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Hydrogen peroxide preoxidation as a strategy for enhanced antimicrobial photodynamic action against methicillin-resistant *Staphylococcus aureus*

Kamila Jessie Sammarro Silva [a,*], Alessandra Ramos Lima [a], Lucas Danilo Dias [b], Mariana de Souza [a], Thalita Hellen Nunes Lima [a] and Vanderlei Salvador Bagnato [a,c]

[a] Environmental Biophotonics Laboratory, São Carlos Institute of Physics (IFSC), University of São Paulo (USP), 13563-120 São Carlos/SP, Brazil
[b] Laboratório de Novos Materiais, Universidade Evangélica de Goiás, Anápolis 75083-515, GO, Brazil
[c] Biomedical Engineering, Texas A&M University College of Engineering, 3127 TAMU, College Station, TX 77843-3127, USA
*Corresponding author. E-mail: kamilajessie@usp.br

KJSS, 0000-0002-6881-4217; ARL, 0000-0001-5810-5137; LDD, 0000-0003-2858-7539; Md, 0009-0006-2753-4872; THN, 0000-0001-6840-3242; VSB, 0000-0003-4833-239X

ABSTRACT

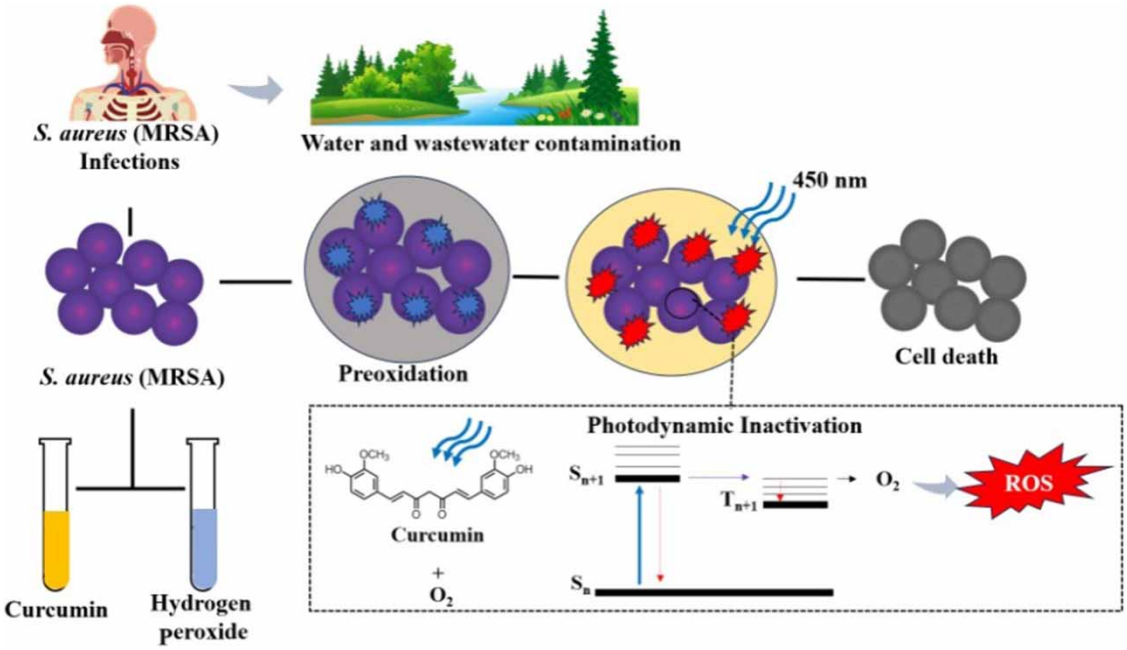
Antimicrobial photodynamic treatment (aPDT) is a photooxidative process based on the excitation of a photosensitizer (PS) in the presence of molecular oxygen, under specific wavelengths of light. It is a promising method for advanced treatment of water and wastewater, particularly targeting disinfection challenges, such as antibiotic-resistant bacteria (ARB). Research in improved aPDT has been exploring new PS materials, and additives in general. Hydrogen peroxide (H_2O_2) a widely applied disinfectant, mostly in the food industry and clinical settings, present environmentally negligible residuals at the usually applied concentrations, making it friendly for the water and wastewater sectors. Here, we explored the effects of preoxidation with H_2O_2 followed by blue light-mediated (450 nm) aPDT using curcumin (a natural-based PS) against methicillin-resistant *Staphylococcus aureus* (MRSA). Results of the sequential treatment pointed to a slight hampering in aPDT efficiency at very low H_2O_2 concentrations, followed by an increasing cooperative effect up to a deleterious point ($\geq$7 $\log_{10}$ inactivation in CFU mL^{-1}), suggesting a synergistic interaction of preoxidation and aPDT. The increased performance in H_2O_2-pretreated aPDT encourages studies of optimal operational conditions for the assisted technology and describes potentials for using the described strategy to tackle the issue of ARB spread.

