lee, 2020; Kim et al., 2017b; Li et al., 2018). Moreover, by having the advantage of penetrating transparent surfaces, light has been proposed for treatments of foods that are packaged with transparent plastic (Hyun and Lee, 2020).

Since common processing technologies have several drawbacks, e.g., thermal inactivation reduces the nutritional value within food products and chemical processing increases the antimicrobial resistance (AMR) of bacterial species, PDI represents an optimal potential solution that suits also the consumers demand for fresh-like products without artificial additives (Devlieghere et al., 2004; Yasmin et al., 2017). PDI using endogenous PS, categorises itself among the novel environmentally friendly (without chemicals) and eco-Journal homepage: <https://www.sciencedirect.com/journal/food-and-bioproducts-processing>

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161 friendly (due to low energy consumption and high durability of LEDs) strategies. Moreover, thanks to their
162 small size, LEDs can be implemented in many existing systems (Srimagal et al., 2016; Subedi et al., 2020).
163 Studies on aPDI have been mainly performed on bacteria in the planktonic form, among which some have
164 highlighted that visible radiation can inactivate planktonic cells, causing considerable reductions (Amin et al.,
165 2016; Kim et al., 2013; Kim et al., 2015; Maclean et al., 2008), while a few witnessed that VIS light promotes
166 growth or has a negligible effect on bacterial suspensions depending on the irradiation range (Ghate et al.,
167 2013; Kumar et al., 2015). Recent works have focussed on the effect on biofilms (Angarano et al., 2020; Ferrer-
168 Espada et al., 2019; Wang et al., 2016), while some other studies have tried to investigate the effect on cells
169 inoculated on abiotic and biotic surfaces, e.g., food contact surfaces and food products (Enwemeka et al., 2008;
170 Haughton et al., 2012; McKenzie et al., 2013). Few studies made a comparison on the impact of the technology
171 applied to different types of bacterial contaminations in the context of food science (Haughton et al., 2012;
172 Hyun and Lee, 2020; Kim et al., 2017b; Murdoch et al., 2012; O'Donoghue et al., 2016). To the best of our
173 knowledge, no studies have been performed with different wavelengths throughout the VIS range against
174 multiple types of bacterial contaminations. Hence, it is important to study the efficiency of different PDI
175 treatments against planktonic cells, individual cells on a surface and biofilms.

176 This study investigates the effect of light inactivation technology by employing four colours of LED light on
177 three different contamination types i.e., (i) in a liquid suspension as planktonic cells, (ii) on an agar surface as
178 individual cells and (iii) on a polystyrene surface in the form of biofilms. The effectiveness of the inactivation
179 technology was assessed based on the number of colony-forming units after treatment. The four LED sources
180 are characterised by different emission spectra in the violet, blue, green and red range of the VIS spectrum.
181 Furthermore, for single cells on agar (i) a stress factor, sodium chloride, was introduced to verify whether the
182 cells were sublethally injured and to better understand the effect of blue light and (ii) the influence of a lower
183 light irradiance was studied. The bacteria used for this study were *Pseudomonas fluorescens*.

184 This research focuses on the three most common types of bacterial contaminations in the food industry context.
185 First of all, the planktonic form is most common in liquid food products (e.g., milk, fruit juice) and water that
186 is used during industrial washing processes. The individual cells which represent the bacteria at the first stage
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of contamination on a surface (abiotic or biotic) are the second type of bacterial contamination assessed for PDI in this research. By applying PDI to individual cells inoculated on an agar surface, the presence of moisture and rich nutrient content are guaranteed, assuring optimal environmental conditions during treatment. Finally, the assessment of PDI against biofilms is of crucial relevance as they represent the most resistant among all types of contaminations. Hydrated biofilms were chosen for this study on one hand to be sure that the cells within the biofilms remained in their optimal state without dehydration occurring and on the other hand because hydrated biofilms that form on wet surfaces of industrial scale washing tanks or equipment are considered highly persistent (Cunault et al., 2018; Fysun et al., 2018; Wijman et al., 2007).

Pseudomonas fluorescens is a well-known food spoiler that has been found in dairy, poultry, meat, fish and fresh-cut vegetable products (Andreani et al., 2019; Cunault et al., 2018; Hyun and Lee, 2020; Pang and Yuk, 2019). Given that it is so widespread, the control of this spoiler has a vast potential for improving food quality and safety, leading to benefits for public health and the food industry. Contaminations of *P. fluorescens* cause economic losses and food wastage. In particular, in 2010, it caused the food discolouration of mozzarella cheese within the dairy industry in Italy and Germany with huge consequences for the food sector (Nogarol et al., 2013). This study shows the potential of PDI treatment for different types of bacterial contaminations and adds to a growing body of research in the field. The primary aim is to understand which contamination types are more susceptible to PDI, in order to determine where the largest application potential of this novel technology lies within the food industry.

2. Materials and Methods

2.1. Experimental design

This study examined the impact of four different colours of LED light on three types of bacterial contaminations: (i) planktonic suspensions (Section 2.3), (ii) individual cells on an agar surface (Section 2.4) and (iii) hydrated biofilms on a polystyrene surface (Section 2.5) at a light source-sample distance of 2.5 cm. Each bacterial contamination type was exposed for 30 min to irradiation of violet, blue, green and red LED light. The irradiated samples were compared to the control, which was kept in the dark during the same time

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212 period. For individual cells on a surface, two additional sets of experiments were performed. In the first set of
213 experiments, the bacteria were treated after inoculation on selective media to assess the impact of an additional
214 stress factor. The selective media were supplemented with 3% (w/v) NaCl. The second set of experiments was
215 carried out for individual cells on a surface with an increased treatment distance of 5.0 cm, to test the effect of
216 a reduced light irradiance. Figure 1 presents the experimental design, highlighting the three types of bacterial
217 contaminations and the four irradiation conditions that were tested against the control. All the experiments
218 were performed at 20°C.

