Pulsed-UV disinfection of Polyvinyl alcohol-based films inoculated with *Bacillus cereus* endospores

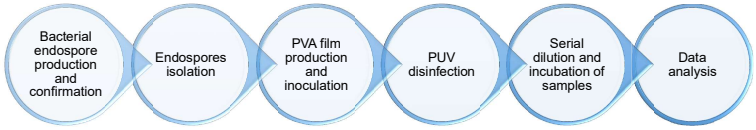
Mabilly C. H. B. Dias[1]; Viviane S. S. Marchi [2]; Michael Nugent [2]; Gavin Burke [2]; Neil. J. Rowan[1];

1. Athlone Institute of Technology, Biosciences Research Institute, Athlone, Co. Westmeath, Ireland
2. Athlone Institute of Technology, Materials Research Institute, Athlone, Co. Westmeath, Ireland.

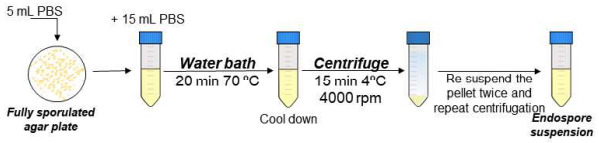
Introduction

Pulsed UV light (PUV) is an emergent non-thermal technology widely used to disinfect food, liquids and surfaces through short but high intensity pulses of polychromatic light (range between 200nm - 1100nm). The PUV is proved to be at least 6 times more efficient against bacteria than continuous UV light due to the peak of power in a short time delivered by the system (Fine and Gervais, 2004). *Bacillus cereus* is a food intoxicant gram-positive bacterium, rod-shaped and endospore forming. Thus, the elimination of bacterial spores is crucial and challenging through the health industry. Some studies had already shown the efficacy of PUV in *Bacillus cereus* disinfection on food, liquid sources and surfaces (Rowan, 2019), being this the first study that relates *B. cereus* disinfection of a polymer and drug candidate via Pulsed UV-light system. This study aims to determine Pulsed UV disinfection efficacy on PVA films with different compositions and analyse how the composition affects efficiency of the process at different fluence values, plus structural changes that the system might cause to the polymer.

Materials and methods



Scheme 1. Workflow of methods.



Scheme 2. Procedures for endospore isolation.

Polyvinyl alcohol hydrogel film production. 500 µL of PVA 2% solution (polyvinyl alcohol Mowiol ® 56-98) with and without curcumin (0.4 mg/mL, previously diluted in 100 µl DMSO) was poured into each well on a 24-well plate and left for drying on the bench for 2 weeks. After drying process, samples were collected for disinfection.

Pulsed Ultraviolet light disinfection. Samples were inoculated with 100 µl of bacterial standard suspension on a 5 cm^2 petri dish, without cover. Pulsed UV Machine (Samtech ® Model PUV-01) coupled to a chamber with Halogen lamp (Everspring ® Ind. Co. LTD Model SA122) was used for disinfection (Scheme 2). The set up was 800 volts at 1 pulse per second, and sample distance from the bulb was 8 cm. Untreated plates were used as controls.

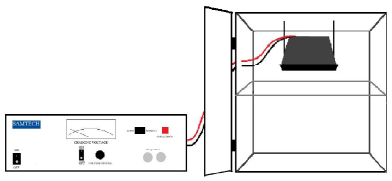


Figure 1. Pulsed UV Samtech® system representation.

Data analysis. Colonies were counted and the results were expressed as 1.5 Log CFU/mL. All analyses were conducted in triplicate at 25 ºC. Log reduction was calculated from the colony count according to the formula Log (N/N0) where N0 is the initial amount of spores inoculated and N number of microorganisms after treatment. All readings and statistical analysis were made with GraphPad Prism 2018.

Results and discussion

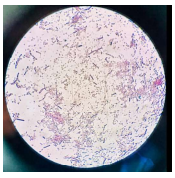


Figure 2. Malachite green dying confirming bacterial endospores.

Figure 3. PVA film sample.

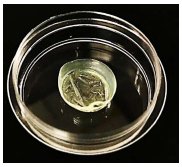


Figure 4. PVA + Curcumin film sample.

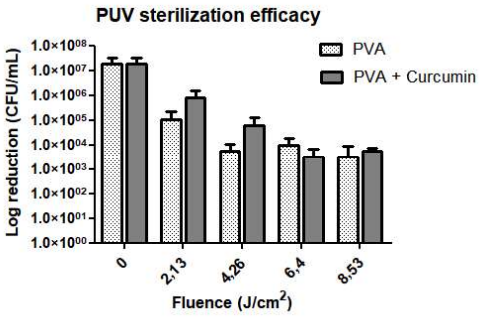


Figure 5. Log reductions of PVA films with and without curcumin after PUV treatment from an initial inoculum of 2.58 x 10^7 CFU/mL. The experiment was conducted on triplicate and error bars indicate confidence level of 95%.

Disinfection efficacy was proven by 3-log reduction with maximum fluence applied and slight decrease on efficacy between disinfection of sample with and without curcumin, probably due to the turbidity caused by the compound. The kinetics appears almost linear with exception of the last set of pulses that shown a statistical plateau of PVA sample and fluctuation between 4.26 J/cm² and 8.53-4.26 J/cm² of Curcumin PVA film. FTIR analysis showed integrity of the polymer and increase of polymerisation with pulses applied.

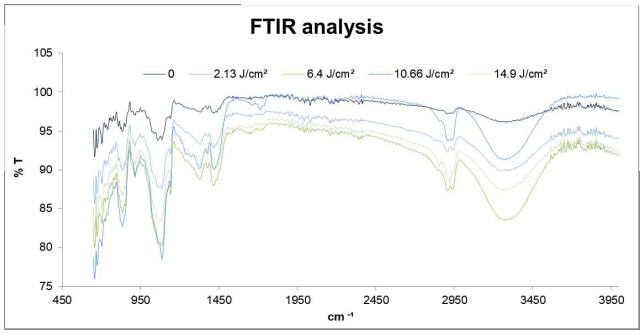


Figure 7. Infra-red spectroscopy analysis of PVA films according to different levels of PUV exposure.

Perspectives

- ✓ *Evaluation of different PVA forms and formulations disinfection;*
- ✓ *Optimization of the disinfection efficacy;*
- ✓ *Structure integrity analysis and characterization.*

References

Neil J. ROWAN (2019). Pulsed light as an emerging technology to cause disruption for food and adjacent industries – Quo vadis?. Trends in Food Science & Technology, Volume 88, 2019, Pages 316-332, ISSN 0924-2244, ttps://doi.org/10.1016/j.tifs.2019.03.027

F. FINE and P. GERVAIS (2004). Efficiency of Pulsed UV Light for Microbial Decontamination of Food Powders. Journal of Food Protection: April 2004, Vol. 67, No. 4, pp. 787-792. https://doi.org/10.4315/0362-028X-67.4.787

THE TIMES THE SUNDAY TIMES GOOD UNIVERSITY GUIDE 2018 INSTITUTE OF TECHNOLOGY OF THE YEAR

AIT Research