Key words: curcumin, disinfection, peroxidation, photodynamic inactivation, resistant bacteria, synergistic factor

HIGHLIGHTS

- Preoxidation with H_2O_2 followed by aPDT led to absence of planktonic MRSA.
- Preincubation with H_2O_2 did not damage curcumin as analyzed by UV–Vis spectroscopy.
- A cooperative effect was obtained for the sequential treatment.

GRAPHICAL ABSTRACT



INTRODUCTION

Antibiotic resistance is a topic of major importance in public health (Salam *et al.* 2023), and it is strongly linked to the environment because of the possible spread of antibiotic-resistant bacteria (ARB) genes. *Staphylococcus* species are attributed to many human infections and are often prevalent in hospital settings, which also make them an important concern in water and wastewater treatment (Oladipo *et al.* 2019). Methicillin-resistant, vancomycin intermediate and resistant strains significantly contribute to the disease burden worldwide (Navidinia *et al.* 2017; Denissen *et al.* 2022). Their detection in hospital effluents has been reported along with various ARB (Tripathi & Tripathi 2017), but also in municipal wastewater treatment plants, recreational waters, and various environmental matrices (Fogarty *et al.* 2015; Santos *et al.* 2020; Rahimi *et al.* 2021).

Antimicrobial photodynamic treatment (aPDT) is a promising alternative to deal with ARB challenge not only in clinical (Soares *et al.* 2022; Piksa *et al.* 2023), but also in environmental applications. One of its major advantages is that aPDT is a multitarget approach that relies on oxidative stress (Almeida 2020). In short, it refers to the use of a photosensitizer (PS) molecule PS when excited by light in the presence of molecular oxygen produces reactive oxygen species (ROS), by the type I mechanism, or singlet oxygen (type II mechanism) (Dąbrowski 2017). aPDT has been widely used in therapies for controlling biofilms and infections in general (Akhtar & Khan 2021; Chen *et al.* 2022). In the environmental sector, it has shown potentials for vector control, as well as water and wastewater treatment (Lima *et al.* 2022a, 2022b; Sarker & Ahn 2022).

aPDT has proved to be effective against several targets, as in bacteria, fungi and viruses (Alves-Silva *et al.* 2023; Gholami *et al.* 2023; Pourhajibagher & Bahador 2023a, 2023b). However, research is still encouraged for improving efficiency and lowering reliance on energy dose and costly PSs. Preoxidation, often carried out by chlorination, is a strategy in this regard, as it has been used to improve efficiency of operational processes in water and wastewater treatment (Xie *et al.* 2016). Alternatively to chlorine, H_2O_2 has been applied as a preoxidant, increasing sludge dewaterability (which correlates to cell death), as well as reducing water color and overall microbial contamination (Wei *et al.* 2019; Silva *et al.* 2022). A paper in dentistry indicated that preincubation with H_2O_2 and riboflavin was an effective method pre-aPDT against planktonic bacteria responsible for oral infections, and led to microbial reduction in multi-species biofilm (Kunz *et al.* 2019). Similarly, studies on methylene blue as a PS assisted by H_2O_2 showed improvements in aPDT against bacteria and fungi of clinical concern (Garcez *et al.* 2011; Yang *et al.* 2019).

Considering environmental applications of aPDT, we recently reported that no combined effects were obtained against *Staphylococcus aureus* using curcumin as a PS under an exposure method with no pretreatment with H_2O_2, but rather spiking the oxidant immediately prior to photodynamic inactivation (Sammarro Silva *et al.* 2023). Results led to antagonistic effects, but the oxidation profile of curcumin in contact with the additive showed stability, which invited further research in H_2O_2 as a preoxidant to aPDT, as explored in peer research with other PS molecules.