220 **Figure 1:** Experimental design on three types of bacterial contaminations: planktonic cells, individual cells on a surface
221 and hydrated biofilms. The samples were treated by exposure to violet, blue, green and red light. The control
222 corresponds with the samples that were stored in the dark. The circles represent the sets of experiments performed on
223 general media: the full black circles and the hollow green ones represent a treatment distance of 2.5 cm and 5.0 cm,
224 respectively. The red crosses represent the set of experiments performed on selective media at a distance of 2.5 cm.

225 2.2. Pre-culture preparation

226 A streak plate of the bacterium *Pseudomonas fluorescens* (ATCC® 13525 culti-loops, Thermo Fisher Scientific,
227 Waltham, United State) was prepared from a frozen stock-culture, stored at -80°C in Tryptic Soy Broth, (TSB,
228 VWR International, Oud-Heverlee, Belgium), supplemented with 20% (v/v) glycerol (VWR International,
229 Oud-Heverlee, Belgium). One colony from the streak plate, stored at 30°C overnight, was transferred into
230 20 mL of TSB growth medium. Afterwards, an incubation period of 24 h at 25°C while shaking (160 rpm)
231 allowed the bacteria in this pre-culture to grow to the stationary phase.

2.3. Planktonic suspension samples

A 100-fold dilution of the pre-culture was made to obtain a suspension of approximately 10^7 CFU/mL. 400 μ L of the suspension was transferred to the surface of a small polystyrene petri dish (50 mm diameter, 8 mm height, Simport, Canada) where a circle of 2.5 cm in diameter was previously marked on the outside of the petri dish. With a sterile inoculation loop, the 400 μ L was spread to guarantee full coverage of the marked area of 4.9 cm². Afterwards, the planktonic samples underwent the light treatment (Section 2.5.) or were left untreated in the dark (control), inside an incubator at 20°C. Experiments were performed in quadruplicate for each of the irradiation conditions (violet, blue, green and red light). For the control sample, 13 independent replicates were performed. Samples were exposed to the light treatments (Section 2.6) and the number of colony-forming units (CFU) was quantified (Section 2.7).

2.4. Individual cells on a surface: Three sets of experiments

On the outside of petri dishes (90 mm diameter, 16 mm height, Simport, Canada) containing 20 mL of Tryptic Soy Agar (TSA, VWR International, Oud Heverlee, Belgium), a circle of 2.5 cm in diameter was marked. 40 μ L of the bacterial pre-culture (10^7 CFU/mL) was inoculated onto the TSA surface, following a similar protocol as Hyun and Lee (2020). With the aid of a sterile loop, the suspension was spread over the circular area of 4.9 cm² that was marked. This inoculum level corresponded to approximately 4.9 log(CFU/cm²). The samples were briefly left to dry under sterile conditions to let the liquid phase evaporate and leave the individual cells on the surface of the TSA. The samples were then immediately irradiated. Eight independent samples were exposed to the irradiation in each condition. For the untreated control, 24 independent replicates were used. Two additional sets of experiments were performed. The first additional set was carried out on selective media composed of TSA supplemented with 3% (w/v) NaCl (Sigma Aldrich, Saint Louis, MI, USA). 4 independent experiments were performed for light irradiated samples. 12 independent samples were used for the control. The second additional set was carried out to test the effect of a reduced light irradiance with a treatment distance of 5.0 cm. The number of independent replicates was 6 for LED-irradiated samples and 18 for control

samples. All the samples were exposed to LED irradiation (Section 2.6) and were successively enumerated (Section 2.7).

2.5. Hydrated biofilms

A 100-fold dilution of the pre-culture was made and 400 μL of 10^7 CFU/mL bacterial suspension was placed in an empty petri dish and spread on a surface area of 4.9 cm^2 , as described in Section 2.3. The petri dishes were closed and placed inside an incubator at 25°C . After 24 h, biofilms had formed at the interface between the suspension and polystyrene surface of the petri dish. The protocol for biofilm growth was based on that of Angarano et al. (2019). The suspension was gently removed with the aid of a pipette. Three rinses with PBS were applied to remove the unattached planktonic cells. Then, 2 mL of PBS was added to the biofilms to avoid dehydration and to simulate biofilms in a wet environment. The presence of a liquid layer on one hand makes sure the cells within the biofilms remain in their optimal state and dehydration would not occur; on the other hand hydrated biofilms that form on wetting surfaces of industrial scale washing tanks or equipment are considered strongly persistent (Cunault et al., 2018). To study the impact of the light on the most resilient biofilm form, hydrated biofilms were used. The experiments were performed in triplicate for each light irradiation condition. Nine replicates were performed for the untreated control. The treatment was performed as described in Section 2.6 and the control sample was stored in the dark at 20°C . After light exposure, the cells within the biofilm were enumerated as described in Section 2.7.

