**Application of an eco-sustainable technology: use of direct and photodynamic UV light for the microbial decontamination on food industries**

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In the first part of the project, a qualitative screening was done to evaluate the inactivation capacity of the blue LED lamps (BL) against several microbes (*E. coli*, *S. aureus*, *L. monocytogenes*, *S.* Enterica, *P. fluorescens, B. subtilis* and *S. cerevisiae*) testing different doses. Secondly, a quantitative assessment of the direct decontamination capacity was done in solid and liquid media testing different conditions (doses, treatment time, lamp’s voltage). Thirdly, the biofilm-forming capacity of microbes on different surfaces was analysed. Biofilms were treated with BL and observed by CLSM. Finally, preliminary activities for the photoactive materials tests were done.

**Applicazione di una tecnologia ecosostenibile: utilizzo della luce UV LED diretta o fotodinamica per processi di decontaminazione microbica nell’industria alimentare**

Nella prima parte del progetto è stato condotto uno screening qualitativo per valutare la capacità di inattivazione microbica delle lampade LED a luce blu (LB) erogando diverse dosi di luce. È stata poi valutata la decontaminazione diretta operata dalla LB, considerando diversi parametri (dose, tempo, voltaggio delle lampade), e la capacità di formazione di biofilm su diverse superfici. I biofilm sono stati sottoposti a trattamento con BL. I danni cellulari sono stati osservati con CLSM. Per la sperimentazione con i materiali fotoattivabili, sono state svolte prove sperimentali per il set-up dei test con LB.

**Key words**: Blue LED light, photodynamic inactivation, biofilm, microbial decontamination.

# **1. Introduction**

In accordance with the PhD thesis project, here are reported the main results of the PhD project:

A1) Assessment of the germicidal effect on planktonic cells and biofilm

A2) Tests with photoactive materials

# **2. Materials and Methods**

The qualitative screening was done by streaking microbes on an agar plate and testing the plate under BL at 405, 420 and 450 nm for 1h at 2, 5 and 10 cm from the lamp to modulate the dose (Cheng et al., 2020). Half of the plate was covered as a control. Before each test, irradiance (*I*) was measured using a probe and this value was used to calculate the dose (*D*), following Equation 1:

*D = I x t*  (1)

Where *D* is the dose (J/cm2), *I* is the irradiance (W/cm2) and *t* is the treatment time (s) (Ghate et al., 2013).

The direct inactivation assessment was done in solid and liquid substrates. In the first case, each tested microbe was serially diluted and plated in an agar plate with the drop plate method (Herigstad et al., 2001). Also in this case, half of the plate was covered as a control. In the direct inactivation assessment in liquid media, a 24-well microtiter plate was used. 1 mL of microbe culture in PBS was inserted in each well and half of the plate was covered as a control. The microbial inactivation was monitored every 15 min for two hours. Tests were done by setting the lamp at 10V and 5V, based on the microbes’ response. For qualitative and quantitative assessment, the temperature was measured during the tests. All the data were expressed as log N/N0, where N is the final microbial population after the BL treatment and *N0* is the initial microbial population.

The biofilm formation capacity was initially assessed with a screening in 96-microtiter wells (Stepanović et al., 2007) and then biofilm formation was tested on glass, stainless steel, and Teflon coupons for 48 hours, 7 and 15 days (Li et al., 2018). Biofilms were treated with BL and the microbial inactivation was monitored by microbial cell count, using the drop plate method, by recovery of the cells before and after the treatment. Damages at biofilm structure and morphology were evaluated by CLSM.

For the photoactive materials, preliminary tests were done to set up the BL treatments since the surface is not homogeneous.

# **3. Results and Discussion**

## **3.1 Qualitative screening**

The qualitative screening of the mentioned microbes evidenced that the light-sample distance of 2 cm was not optimal for the BL treatment since it increased the dose and so the final temperature in 60 min reached > 50°C in all of the cases with some exceptions exceeding >70°C. This screening evidenced conditions where the microbial inactivation occurred due to T effect (Figure 1. a.) or where was T not influenced (Figure 1. b.). Furthermore, it is underlying how the wavelength effect is dependent on the microbe that is used.