Taking this approach with curcumin is a timely effort for aPDT applications in water matrices because it consists of a natural-based PS with low toxicity photodegradation products (Dias *et al.* 2020; Lima *et al.* 2022b). As for the additive, peroxidation is a popular decontamination practice using standalone H_2O_2, e.g., in the pharmaceutical industry, clinical environments, aquaculture, agriculture and poultry, food industry, and water treatment (Silva & Sabogal-Paz 2022). Therefore, the aim of this follow-up study was to assess the effects of preoxidation with H_2O_2 in the efficiency of blue light-mediated aPDT using curcumin against methicillin-resistant *Staphylococcus aureus* (MRSA).

METHODS

Chemicals

H_2O_2 dilutions were prepared from a 29% stock solution (Synth®, Brazil). Aliquots from the stock solution were analyzed prior to each experiment. Quantification was performed by the colorimetric method using ferric thiocyanate (Vacu-Vials® kit, Chemetrics, USA, $\lambda = 470$ nm). H_2O_2 dilutions were prepared immediately before each assay. The PS used in this study was synthetic curcumin in powder (PDT Pharma®, Brazil), and it was applied in solution first diluted in ethanol (Analytical Standards) (Êxodo® Científica, Brazil), then in distilled water to our work concentrations.

Target organism

Inactivation assays were performed against MRSA (clinical isolate). A stock suspension previously stored at −20 °C in glycerol (Êxodo® científica, Brazil) was seeded onto Petri dishes with brain heart infusion (BHI) agar, then incubated at 37 °C for 24 h. Colonies were resuspended in BHI broth, homogenized, then incubated at 37 °C for 18 h. Growth was monitored by optical density ($\lambda = 600$ nm, Bel Photonics spectrophotometer) until the mid-log phase, point at which suspensions were purified in sterile phosphate saline buffer (PBS, pH 7.4). Purification was performed by centrifugation at 3,000 rpm for 15 min (Eppendorf 5702 centrifuge). After inactivation assays, viable colonies were quantified by serial dilutions in PBS, spread onto BHI agar and grown for 18 h at 37 °C. Final counts were considered in CFU mL^{-1} and inactivation values were calculated as described further.

Experimental setup

The experimental setup comprised two stages: incubation and irradiation, which is the standard protocol for aPDT. Enhanced treatments included the presence of H_2O_2 during incubation with the PS, which may be considered a preoxidation step. All aPDT assays were carried out in 24-well plates illuminated by a light-emitting diode (LED) device equipped with 24 blue LEDs (Biotable®, $\lambda = 450$ nm), leading to an average irradiance of 41.05 mW cm^{-2} at the tested water level. Applied fluence was 50 J cm^{-2}, adapted from peer research against MRSA (Almeida *et al.* 2017). The emission spectrum of the Biotable® device is provided in the supplementary file. In order to maintain the water head, sample volumes of equal proportions would always lead to a total of 750 μL, accounting for the combinations of H_2O_2, curcumin, MRSA suspensions, and autoclaved distilled water when necessary. Selected concentrations for mapping H_2O_2 effect were defined according to previous studies in combined physicochemical treatment (Sammarro Silva *et al.* 2022), and combined aPDT with curcumin, specifically (Sammarro Silva *et al.* 2023).

The PS was incubated with bacteria, along with different concentrations of H_2O_2 for 30 min in static conditions and protected from light, at room temperature. This stage consisted of preoxidation, as aPDT only takes place when the PS is irradiated. A simplified scheme of methodological steps is shown in Figure 1. The operational conditions for the incubation step and illumination were the same for aPDT and enhanced aPDT. The differential in sequential treatment was the addition of H_2O_2. Microbiological examinations were readily performed after irradiation in all aPDT assays.

Oxidation tests were also carried out in order to identify the contribution of H_2O_2 action as an individual disinfection method (as per the preoxidation represented in Figure 1). Experimental setup was the same as for sequential treatments and undertook 30 min exposure time of bacteria to a range of H_2O_2 concentrations. Identical oxidation assays were performed with an additional contact time, in order to compensate for possible prolonged exposure in enhanced aPDT tests