2.6. Light source and treatment

Irradiation was performed using four light-emitting diode devices of different colours (LED, Aviat Network Inc., Santa Clara, California). The devices were equipped with an internal reflector and lens, which improved the uniformity of the treatment on the circular irradiated area of 2.5 cm^2 at distance 2.5 cm. Specifically, violet, blue, green and red LEDs were used as light sources for the irradiation treatment. The LEDs were optically characterised in terms of spectrum and emission. The emitted irradiance was measured using the spectroradiometer BTS256-EF (Gigahertz-Optik GmbH, Germany) and corresponded to 55.4, 170.2, 27.5 and 133.9 mW/cm^2 for violet, blue, green and red, respectively. The LEDs' optical parameters, namely the peak

281 wavelength (λ_{em}) and the full width at half maximum ($\Delta\lambda$), are reported in Table 1. The LEDs' spectra are
282 reported in Figure 2. The treatments were performed for 30 min and the total dose received by the biological
283 samples is also reported in Table 1 and calculated as follows (Maclean et al., 2009):
284 Total dose [J/cm^2] = Irradiance [W/cm^2] $\cdot$ t_{tot} [s]
285 where t_{tot} corresponds to the total exposure time. The total dose represents the total energy delivered to the
286 irradiated area. The distance between the sample (target) and the light source was set to 2.5 or 5.0 cm (the
287 latter was only applied to individual cells on a surface, as explained in Section 2.4.).
288 The treatment was performed under sterile conditions inside an incubator to keep the temperature constant
289 at 20°C. Samples were separated with a dark opaque panel to ensure that they only received the selected
290 wavelength radiation.

LED colour	λ_{em} [nm]	$\Delta\lambda$ [nm]	Dose [J/cm^2] at distance	
			2.5 cm	5.0 cm
Violet	401	14	99.7	24.9
Blue	470	26	306.3	76.6
Green	522	35	49.4	1.4
Red	644	21	241.1	60.3

291

292 **Table 1.** Light characterisation of violet, blue, green and red LEDs. Peak wavelengths (λ_{em}), full width at half maximum
293 ($\Delta\lambda$) and calculated doses for 2.5 and 5.0 cm of distance.

294

295

Figure 2. Normalised emitted irradiance of the LEDs. The emission spectra of the coloured LEDs, namely violet, blue, green and red, showing the peak wavelengths, the narrow spectral emitted ranges and the wavelength ranges corresponded to the tails of the emission.

2.7. Microbiological analysis of planktonic cells, individual cells on a surface and biofilms

The number of bacteria was determined after irradiation for each experiment. For the quantification of the planktonic suspension samples, serial dilutions were prepared immediately after the light treatment (or dark storage for the control). Afterwards, the dilutions were plated on general media (TSA) following the standard viable plate counts procedure (Herigstad et al., 2001). The cell densities were calculated for comparison among the different irradiation conditions.

Regarding the quantification of the individual cells on a surface, the plates were placed inside an incubator after the treatment. This allowed the surviving cells to grow to colonies on the general or selective media. Afterwards, the colonies were enumerated.

The quantification of the hydrated biofilms needed some additional steps. The cells were first mechanically removed from the polystyrene petri dish with a sterile scraper (Carl Roth, Germany). Afterwards, the solution was homogenized with the aid of a pipette. Finally, the cell densities were quantified by means of serial dilutions and viable plate counts.

All agar plates that were used for cell quantification were placed in an incubator at 30°C for 48 h. After growth to colonies, mean counts for each light treatment were calculated and expressed in CFU per millilitre (CFU/mL) with respect to the surviving cell number of *P. fluorescens* in planktonic suspension, and in CFU per area (CFU/cm²) with respect to *P. fluorescens* surface inoculated cells and biofilms. These values were log₁₀ transformed before comparison.

2.8. Statistical analysis

To statistically compare the data, a one-way analysis of variance (ANOVA) was used. A multiple comparison test performed using the *multcompare* function implemented in MATLAB R2016a (The Math Works, Inc.). The number of replicates, carried out for each set of experiments, was considered during the statistical analysis for Journal homepage: <https://www.sciencedirect.com/journal/food-and-bioproducts-processing>

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control, violet, blue, green and red LED irradiated samples. A p-value lower than 0.05, corresponding with a level of confidence of more than 95%, was considered to demonstrate a significant difference.

3. Results

The findings are divided into three main sections according to the three types of *P. fluorescens* contaminations that were considered: planktonic cells, individual cells on a surface and hydrated biofilms. The impact of the four different light sources was studied at a distance of 2.5 cm between the samples and the light source. In Section 3.2, related to individual cells on a surface, the influence of a 3% (w/v) NaCl containing TSA (selective media) and a reduced light irradiance (distance of 5.0 cm) were investigated as well.

3.1. Impact on planktonic cells

Following the irradiation treatments with different light sources on planktonic cells, the number of viable cells was quantified. The quantities of surviving cells are represented for each treatment in Figure 3. The first bar on the left represents the control (without irradiation). Only for the treatment with violet light, a significant reduction in the population size was found. This reduction corresponded with approximately a 0.5-log average decrease in the number of viable cells.

Figure 3. Bar chart related to the treatment of planktonic bacteria by means of LEDs of different colours. From left to right data represent the control (dark condition), violet, blue, green and red irradiation. The error bars represent the standard deviation on 13 independent tests for the control and 4 independent tests for light-treated samples. Bars bearing different lower case letters differ significantly with a confidence level of 95%.

