

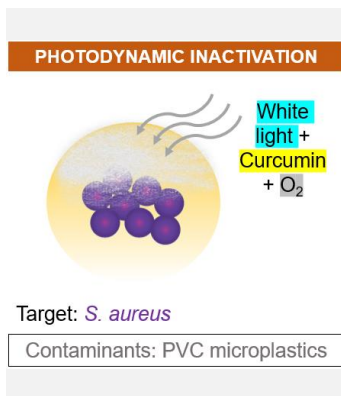
Influence of PVC microplastics on photodynamic inactivation of *Staphylococcus aureus*

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Here we showed that even though photodynamic action is effective against planktonic *Staphylococcus aureus* and increases performance according to curcumin concentration, the presence of PVC MPs impairs its efficiency. We attributed this to the umbrella effect, which is protective towards microorganisms and hinders both light delivery and exposure to the photosensitizing molecule. Photodynamic inactivation (PDI) is therefore a potential tool in water and wastewater disinfection, but contamination scenarios must be taken into account, including MP pollution. Future studies may include real-life concentrations and environmental matrices.

Introduction

The presence of microplastics (MPs) in various water sources has become a growing challenge to water and wastewater treatment technologies [1]. This has brought attention towards how unit processes respond to contamination scenarios, especially light-based treatments such as UV, in which transmittance and diffraction effects may interfere in treatment performance [2].

PDI has gained attention in environmental applications [3] and also consists of a photonic treatment, whose impacts of MPs are yet to be evaluated. PDI refers to the use of a photosensitizing substance (PS) that combined with a light source and oxygen molecules produces reactive oxygen species (ROS) and singlet oxygen, leading to cell death in the target organism. [4]. Thus, this study aimed to assess the effects of MPs, taking polyvinyl chloride (PVC) as reference, on the photodynamic process against *Staphylococcus aureus* using curcumin as PS.

Material and Methods

Experiments were carried out in batches in-vitro. We used 24-well plates filled up to a total of 750 μ L each well, comprising equal parts in volume of: the *S. aureus* suspension (ATCC® 25923, $\sim 10^8$ CFU/mL), PS solutions (3X) and MP suspension (3X) at different concentration combinations. Illumination was performed under a fixed energy dose of 5 J/cm² provided by a white LED light irradiation source (Biotable®, 12.7 mW/cm²).

The PS under study was curcumin powder (PDT Pharma, Brazil) diluted in ethanol (99.7%, stock solution), then autoclaved distilled water for work solutions [5]. MP concentration was based on

extrapolated values of contamination scenarios [2]. Viable colonies were quantified by growth on brain-heart infusion agar incubated at 37 °C. We analyzed log-reduction value results for normality using Lilliefors-L test (95% confidence interval), followed by ANOVA and Tukey's pairwise test for each group of curcumin concentration.

Results and Discussion

Figure 1 shows results obtained for *S. aureus* log removal by PDI (dashed columns) and PDI in the presence of PVC. The photodynamic process, as expected, was effective for bacterial inactivation and was concentration-dependent to the PS, which may be attributed to an increase in ROS formation [6].

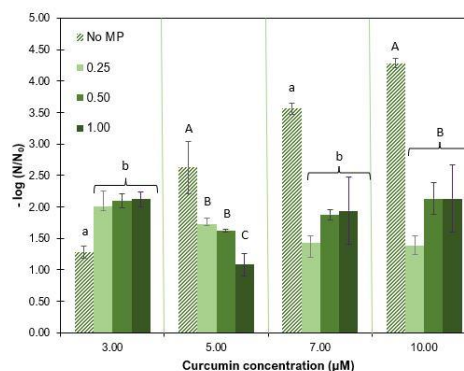


Figure 1. Photodynamic inactivation of *S. aureus* incubated with different concentrations of curcumin and MP (PVC microplastics). Dashed column refers to control PDI efficiency (no MPs added to the sample). Error bars display the standard deviation (n=4).

The presence of microparticles hindered PDI to a similar level among different concentrations of PVC. Though results may suggest a recovery in PDI performance (but still not reaching the values obtained in absence of MPs in the matrix), this was not confirmed by statistical analysis in the tested conditions.

The reduced performance of PDI in the presence of the microparticles related to the umbrella effect described in the literature [7], which consists of a reduced delivery of the disinfectant to the microorganism (either energy – by reduced light transmittance) or the chemical agent (by bacterial enriched to the MP surface).

Conclusions

Photodynamic inactivation using curcumin is a potential tool for bacterial removal considering *S. aureus* as a target, but the presence of PVC microparticles at the tested concentrations impaired its performance. Further studies are recommended to determine what are the mechanisms involved in MP interference in PDI and whether it happens at real-life contamination scenarios.

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References

- [1] Oladoja, N.A.; Unuabonah, I.E. The Pathways of Microplastics Contamination in Raw and Drinking Water. *Journal of Water Process Engineering*, 2021, 41, 102073
- [2] Manoli, K.; Naziri, A.; Tfofi, I.; Michael, C.; Allan, I.J.; Fatta-Kassinos, D. Investigation of the Effect of Microplastics on the UV Inactivation of Antibiotic-Resistant Bacteria in Water. *Water Research*, 2022, 222, 118906,
- [3] Lima, A.R; Dias, L.D ; Garbuió, M; Inada, N. M ; Bagnato, V.S . A look at photodynamic inactivation as a tool for pests and vector-borne diseases control. *Laser Physics Letters*, 19, 025601, 2022.
- [4] Dąbrowski, J.M. 2017 Reactive Oxygen Species in Photodynamic Therapy: Mechanisms of Their Generation and Potentiation, in, pp. 343–394.
- [5] Sammarro Silva, K.J.; Lima, A.R.; Dias, L.D.; De Souza, M.; Nunes Lima, T.H.; Bagnato, V.S. Hydrogen Peroxide Preoxidation as a Strategy for Enhanced Antimicrobial Photodynamic Action against Methicillin-Resistant *Staphylococcus aureus*. *Journal of Water and Health* 2023, 21, 1922–1932.
- [6] Sammarro Silva, K.J.; Lima, A.R.; Correa, T. Q.; Dias, L.D.; Bagnato, V. S. Hydrogen peroxide as an additive to curcumin on the photodynamic inactivation of bacteria: a preliminary study. *Laser Physics*, 2023, 33 085601
- [7] Shen M, Zeng Z, Li L, Song B, Zhou C, Zeng G, Zhang Y, Xiao R. Microplastics act as an important protective umbrella for bacteria during water/wastewater disinfection. *Journal of Cleaner Production*. 2021 Sep 15;315:128188.