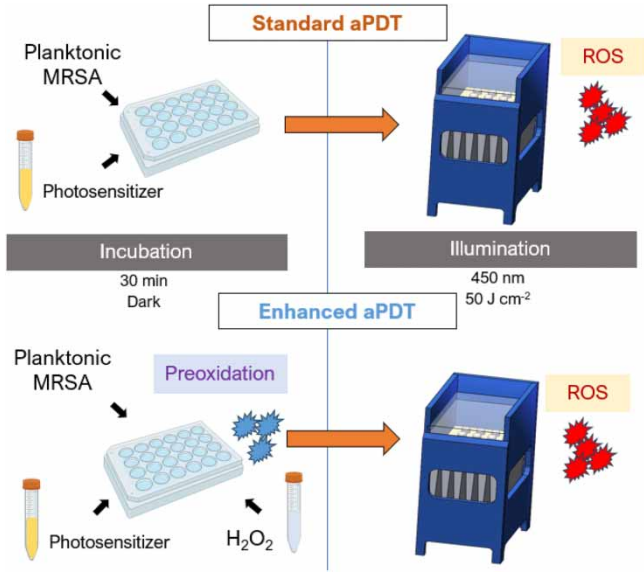


Figure 1 | Simplified scheme of the aPDT against MRSA compared to H_2O_2-assisted aPDT.

(preoxidation + illumination time), as no quenching of residual H_2O_2 was done. Selected concentrations for standalone oxidation test were result-oriented, based on the obtained performances of H_2O_2-assisted aPDT. These blocks of experiments were performed in absence of direct light, also in multi-well plates that were kept incubated in the dark at room temperature and static conditions during contact time.

The inactivation performance (Y) was evaluated in terms of relative log-reductions of the ratio of remaining colonies (N) (CFU mL^{-1}) to N_0 (initial concentration of the inoculum). A synergy factor (SF) was calculated by Equation (1) for each combination of independent variables, following an adaptation of peer literature (Cortina-Borja *et al.* 2009). If a cooperative performance takes place, SF > 1, whereas SF < 1 suggests competitive effects. Y_{12} refers to the inactivation obtained for sequential treatment (preoxidation followed by aPDT), Y_1 is the aPDT efficiency as a single factor (main treatment), and Y_2 refers to the log-reduction found for each oxidation test, relying exclusively on H_2O_2. An ideal *SF* was calculated for oxidation results in 30 min suggesting no prolonged action of H_2O_2 in solution, but we also evaluated synergistic factors accounting for possible extended oxidation due to the light exposure period with no quenching. *SF*s for combinations that led to deleterious effect were accounted for in terms of the referred remaining colonies (and their possible absence) against N_0. In these scenarios, note that SFs could be higher, as they were limited by the order of magnitude of the inoculum.

$$\mathrm{SF} = \frac{Y_{12}}{Y_1 \times Y_2} \tag{1}$$

PS internalization estimates

Aliquots of MRSA, grown and purified identically to the inactivation assays, were incubated for 30 min in different test groups: internalization (IT), which comprised bacteria suspended in curcumin (10 μM) and water, and curcumin and H_2O_2 (0.03%), and PS control, that referred to a curcumin solution (10 μM) in distilled water. Combinations considered equal parts of each medium (1 mL) and proportionate dilutions, as the PS solution was originally prepared at 3 ×.

After the IT period, the samples were centrifuged (Eppendorf centrifuge 5702) at 3,000 rpm for 15 min. Two mL of the supernatant was added to a quartz cuvette (10 mm optical path) and subjected to UV–Vis absorption spectrophotometry (Varian Cary Bio 50).

Curcumin peroxidation estimates

Possible peroxidation of the PS was assessed by monitoring the absorbance at 430 nm of different combinations of curcumin and H_2O_2 at various concentrations. This wavelength was chosen based on ultraviolet–visible (UV–Vis) absorbance

spectroscopy of curcumin solutions, described in the supplementary file. The exposure time was selected in order to simulate preincubation performed in enhanced aPDT assays (30 min), as well as prolonged contact time, accounting for irradiation period. Test concentrations of the additive were selected in an exploratory manner, starting out from the lowest (0.01%), the highest (0.30%), and a few intermediate ones. Absorption spectrophotometry was performed in a 10 mm optical path (Varian Cary Bio 50 spectrophotometer). The experiment was performed for $n = 3$, and results were analyzed by t-test ($\alpha = 0.05$) against a given mean value (standalone PS).