3.2. Impact on individual cells on a surface

The same treatment was performed on cells of *P. fluorescens* that were spread on a TSA surface. The data are displayed in Figure 4 (black bars correspond to the distance of 2.5 cm between the light source and sample). Blue, green and red light did not affect the population density, compared to the control samples. On the other hand, an inactivation of about 3-log of the population density was observed for violet exposure. The high error bar for the treatment at a distance of 2.5 cm with violet light is due to the absence of any surviving cells in some of the samples while other samples showed a few survivors. The few cells that formed colonies were at the edge of the treated area, where the intensity of the treatment was slightly lower. Because of the strong inactivation obtained with violet light, the sample-LED distance was increased to 5 cm to reduce the irradiance (i.e., the number of photons impacting the cells). Data are displayed in the same bar chart for all light

351 treatments (green bars, Figure 4). The inactivation due to violet light is weaker when the sample-LED distance
352 is longer. However, a significant reduction of the microbial population was still found. A reduction of 2.2-log
353 was observed at 5.0 cm compared to 3.7-log at 2.5 cm. Additional experiments were performed to ensure that
354 the reduction in CFUs was not related to the alteration or deterioration of the TSA due to exposure to the light
355 sources. Therefore, preliminary experiments were performed in which cells were inoculated on regular TSA
356 and TSA that had been irradiated for 30 min. The data (not showed) demonstrated that the irradiation of the
357 TSA surface did not affect the development of colonies on the agar surface.

358
359 **Figure 4.** Effect of four colours of LED irradiation on individual *P. fluorescens* cells on a TSA surface at a treatment distance
360 of 2.5 cm (black) and 5.0 cm (green). The bar chart shows the control (no irradiation) and violet, blue, green and red light
361 irradiated samples. The error bars represent the standard deviation on 24 and 18 independent replicates for the control
362 and 8 and 6 independent replicates for the light-treated samples for a distance of 2.5 cm and 5.0 cm, respectively. Bars
363 bearing different lower case letters represent significantly different data at a confidence level of 95%.

364 After 24 h, careful observation of the size of the developed colonies demonstrated smaller colonies for the
365 samples that were irradiated by blue light compared to green and red light (Figure 5). For this reason, the same
366 inactivation experiment was carried out with cells spread on TSA containing 3% (w/v) NaCl, which was used
367 as a selective medium. TSA containing 3% (w/v) NaCl was previously tested on untreated (healthy) cells and
368 did not affect the cell growth (control in Figure 6). Cells were irradiated with violet, blue, green and red light
369 after inoculation on this selective medium. It was observed that the bacteria irradiated by violet and blue light
370 could not form colonies on the selective agar as the samples showed no growth (not detectable), while red and
371 green treated samples demonstrated not to be affected.

372
373 **Figure 5.** Colonies that have formed from *P. fluorescens* cells spread on TSA agar after 24 h incubation following (a) no
374 exposure (control) and exposure with (b) violet, (c) blue, (d) green and (e) red light.

375

Figure 6. Effect of four colours of LED irradiation on individual *P. fluorescens* cells on the surface of TSA. Comparison between TSA with (red) and without (black) the addition of 3% (w/v) NaCl acting, respectively, as selective and general medium. The error bars represent the standard deviation on 24 and 12 independent replicates for the control and 8 and 4 independent replicates per light treated sample on, respectively, general medium and selective medium. Bars bearing different lower case letters represent significantly different data with a confidence level of 95%. For violet and blue exposure, no cells were detected when grown on the selective medium (Not Detected, ND).

3.3. Impact on hydrated biofilms

The 24 h grown biofilms were hydrated by a small layer of PBS covering them during the treatment. The results, as displayed in Figure 7, demonstrate that no bacterial inactivation was observed after violet, blue, green or red light exposure compared to the control. The population densities within the biofilms were not influenced by the irradiation of any of the light sources.

Figure 7. Cell densities within the hydrated biofilms after treatment with violet, blue, green and red light compared to the control (without irradiation). The error bars represent the standard deviation on 9 independent replicates for the control and 3 independent replicates for the light-treated biofilms. Columns bearing different lower case letters represent significantly different data at a confidence level of 95%.

392 4. Discussion

393 Whilst the available literature, focusing on the effect of light on bacteria, demonstrates the efficiency of shorter
394 wavelengths within the visible light spectrum (< 460 nm), results based on longer wavelengths (up to 800 nm)
395 are relatively scarce and the reported effects are more contradictory (Ghate et al., 2013; Ghate et al., 2015; Kim
396 et al., 2013; Kumar et al., 2015; Schmid et al., 2019). This study adds new knowledge on the efficacy of PDI by
397 investigating the effect of four coloured LEDs within the visible region on three different contamination types
398 of *P. fluorescens*: planktonic cells, individual cells on a surface and hydrated biofilms. The discussion is divided
399 into paragraphs focussing on the impact of the different wavelengths. The impact of violet light (401 nm) is
400 first explained, then blue light (470 nm) and, finally, green (522 nm) and red light (644 nm) are jointly
401 discussed, each time taking into account the three different bacterial contamination types.

402 The efficiency of PDI depends on some intrinsic factors, e.g. the presence of endogenous photosensitizers (PSs)
403 and their probability of being photoexcited, and on some extrinsic factors such as the exposure time, the total
404 dose, the temperature and the target sample. These factors are discussed in Section 4.4. Finally, the versatility
405 and the potential applications of PDI are discussed in Section 4.5.

