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Inactivation of *Candida albicans* in Water Using Advanced Oxidation Processes †

Ana Gomes [1], Ana Sampaio [2,*], Sara Silva [2], José R. Fernandes [3], José A. Peres [1] and Marco S. Lucas [1]

[1] Chemistry Center of Vila Real (CQVR), Chemistry Department, ECVA, University of Trás-os-Montes and Alto Douro (UTAD), Quinta de Prados, 5001-801 Vila Real, Portugal; ana.filipa111@hotmail.com (A.G.); jperes@utad.pt (J.A.P.); mlucas@utad.pt (M.S.L.)

[2] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), ECVA-UTAD, Quinta de Prados, 5001-801 Vila Real, Portugal; sarasilva042@gmail.com

[3] Chemistry Center of Vila Real (CQVR), Physics Department, ECT, University of Trás-os-Montes and Alto Douro (UTAD), Quinta de Prados, 5001-801 Vila Real, Portugal; jraf@utad.pt

* Correspondence: asampaio@utad.pt

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Abstract: Pathogenic microorganisms such as bacteria, viruses, fungi and protozoa have played a central role in the safety of drinking water, since they spread easily in the water network, constituting a health risk for human and animals. Currently in water treatments, Advanced Oxidative Processes (AOPs) have been increasing importance in the microbiological disinfection of water. The present study aimed to inactivate *C. albicans*, a commensal yeast species in Vertebrates that can cause disease, using AOPs. To achieve this objective, a powerful oxidant (hydrogen peroxide) was combined with UV radiation to promote the inactivation of *C. albicans*. Initially, the inactivation capacity of the H_2O_2 was assessed and it was verified that the application of 2.5 mM, 5 mM and 10 mM H_2O_2 reached a cell reduction of 3 log after 180, 360 and 300 min, respectively. Subsequently, the combination with UV-A radiation (λ = 365 nm) proved to be even more promising, as the H_2O_2 + UV-A system, using the same H_2O_2 concentrations, reached an inactivation of 3 log after 240, 180 and 60 min, respectively. These results support that UV-A radiation promotes the generation of hydroxyl radicals, which have a higher oxidation potential (2.8 eV) comparatively to the H_2O_2 (1.8 eV), responsible for the inactivation of *C. albicans* cells. Thus, UV-A/H_2O_2 process can reduce this microorganism in an aqueous matrix, avoiding potential hazard to human and animal health.

Keywords: AOPs; *C. albicans*; human health; microbiological disinfection; UV-A radiation

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1. Introduction

Approximately 1.7 million deaths per year worldwide, particularly in immunocompromised individuals, are caused by fungal infections [1]. Since the 1970s, infections caused by the genus *Candida* have steadily increased, due to the increased risk of opportunistic infections, the improvement of clinical procedures that identify fungi that cause nosocomial infections, as well as the development of antifungal resistance to prolonged exposure treatments [2,3].

In recent years, different countries and international organizations have legislated or published guidelines to regulate the reuse of treated wastewater [4–6]. These laws, or guidelines, consider the type of water reuse (urban, agricultural, industrial, recreational or environmental) and establish the maximum acceptable concentration of contaminants. The increasing demand for water and the scarcity of available water sources boosted the treatment and reuse of wastewaters [7]. The advanced oxidation processes (AOPs) generated reactive free radicals, the most important of which is the hydroxyl radical ($HO^\bullet$)

with an oxidizing power of 2.80 V, which react with non-selective organic compounds [8,9]. The HO• radical has numerous advantages, including not being toxic or corrosive, not generating waste, and having a very limited lifespan [10,11].

In this work it was decided to test the effect of H_2O_2 and H_2O_2/UV-A, as hydrogen peroxide has been widely used in the removal of low levels of pollutants from wastewater (chlorine, nitrites, sulphites, hypochlorites, etc.) and as disinfectant. As mentioned by [12] the H_2O_2/UV process initially occurs in the photolytic degradation of hydrogen peroxide, through the scission of a H_2O_2 molecule that produces two hydroxyl radicals.

The main objective of this research is to evaluate the inhibition potential of *C. albicans* in water samples, through the addition of a powerful oxidant (hydrogen peroxide) and combining the effect of this oxidant with UV-A radiation.

2. Materials and Methods

2.1. Microorganism and Reagents

For the inactivation study, cells of the strain *C. albicans* ATCC 90028 were grown on yeast malt extract agar (YMA). Hydrogen peroxide (H_2O_2) was purchased from Labkem. All reagents used were analytical grade. The inoculum was prepared from a culture of *C. albicans* with 48 h of growth, in which a loopful was suspended in 5.0 mL of sterilized saline solution (0.85% NaCl). The turbidity of the suspension was adjusted to 0.5 on the McFarland scale (1.5×10^8 CFU/mL).

2.2. UV-A LEDS

All the experiments were carried out in a self-designed lab-scale reactor with 110 cm^3 of capacity. The UV-A LEDs system was composed by 12 Indium Gallium Nitride (InGaN) LEDs lamps (Roithner APG2C1-365E LEDs) with a λ_{max} = 365 nm. Each UV-A LED has a nominal consumption of 1.4 W when the current is 350 mA with an optical power of 135 mW and an opening angle of 120°, eliminating shadow zones. The radiation was emitted in continuous mode for all the 12 UV-A LEDs being controlled by a power MOSFET in six different current settings, resulting in irradiance levels from 16 up to 85 W m^{-2} measured at 5 cm distance with a UVA Light Meter (Linshang model LS126A). The UV-A LEDs system was located 5 cm above the solution surface in a parallel position.