RESULTS AND DISCUSSION

Enhanced aPDT efficiency

Figure 2 displays results for H_2O_2-assisted aPDT. As shown by the orange hatched column, standard aPDT led to a final threshold of remaining colonies of 5.72 ± 0.19 $\log_{10}$ CFU mL^{-1}, which refers to an approximate $\log_{10}$ inactivation of $Y_{aPDT} = 2.01 \pm 0.19$. At first, adding H_2O_2 to the aPDT process hindered its efficiency, as the obtained inactivation levels were $Y_{0.01\%} = 0.25 \pm 0.05$, and $Y_{0.03\%} = 1.08 \pm 0.08$. However, a breakpoint to H_2O_2-enhanced action in aPDT was identified at 0.04%, followed by complete inactivation of MRSA, given that absence of microorganisms was found when incubating samples at concentrations $\geq$0.05% H_2O_2. Though details on this change from antagonistic to synergistic effects are further quantified by SFs in Table 1, it should be noted that previous research has reported hindered aPDT activity in the presence of this oxidant (Sammarro Silva *et al.* 2023). H_2O_2 inactivation requires high CT values (concentration $\times$ time) and, in the above referenced work, H_2O_2 exposure was short in terms of time (immediately before illumination). As for the present study, aPDT performance decreased at lower H_2O_2 concentrations (also low CT values). Similarly, peer research has quantified ROS in aPDT carried out after preoxidation with H_2O_2 and determined a lower production of reactive radicals, which suggests that the presence of H_2O_2 in solution may alter oxidation damage in aPDT (Garcez *et al.* 2011). At higher CT values, however, standalone H_2O_2 plays an inactivation role as a single factor, and this may be additive (and synergistic) to aPDT, as discussed further.

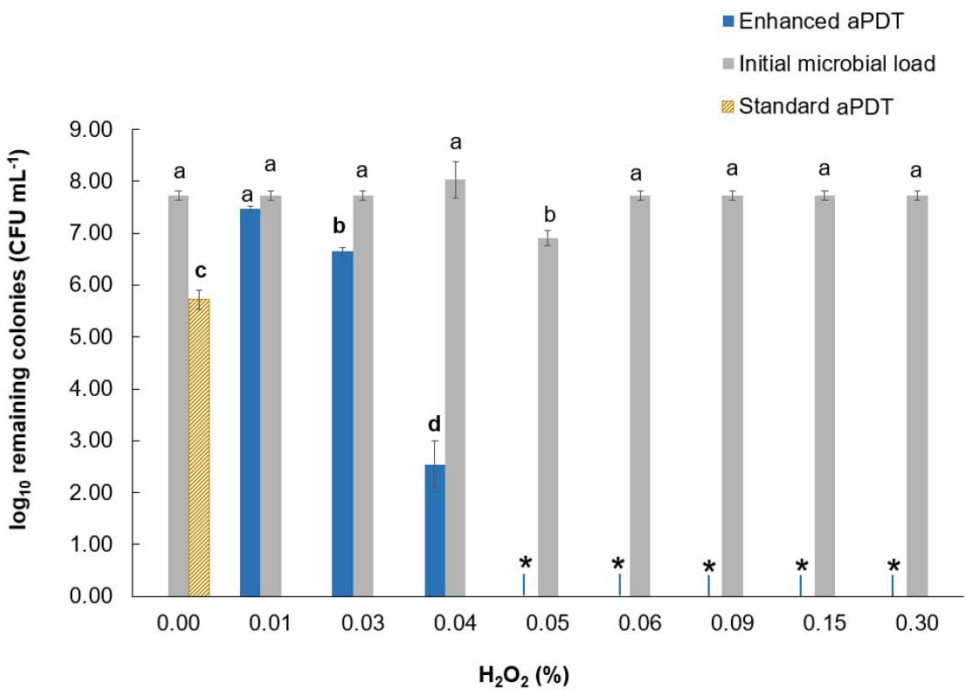


Figure 2 | Remaining MRSA culturable colonies after H_2O_2-assisted aPDT using curcumin (450 nm, 50 J cm^{-2}). *Notes:* The hatched column refers to standard aPDT. Different lowercase characters point to statistically different groups ($\alpha = 0.05$, one-way ANOVA, Tukey's post hoc). Bold lowercase letters show statistically significant log-removal against a group's specific inoculum. Asterisks point to experimental conditions with no colonies detected.