406 The PDI inactivation effect depends on the way the treatments have been performed, drawing a lot of attention
407 on parameters such as volume, agitation, bacterial growth phase, bacterial species, light source and irradiation
408 time. Notwithstanding the uncountable protocols and different research conditions in literature, a
409 comparative study that investigates four different LED light sources interacting with the bacterial cells of three
410 different types of contaminations has not been published yet. Moreover, among the published studies on PDI,
411 less than half dealt with bacterial species that are relevant for the food science or which are the causative agents
412 of foodborne outbreaks (Haughton et al., 2012; Kim et al., 2015). Specifically, *P. fluorescens* has been considered
413 only a few times with respect to PDI (Angarano et al., 2020; Cunault et al., 2018; Hyun and Lee 2020; Pang and
414 Yuk, 2019).

415 4.1. Effect of violet light

416 Violet light peaking at 400 nm has been widely investigated against planktonic suspensions of different
417 bacterial genera. The last decades witnessed an increased number of publications concerning its potential and
418 confirming that it reduces the number of viable cells in liquid suspensions of *Listeria*, *Salmonella*, *Staphylococcus*,
419 *Pseudomonas*, *Escherichia* spp. and many other species (Ghate et al., 2019; Haughton et al., 2012; Kumar et al.,
420 2015; Murdoch et al., 2012).

421 The results were able to demonstrate a slight but significant inactivation of the planktonic *P. fluorescens* cells
422 due to the irradiation with violet light (Figure 3). The effect on individual cells on a surface was proven to be
423 even stronger (Figure 4), but the violet light had no effect on hydrated biofilms (Figure 7). To the best of our
424 knowledge, even though few studies compare different types of contaminations using violet light (Haughton
425 et al., 2012; Murdoch et al., 2012), none of them considered biofilms in the comparison. For this reason, a
426 detailed analysis, in which the authors make comparisons with other studies, is included below for the three
427 types of contaminations.

428 The studies on planktonic bacteria are numerous. Different species are analysed but there are none on *P.*
429 *fluorescens* and LEDs at 400 nm. Within the field of food science and by applying a protocol similar to that in
430 the current study, Kim et al. (2015) investigated the impact of light from a narrow-emitting 405 nm LED on
431 *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus* at a concentration of 10⁸ CFU/mL and they
432 found reductions of 1.0 to 2.0 log in bacterial suspensions after 7.5 h of light exposure (total dose of 486 J/cm²).
433 The comparable reductions underline that bacterial suspensions can be light inactivated when violet light is
434 used. In the present research, mature cells were irradiated after the bacterial suspension reached the stationary
435 phase. The findings highlighted the potential of the PDI technology for the decontamination of liquid in the
436 food industry context such as liquid food products or washing water, in order to avoid infections. The
437 experiments of Kim et al. (2015) were performed at 4°C, compared to 20°C used in the present study. This
438 demonstrates that bacterial inactivation of planktonic bacteria with violet light can be achieved at various
439 temperatures, underlining the wide applicability of the inactivation technology within the food industry. A
440 higher inactivation (up to 5-log) was achieved for planktonic cells with a concentration of 10⁵ CFU/mL by
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441 Murdoch et al. (2012). However, the bacterial suspensions (*Salmonella* Enteritidis, *Shigella sonnei*, *L.*
442 *monocytogenes*, *Escherichia coli* and *Mycobacterium terrae*) were stirred during irradiation and this might
443 contribute to the difference in results (Murdoch et al., 2012). Finally, although Kumar et al (2015) found
444 reductions up to 1-4 log for a concentration of 10^6 CFU/mL, depending on the bacterial species and the
445 temperatures during violet exposure (with doses up to 600 J/cm^2), the authors claimed *Pseudomonas aeruginosa*,
446 which belongs to the same genus of *P. fluorescens*, to be less susceptible to violet light treatment. This claim
447 was also made on the basis of a number of other studies on *P. aeruginosa* with 400 nm where it was found that
448 a dose $\leq 100 \text{ J/cm}^2$ was needed to achieve a reduction of 1 log, which is in accordance with the 0.5-log reduction
449 observed in the present study (Hessling et al., 2017).

450 Regarding the inactivation of the individual cells on agar when irradiated with 400 nm light, only a few studies
451 have been found with a comparable scope. The research group of Kim et al. (2017b) investigated the impact of
452 light at 405 nm on *E. coli*, *Salmonella* Typhimurium and *L. monocytogenes* as individual cells on a surface
453 following a protocol that is comparable to the one used in the present research. The authors irradiated the cells
454 after inoculation on fresh mango fruits rather than on TSA. They investigated different temperatures, among
455 them 20°C , and found after 48 h of exposure (i) a 1.2 log reduction for *S. Typhimurium*, (ii) inhibition of growth
456 for *E. coli* and (iii) a 3-log reduction for *L. monocytogenes*. The total irradiation dose was 1.7 kJ/cm^2 , ten times
457 higher than the one used in the present study, but was spread out over a much longer period of time. This
458 long time period could explain the comparable inactivation that was achieved, even though the treatments in
459 the present study had a lower total irradiation dose. A similar study performed on agar-inoculated cells has
460 been conducted by Enwemeka et al. (2008). The authors spread a bacterial suspension on a circular surface of
461 3.5 cm in diameter. The bacteria that were subjected to the treatment were two different strains of methicillin-
462 resistant *Staphylococcus aureus*. An inactivation of just over 1 log was obtained when bacterial cells were
463 exposed to 60 J/cm^2 of radiation at 405 nm in a period of less than 10 min. Finally, after seeding *S. Enteritidis*,
464 *S. sonnei*, *L. monocytogenes*, *E. coli* and *M. terrae* on agar, Murdoch et al. (2012) observed a total inactivation (1-
465 log reduction) when samples were irradiated with a dose up to 1.9 kJ/cm^2 and a treatment time up to 45 min.