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| a.b. |

**Figure 1** *a. L. monocytogenes*, *S.* Enterica 2 cm 405 nm; *b. L. monocytogenes*, *S.* Enterica 5 cm 405 nm.

**3.2 Quantitative assessment: cells**

As evidenced by quantitative screening, the 2 cm lamp-sample distance was not optimal. Therefore, the quantitative tests on solid media proceed with light-sample distances of 5 cm and 10 cm. Tests evidence that at 5 cm almost all the microorganisms were completely inactivated with no temperature effect with using all the wavelengths. An exception was for *L. monocytogenes* and *P. fluorescens*, which were affected by the high temperature (ca. 48°C). Indeed, at 10 cm microbes follow different inactivation based on the wavelength that was used and the emitted doses. For instance, *S. aureus* was less affected by 450 nm (-1.61±0.70, D=198 J/cm2, T60=36.5°C), while at 405 and 420 nm the inactivation was -4.76± 0.87 (D=144 J/cm2, T60=34.7°C) and -3.02±0.48 (D = 162 J/cm2, T60=37.5°C), respectively. *L. monocytogenes* was reduced up to -1.91 ± 0.28 (D=216 J/cm2, T60=39.4°C), -7.34 ± 0.30 (D=198 J/cm2, T60=37.1°C) and -4.31 ± 0.43 (D=198 J/cm2, T60=37.7°C) at 450, 405 and 420 respectively. In general, all microbes tested were less susceptible to 450 nm, with some exceptions. For instance, *S.* Enterica shows poor inactivation at all the wavelengths (max inactivation -1.31 ± 0.13 at 420 nm, D= 180 J/cm2, T60=37.1°C).

**3.3 Quantitative assessment: cells in suspension**

Tests in the liquid substrate were done on *E. coli*, *S.* Enterica, *L. monocytogenes* and *S. cerevisiae*. *E. coli* irradiated with 405 nm (10 V) was reduced up to -4.81±0.95 in 90 min (T90 =39.9; Dose = 280 J/cm2), -3.81±0.47 at 420 nm (T90 =39.4°C; Dose = 280 J/cm2) and -1.82 at ±1.04 at 450nm (T90 =38.7; Dose = 280 J/cm2), while the dark controls remain almost constant. *S.* Enterica was more resistant to BL. In fact, at 405 the reduction was -2.08 ± 1.10 after 120 min (T120 =43.5; Dose = 360); however, at 420 nm and 450 nm, the reduction was < 1 log cfu/mL. *L. monocytogenes* showed better results by setting the light voltage at 5V. Using the 405 nm lamp, it decreased up to -3.15 ±0.47 after 120 min (T120 =31.6; Dose = 324 J/cm2). Also in this case, the lower effect was seen for 450nm lamp, -1.08±0.01 after 120 min (T120 =31.5; Dose = 324). *S. cerevisiae* after 120 min decreased up to -1.23±0.26 (T120 =40.4; Dose = 396 J/cm2), -1.41±0.07 (T120 =44.1; Dose = 360 J/cm2) and -1.43±0.23 (T120 =40.5; Dose = 432 J/cm2) for 450, 405 and 420 nm, respectively. However, the dark control decreased after 120 min -1.12±0.26, -0.86±0.19 and -1.56±0.37 at 450, 405 and 420 nm, respectively. This was probably because the temperatures reached were 33.4, 32.1 and 34,5 °C at 450, 405 and 420 nm, respectively. Also, the BL has a different efficacy on microorganisms, depending on the microbial species. Moreover, by CLSM observations of biofilms, the BL affected the biofilm’s structure formation and produced cellular damage on planktonic cells (study in progress).

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