Table 1 | MRSA inactivation by standalone H_2O_2 action at different exposure times and respective synergistic factors (*SFs*)

	Oxidation	Extended oxidation		
H_2O_2 (%)	$Y_{30\ min}$	$Y_{50\ min\ 18\ s}$	$SF_{30\ min}$	$SF_{50\ min}$
0.010	0.35 ± 0.27	0.73 ± 0.08*	0.35	0.17
0.030	0.55 ± 0.36	0.90 ± 0.08*	0.98	0.60
0.040	0.65 ± 0.14*	1.57 ± 0.09*	4.32	1.79
0.050	0.56 ± 0.08*	1.33 ± 0.09*	6.10	3.00
0.060	0.19 ± 0.07	1.38 ± 0.05*	20.34	2.78
0.090	0.43 ± 0.13	1.57 ± 0.09*	8.85	2.45
0.100	0.60 ± 0.10*	1.40 ± 0.09*	6.71	2.85
0.150	0.52 ± 0.08	1.70 ± 0.17*	7.46	2.26
0.300	4.23 ± 0.21*	3.41 ± 0.17*	0.91	1.13

Notes: Y refers to -log (N/N_0). SD is the standard deviation. NM indicates not measured conditions. Asterisks indicate significant reduction in CFU mL^{-1} against the referred inoculum ($\alpha = 0.05$). SF values were calculated considering aPDT log-removal of $Y_{aPDT} = 2.01 \pm 0.19$.

As for reports on conventional aPDT against MRSA, studies have shown that it is more resistant to inactivation than the methicillin-sensitive strain. By using an edible die as PS (sodium copper chlorophyllin) under red light (30 J cm^{-2}), a study found that efficiency was concentration-dependent regarding the PS, but even higher concentrations (20 μM, for instance) did not reach complete MRSA inactivation (Caires *et al.* 2020). Similarly, a study on phototoxic effects of curcumin against bacteria screened concentrations and light doses over the sensitive strain, in order to define experimental conditions for the photokilling of MRSA, and only the highest tested concentration of the PS (20 μM) was effective against the resistant strain, at a 37.5 J cm^{-2} fluence of blue light (Ribeiro *et al.* 2013).

Regarding improved methods of aPDT, an in vitro study including potassium iodide and iodopovidone as additives to a porphyrin formulation led to substantial increase in MRSA inactivation (Braz *et al.* 2020). In that paper, the additives were incubated with the PS for 10 min prior to photodynamic assays, inviting for research in sequential aPDT. A similar study in photodynamic inactivation of MRSA achieved an increase in efficiency in 5-log_{10}, under 100 J cm^{-2} (laser, near infrared light), applying indocyanine green (ICG) as a PS. It should be noted that, in this paper on ICG, the tested H_2O_2 concentration was 0.1% (Wong *et al.* 2018), higher than the breakpoint we found for curcumin. This not only substantiates the positive impact of pretreatment to aPDT, but also endorses the importance of screening tests for optimizing operational conditions. These will lead to a reduced dependence on light and the PS concentration, based on the selection of parameters considering the three independent factors aimed at minimum microorganism survival. Overall, the improved inactivation followed by deleterious effect found in our present study point H_2O_2 preoxidation and aPDT as a promising method for disinfection and tertiary treatment against ARB.

Oxidation as a single factor and analysis of synergistic effects

Details of H_2O_2 contribution for 30 min incubation and extended contact time are laid out in Table 1 along with their respective *SF*s. As expected, prolonged exposure contributed to an increase in bacterial inactivation, but, as we worked with low H_2O_2 concentrations, this reduction is not very pronounced.

When considering oxidation performance as a single factor, MRSA log-reductions met expectations for standalone liquid H_2O_2, because this oxidant requires high CT values for drops in microbial load (Silva & Sabogal-Paz 2021). Against MRSA, most published research relies on different application methods such as dry mist (AHP), vaporized H_2O_2 (VHP) and peroxygen-based formulations applied as either AHP or VHP for surface disinfection. A study testing inactivation efficiency using contaminated carrier disks, for instance, targeted MRSA using 5% H_2O_2 AHP for 2.5 h (Piskin *et al.* 2011). Also applying AHP, similar research tested swabs from high-touch surfaces in clinical environments with patients with known multi-resistant organisms colonization following manufacturer's instructions for Deprox AHP, but operational details are not available, though there was a decrease in rates of contaminated surfaces after disinfection (McKew *et al.* 2021). Similarly, an automated VHP system was tested at 5, 10, and 35% (w w^{-1}) against MRSA for 40 min exposure followed by 200 min dwell time, also for surface disinfection (Murdoch *et al.* 2016). It is therefore challenging to extrapolate such results to our experimental content.