466 All the studies witness a considerable inactivation towards a wide spectrum of bacteria, confirming the present
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467 results obtained. To the best of our knowledge, no studies have considered light treatments on *P. fluorescens*
468 that can be directly compared with the presented findings on individual cells on agar. However, since (i)
469 Enwemeka et al. (2008) used a lower dose and a shorter time (60 J/cm² and 10 min), (ii) Kim et al. (2017b) used
470 a high dose and a long time (1.7 kJ/cm² and 48 h) and (iii) Murdoch et al. (2012) used high doses and moderate
471 time (1.9 kJ/cm² and 45 min), the best performance of PDI with violet light against *P. fluorescens* are obtained
472 in the current study. Indeed, the violet light was applied for a short time of 30 min and with a low dose
473 (approximately 100 J/cm²) causing a high inactivation for *P. fluorescens* (3.7 and 2.2 log for the highest and
474 lowest dose of violet light respectively).

475 The last contamination types that were examined in this study were the hydrated biofilms, whose outcomes
476 are in contrast with earlier findings. In a previous research, McKenzie et al. (2013) irradiated 24h-grown
477 biofilms of *E. coli*, *P. aeruginosa*, *L. monocytogenes* and *S. aureus*, grown on acrylic and glass surfaces with violet
478 light peaking at 405 nm. They found a reduction of 5 log on the acrylic surface and 7 log on the glass surface
479 after irradiating with 140 mW/cm² for 60 min (total dose of 504 J/cm²). Similar works using different light
480 sources, irradiation times and protocols for biofilm growth found a reduction in the population density as well
481 (Ferrer-Espada et al., 2019). Despite applying a similar treatment as McKenzie et al. (2013), no reduction is
482 observed in this study when hydrated biofilms are irradiated with violet light for 30 min. The authors believe
483 that the hydration of the biofilms plays an important role in the impact of the photoinactivation process. It
484 should be considered that the common procedure that is used for biofilm photoinactivation, follows these
485 steps: (i) biofilm growth, (ii) rinsing the biofilm and (iii) light exposure. The layer of liquid that was added in
486 the current research prevented drying of the biofilms during the light irradiation. Angarano et al. (2020)
487 observed an inactivation of about 0.4 log after 30 min for untreated *P. fluorescens* biofilms. This inactivation
488 continued when storing the biofilms for longer periods of time and was found to be due to the dehydration of
489 the biofilms. Also Pang and Yuk (2019) studied the desiccation of biofilms of *P. fluorescens* and *L. monocytogenes*
490 and they observed 2 to 3 log reduction within 5 days due to mere desiccation. Based on this, it is possible to
491 claim that the dehydration applies an additional stress to the biofilms. This additional stress is likely to enable
492 or enhance the inactivation of biofilms due to light treatment. However, biofilms that grow in a food
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processing environment will often develop on surfaces that are frequently wetted or in generally humid areas, as they require the presence of some water to develop. Therefore, it is essential to test the PDI effect on hydrated biofilms that represent the worst case scenario for biofilm inactivation, because the liquid layer may offer some protection of the cells to the PDI treatment.

4.2. Effect of blue light

Whilst violet light is generally peaking at 400 to 405 nm, the definition of blue light in literature is much wider, as it can range from 420 to 470 nm. Based on the literature, blue light can have a strong or weak effect depending on the type of emitting light source and the bacterial strain. As can be seen from the results in Figure 3, 4 and 7, the blue light did not have any effect on *P. fluorescens* as planktonic cells, individual cells on a surface or hydrated biofilms when enumerating on general media. However, based on the results in Figure 5, it was observed that the colonies grew much smaller after surface inoculation when treated with blue light compared to those treated with green or red light. The smaller colony size was caused by the need for a longer recovery time after the light treatment. The comparison of the population density (CFU/area) on TSA (general medium) and on 3% (w/v) NaCl supplemented TSA (selective medium) evidenced that the entire population that underwent blue light exposure was sublethally injured (Figure 6). Consequently, these cells did not grow on TSA containing the additional stress factor, NaCl. This is an important aspect to be considered. Generally, in the context of microbial food safety and quality, multiple stress factors (osmotic pressure, pH, temperature variation) are combined for preservation purposes. These additional stress factors, which are a property of the food product or process, could also work in synergy with PDI, as demonstrated in the present research. In fact, not only the NaCl concentration but also the pH, temperature and chemical products can alter the bacterial membrane, facilitating the interaction of photons with endogenous PS. Hyun and Lee (2020) took an alternative approach to demonstrate the potential of blue light radiation on *P. fluorescens*. By applying very long treatment times of 2 days for suspensions and 7 days for surfaces, they were able to achieve inactivation. Specifically, their study achieved an inactivation of 1.32 log for cells in suspension, 1.60 log for cells on agar and 1.85 log for cheese inoculated cells. The authors stated that deformations and damage to the bacterial cell

membrane took place. In the context of employing blue light PDI within the food industry, the advantage is that it is a mild technology that is claimed not to disintegrate proteins and other nutrients, unlike conventional food preservation processes (Ghate et al., 2019; Kim et al., 2017b).