Though individual H_2O_2 action is low, SFs listed in Table 1 point it to a promising preoxidant to aPDT and quantify data on 0.04% as the breakpoint concentration for favorable inactivation, as shown in Figure 2. Such favorable results for sequential action stand out even more when we consider the very low concentrations selected for H_2O_2. In environmental applications, these may be considered insignificant, based, for instance, on the exemption in the list for tolerance in terms monitoring and quenching residuals in food decontamination (as long as application is $\leq$1%) (USEPA 2002).

Similarly, curcumin is considered an environmental-friendly PS that will be consumed in the course of aPDT action, and, when applied in the very low concentration proposed for the H_2O_2-enhanced treatment, does not lead to any increase in color. Moreover, its possible residual byproducts are safe, as shown in aquatic ecotoxicity assessments previously published, where authors showed that any photobleaching intermediates do not present toxicity potential against green algae, fish, nor *Daphnia* (Lima *et al.* 2022b), also confirmed by bioassays performed against non-target organisms (Venturini *et al.* 2020), indicating that curcumin-driven aPDT does not raise ecotoxicity issues.

Considerations on PS uptake

Figure 3 displays the absorbance profiles of the PS in solution, as well as its presence in MRSA suspension after incubation, and with H_2O_2. In the present research, IT estimation tests were not aimed at analytically quantifying PS uptake, but rather providing overall suggestions of the possible mechanisms involved in the performance of H_2O_2 preoxidation followed by aPDT. Detailed studies in dye IT are encouraged, to provide accuracy in quantifying the PS incorporation phenomena. In UV–Vis absorbance spectroscopy, though other mechanisms of curcumin removal may be involved (e.g., cell adhesion, adsorption, etc.), it is expected that, due to PS uptake, lower concentrations of curcumin are found in bacterial suspensions, when compared to the solution. The graph shows that curcumin IT was similar in both incubation conditions, and the presence of H_2O_2 did not lead to a considerably higher increase in PS uptake. This suggests that the mechanisms involved in synergistic aPDT do not necessarily refer to an increase in PS incorporation for MRSA in the selected conditions, as per estimates based on UV–Vis spectrophotometry, though research in H_2O_2 incubation prior to aPDT using methylene blue has hypothesized so (Garcez *et al.* 2011; Garcez & Hamblin 2017), under different operational settings.

Assuming that there was no increase in IT in H_2O_2-pretreated aPDT, we may infer that synergistic effects were obtained based on preoxidation as a single factor, as previously shown, followed by an improved photodynamic inactivation on remaining organisms, possibly more fragile. We believe that this synergistic effect obtained in sequential treatment is promising for environmental applications, where challenging targets persist through the treatment train, but also interesting future research in aPDT in general, e.g., control of biofilms and infections.

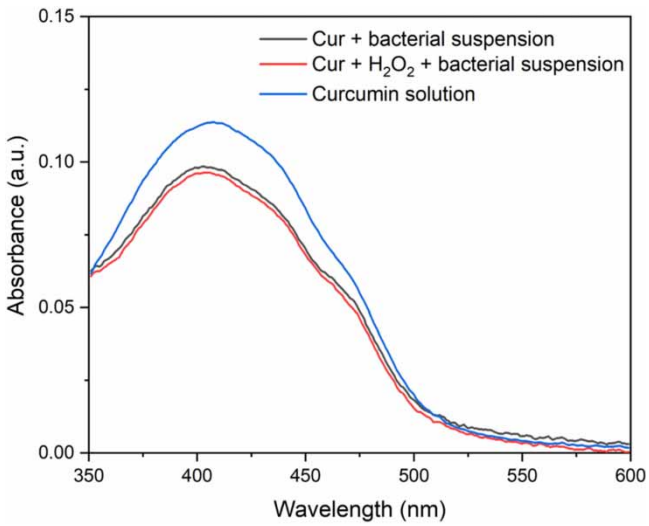


Figure 3 | Absorbance profiles of non-internalized curcumin in MRSA suspensions after different incubation settings (30 min). *Note:* Cur = curcumin.

Table 2 | Mean absorbance values at 430 nm (a. u.) for mixtures of 10 μM curcumin and H_2O_2 as a function on time

H_2O_2 (%)	30 min	*p*-value	50 min	*p*-value
0.010	0.07298 ± 0.0004	0.5415	0.07137 ± 0.0008	0.1367
0.040	0.07976 ± 0.0022	0.1856	0.08268 ± 0.0001	**<0.001**
0.060	0.08550 ± 0.0081	0.1587	0.08584 ± 0.0125	0.2786
0.300	0.08395 ± 0.0074	0.1669	0.09399 ± 0.0003	0.2005

Notes: *p*-values stand for *t*-test against a given mean of 0.07278, which refers to standalone curcumin at similar conditions. Confidence level is 95%. Results in bold indicate significant differences in means.