The outcomes of the present study related to blue light underline that a short exposure can at least inhibit microbial growth. Moreover, in synergy with other stress factors, blue light can be sufficient to obtain significant inactivation of cells on a surface. The performed experiments have a number of similarities with O'Donoghue et al. (2016), who found a total growth inhibition when *L. monocytogenes* was irradiated on agar and a partial inhibition when these bacteria were irradiated in suspension. Unlike the short treatment of 30 min, used in this study, the authors exposed the bacteria overnight to 470 nm and irradiances of 2 mW/cm².

4.3 Effect of red and green light

The experiments performed with red and green light did not demonstrate any inhibition of the bacterial populations. The literature in this area is somewhat contradictory because (i) some authors demonstrated stimulated growth of planktonic cells and biofilms (Ghate et al., 2013; Yu and Lee, 2020), (ii) some showed no effect (Kim et al., 2013; Lui et al., 2016; Schmid et al., 2019) and (iii) others claimed reduction (Ghate et al., 2013; Kim et al., 2013; Kumar et al., 2015) when red or green irradiation was used. These wavelength ranges are still interesting because they correspond to secondary absorption peaks (Q bands) or tails of the endogenous PSs. Consequently, they might promote reactive oxygen species formation (Hessling et al., 2017; Hoenes et al., 2020; Orlandi et al., 2018; Schmid et al., 2019). Based on our findings, no effects were observed on the population size of planktonic cells, individual cells on a surface and biofilms, making the radiation very inadequate for PDI of *P. fluorescens* promoted by endogenous PSs. The technique could be exploited with the addition of exogenous PS (riboflavin, erythrosine, etc) in future research. Some examples, both in accordance or in contrast with the present study, are discussed. Orlandi et al. (2018), in a study focused on medical applications, used two mutants of *P. aeruginosa*, in one of them suppressing the expression of Catalase A, and investigated the effect of blue, red and green light. Although their purpose was to understand the role of Catalase A in the PDI process, they found red and green radiation to be ineffective, which is in accordance with this study

(Orlandi et al., 2018). The work of Kim et al. (2013) demonstrated in contrast to the present findings that green light peaking at 525 nm caused the reduction of both cells on agar (less than 0.4 log, depending on the bacterial strains: *Pseudomonas gengivalis*, *S.aureus* and *E.coli*) and in suspension (0.3 log reduction) for 8 h of exposure time. In accordance with the present study, no effect was observed for red light peaking at 625 nm (Kim et al., 2013). Finally, in a work in which white light was used instead, the authors noticed a reduction of spores and vegetative bacterial cell concentrations (methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, multidrug-resistant *acinetobacter* and *Clostridium difficile*) on formica substrate (Rutala et al., 2018). Based on the findings of the current research, it is expected that the wavelength ranges that are part of the white light having an inhibition effect on bacteria are the ones characterised by shorter wavelengths, corresponding to high frequencies and energy.

4.4. Factors influencing the PDI performances: intrinsic and extrinsic factors

The PDI efficacy is influenced by several factors, among those the endogenous photosensitizers' presence within bacterial cells, which are considered an intrinsic factor. The wavelength (extensively described in Section 4.1, 4.2 and 4.3), the treatment time and the total dose used for the PDI treatment belong, instead, to the extrinsic factors.

Although this study is not aimed at the mechanistic understanding of PDI, a brief explanation of the reason why certain wavelengths (specific photon energies) can achieve inhibition or inactivation is included. Some bacteria produce endogenous PSs, which are molecules with cyclic structures belonging to the categories of flavin and porphyrins. These are the well-known, but other photosensitizers may also be involved in the process (Hessling et al., 2017; Hoenes et al., 2020). Photosensitizers have the capacity to absorb light. Their main absorption peaks correspond to the violet-blue range. In the presence of oxygen-containing species, the light absorption results in the formation of reactive oxygen species. These reactive oxygen species are cytotoxic and cause damage to the bacterial cells or an impairment of their functionalities (e.g., ion pumps). Studies on *Pseudomonas* spp. demonstrated that this genus produces coproporphyrin II, protoporphyrin and

uroporphyrin III (Amin et al., 2016; Dai et al., 2013; Sailer et al., 1997). Therefore, it is possible to hypothesise that the photoinactivation of *P. fluorescens* is due to these species.

Among the multiple studies focused on PDI the treatment time is very variable, from 2 min to 7 days. Of course, the choice depends on the selected light source and the type of application. For instance, a powerful laser beam will require a shorter treatment time compared to LEDs that have a lower irradiance. This study focussed on relatively short treatments (30 min) with LEDs. This option of short treatment times and a widely available, inexpensive light source make the results of this research highly relevant for implementation in food processes. This approach is new in the context of microbial food quality and safety. In fact, even if PDI has already been proposed for, e.g., fresh-cut products or cheese, previous research focused mainly on preservation and long-time exposure up to 72 h (Hyun and Lee, 2020; Kim et al., 2017a, 2017b; O'Donoghue et al., 2016).

The outcomes of this research demonstrate on one hand that 30 min of treatment can significantly inactivate (using violet light) or at least sublethal injure (with blue light) microorganisms when individual cells are present on surfaces. On the other hand, the results also show that the dose required is relatively low compared to other studies where a similar inactivation effect was observed. Murdoch et al. (2012) applied a dose that was over 10 times higher to inactivate the population density below the detection limit. Also in other studies of Enwemeka et al. (2008) and O'Donoghue et al. (2016) that dealt with different bacteria and applications, the treatment dose was about 5 to 10 times higher. These two aspects (short exposure time and low intensity) translate into lower energy consumption and a rapid method, which is extremely desirable for industrial applications (Enwemeka et al., 2008; O'Donoghue et al., 2016).