Curcumin peroxidation estimates

Quenching of H_2O_2 radicals is a topic that must be considered to determine whether there are any residuals to be quenched by an external agent or the substrate itself. As shown by similar studies in sequential aPDT, their presence may reduce the production of other radicals (Garcez *et al.* 2011; Garcez & Hamblin 2017). Also, organic matter in solution acts as oxidant demand at the first minutes of exposure (Freitas *et al.* 2021; Silva *et al.* 2022).

Table 2 displays data on curcumin peroxidation monitoring at 430 nm, considering different exposure times and concentrations of H_2O_2. In general, no significant differences in means were found against the PS under peroxidation stress, which suggests that the presence of H_2O_2 in solution did not damage curcumin. The only situation in which $p < 0.05$ was at 0.04% H_2O_2 during prolonged oxidation, which we may attribute to various reasons such as operational error, curcumin solubility, or randomness. These may be considered positive results, because they suggest that the presence of the oxidant during incubation does not impair the integrity of the PS molecule, and, therefore, the performance of aPDT, and, when looking at the oxidant demand, it does not compete with bacteria.

LIMITATIONS AND PROSPECTS

Results found for preoxidation with H_2O_2 led to promising takes for disinfection applications and infection control, as it even caused a deleterious effect, as shown in Figure 2. This absence of microorganisms, however, must consider inherent limitations in the detection method of viable bacteria. The quantification protocol requires extrapolations for determining the microbial concentration in CFU mL^{-1}. Future work to delineate optimal operational conditions may shed light onto details for the H_2O_2 concentration dependence and include additional quantifiers for bacterial viability.

Further research must comprise information on survival of pathogens from different core groups (e.g., viruses, protozoa, helminths, etc.), so that infection risk will be accurately calculated (Mraz *et al.* 2021). Along with the target challenge, water quality plays a key role in oxidation performance (Silva *et al.* 2022), and will affect the efficiency of both the pretreatment and aPDT. Likewise, removing biofilms and preventing biofilm formation also represent challenges to disinfection. Given recent studies in aPDT for clinical applications against biofilms (Akhtar & Khan 2021; Alagha & Gülsoy 2023), there are promising perspectives to it in environmental applications that may be further assessed in enhanced treatment (Yang *et al.* 2019). This also brings perspectives toward kinetics studies, modeling and in silico assessments.

As for aPDT as a single factor, though curcumin is known as an environmentally safe PS molecule (Dias *et al.* 2020; Lima *et al.* 2022b), it is crucial that no residuals or photoproducts are present in treated samples, particularly considering the organic input. Future work on optimal operational conditions must therefore consider mineralization of the PS. Including the pretreatment step with H_2O_2 may lower required concentrations of the PS (or energy input) and this should be considered a potential avenue for work aimed at more sustainable technologies in advanced water and wastewater treatment. These may even include tests on full spectrum of visible light- or solar-based systems.

CONCLUSIONS

The stated objective of this study was to provide a follow-up on the effects of H_2O_2 as a pretreatment to aPDT against bacteria. Though recent research showed antagonistic effects in combined aPDT, our newly proposed method led to cooperative effects. The method consisted of preoxidation by incubating the PS along with the target (planktonic MRSA) prior to photodynamic assays, carried out using curcumin under blue light. Here, we showed that it acts in a synergistic manner, though leading to some slight impairing effects in lower doses, but with a breakpoint substantially favorable to aPDT at 0.04%

H_2O_2, followed by complete bacterial inactivation, a deleterious effect that is really promising for advanced treatment in water and wastewater matrices.

The proposed approach for sequential treatment (tested in vitro at this point) relies on environmental-friendly solutions for both applied chemicals, and their photoproducts. Also, describing an increased efficiency of aPDT invites research in optimal conditions for the assisted technology, aiming to reduce reliance on light dose, and its associated costs, as well as the PS. Our results, therefore, describe potentials for using H_2O_2 preoxidation as a strategy to improve aPDT performance and tackle the issue of ARB in environmental matrices.

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ETHICS IN RESEARCH STATEMENT

MRSA isolates were obtained from samples of a previous clinical study on pharyngotonsillitis carried out with permission from the ethics committee CAAE number: 83082018.4.0000.8148 (Brazil).

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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