4.5 PDI versatility and potential application

The most interesting feature of PDI is its versatility: it can be applied (i) at several temperatures and (ii) to different target surfaces.

The current study was performed at 20°C and demonstrated an unquestionable potential for the use of PDI on surfaces at this temperature. Other works demonstrated that an even better effect can be achieved at lower

592 temperatures (4°C and 10°C) (Kim et al., 2017). The reduction observed when *P. fluorescens* is exposed to violet
593 light on or in general media should be further investigated to assess the potential for real food matrices.
594 Perhaps a combination of temperature and light can be suggested as a synergetic strategy.

595 PDI is suitable for those applications involving target surfaces that are not compatible with harsh treatments.
596 Examples of such surfaces in the food industry would be ready-to-eat foods, e.g., fresh fruits and vegetables.
597 These food products could even be treated when already inside transparent packaging. Whilst having an
598 inhibitory effect on the microorganisms, PDI is claimed to guarantee the conservation of nutrient content and
599 organoleptic properties of food products (Hyun and Lee 2020; Kim et al., 2017b). It does not cause food colour
600 deterioration while being suitable for extending the shelf-life of food products by reducing the bacterial load
601 on the surface (Kim et al., 2017b).

602 Although further research is required to investigate the efficacy on different food matrices, the current study
603 underlines the potential of this technology, specifically against individual cells on a surface. It demonstrated
604 that violet and blue light are most effective against individual cells on a surface compared to planktonic cells
605 or hydrated biofilms. So far, individual cells on a surface have only been considered as contamination type by
606 a limited number of studies on PDI. Most of those have focused on PDI applied to planktonic cells and
607 biofilms, which represent a major issue as well, but are less susceptible targets for light irradiation (Angarano
608 et al., 2020; Ferrer-Espada et al., 2019; Ghate et al., 2019; McKenzie et al., 2013; Wang et al., 2016).

609 The inactivation of *P. fluorescens* that was achieved for planktonic cells (0.5 log reduction) compared to
610 individual cells on a surface (3.7 log reduction) indicated that the direct exposure of the bacteria to the photons
611 has to be taken into account. The photoexcitation of endogenous PSs is a probabilistic process that depends on
612 the number of photons reaching the bacterial cells directly. The probability of the photons reaching the
613 bacterial cells and interacting with the PS is lower for cells in a suspension than for those that are spread out
614 on a surface. In fact, the liquid barrier between the light source and the cells causes diffusion of the light and
615 thereby decreases the probability of photons reaching the cells. This means that the amount of interaction
616 between photons and endogenous PSs will be lower for cells in suspension than for individual cells on a dry
617 surface. The same comparison can be made between individual cells on a surface and the hydrated biofilms.

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618 Next to the liquid layer that is similar to that of the planktonic cells, the hydrated biofilms are also protected
619 by the extracellular polymeric substances (EPS) matrix. This layer of EPS protects the cells to a certain extent
620 from the interaction of the photons with the endogenous PSs. Since the photoinactivation is based on a
621 probabilistic process, the excitation of the PSs depends on the flux of photons but even more on the actual
622 amount of photons reaching the target (endogenous PSs). The more the target is hit, the more reactive oxygen
623 species are produced (Aveline, 2001).

624 5. Conclusions

625 This research focussed on the effects of four different colours of VIS light on three different types of *P.*
626 *fluorescens* contaminations. This broad investigation of the potential of VIS light PDI on *P. fluorescens*
627 contaminations has led to a number of interesting conclusions with respect to the application potential of this
628 novel technology for the food industry. The results demonstrated that violet was the only colour of light to
629 achieve any inactivation for planktonic *P. fluorescens* in a suspension. The limited extend of the inactivation
630 would make it unsuitable to replace, e.g., pasteurisation processes or applications in which UV light is
631 currently used. However, violet light has potential for the treatment of areas that are expected to be wet or
632 humid with continuous lighting or at least for long time periods such as overnight. For individual cells on an
633 abiotic TSA surface, violet light was also the only colour that could achieve inactivation if the cells were at
634 optimal conditions. Considering that it is a mild treatment, violet light was even found to be quite effective
635 with an inactivation of about 3.7 log within 30 min. When adding a stress factor to the environment, both violet
636 and blue light were able to achieve high inactivation of the individual cells on a surface, to levels below the
637 detection limit. This demonstrates the great potential of violet and blue light to inactivate contaminations on
638 surfaces of food products that contain other stress factors (e.g., low water activity foods) or on food contact
639 surfaces that are kept mostly dry. Since cleaning methods for food contact surfaces require the drying of these
640 surfaces, these results indicate the potential of adding a violet or blue light treatment as a decontamination
641 step. Finally, for the hydrated biofilms grown on polystyrene surfaces, no inactivation could be achieved with
642 any of the light sources. These biofilms are assumed to be protected from the light irradiation by both the EPS
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643 matrix and the liquid layer that covers the biofilm. These results, further narrow down the scope of
644 applications for PDI treatments as it can be stated that VIS light is more suitable as a technique to prevent
645 microbial proliferation by intensive implementation than as a method for disinfecting developed microbial
646 populations.

647

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