2.3. Experimental Procedure

The cells inactivation process was carried out in a 500 mL reactor. Figure 1 shows the experimental procedure used for *C. albicans* inactivation. The microbial suspension was added to the reactor with 200 mL of saline solution (0.85% NaCl), a solution that preserves yeast cell homeostasis to obtain a microorganism concentration of 10^5 colony forming units (CFU)/mL.

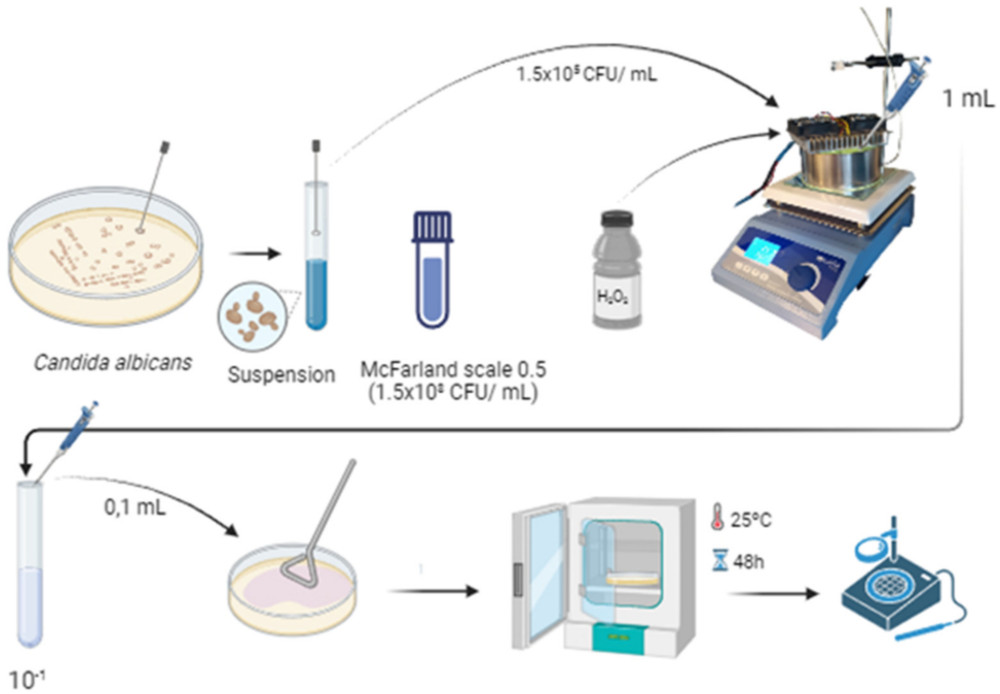

Figure 1. Experimental setup used for *C. albicans* inactivation by UV-A/H_2O_2.

Initially experiments with the addition of H_2O_2 were carried out. Three different concentrations of H_2O_2 were evaluated under the same experimental conditions: 2.5 mM, 5 mM and 10 mM. In a second round of experiments, UV-A LEDs were added to the H_2O_2 concentrations previously tested. During all the oxidation processes the reaction temperature was recorded and samples were taken along 360 min. Microbiological analyzes were performed by the spread plate method (Standard Method 9215C, [13]) after 10^{-1} dilution in tubes with saline solution (0.85%). After incubation at 25 °C for 48 h, colonies were quantified and the results expressed in log CFU/mL.

The entire procedure was performed under total aseptic conditions in a Biosafety BSL2 chamber, in order to avoid any type of contamination.

2.4. Statistical Analysis

The data were analyzed using the EXCEL 2011 and OriginLab 2019 (Northampton, MA, USA) software. The analysis involved descriptive statistics (means and standard-deviation), and the one-way ANOVA with the post-hoc Tukey test.

3. Results and Discussion

Table 1 shows the results of the inactivation of *C. albicans* after the application of H_2O_2 and the combination of H_2O_2 with UV-A LED radiation.

As shown in Table 1, the efficacy of H_2O_2 against *C. albicans* is time- and, in a less extent for the tested concentration range, dose-dependent. The ANOVA—One Way analysis did not found differences ($p > 0.05$) between both controls (without H_2O_2 versus without H_2O_2 + UV-A). As the concentration of H_2O_2 rises, oxidative stress increases, triggering responses from *C. albicans* cells. In fact, this species is well adapted to oxidative stress induced by macrophages that includes an enzymatic arsenal and morphological changes [14,15]. Also, in the presence of H_2O_2, *C. albicans* presents a rough and wrinkled surface, according to images obtained by SEM, indicating that H_2O_2 can damage cell wall and cell permeability [16]. In another study where peroxymonosulphate (PMS) combined with UV-A LED was used for inactivate *C. albicans*, the authors reported that due to its greater resilience to oxidative stress, higher doses (5 mM) were required [17]. *C. albicans* cells appear to be more resistant to H_2O_2, cationic stress and disinfectant agents than *C. auris* [18].

The application of H_2O_2 at a concentration of 2.5 mM or above achieved 3-log inactivation of *C. albicans* after 180 min of treatment (Table 1). Punctually, and at concentrations 5 mM and 10 mM, and after 180 min of treatment, was noted growth (corresponding to 1 UFC/plate) that which may indicate differences among *C. albicans* cells to oxidative stress caused by H_2O_2.

Table 1. *C. albicans* inactivation (log CFU/ mL) time dynamics by H_2O_2, alone and combined with UV-A ($\bar{x} \pm$ sd). n.d.—not detectable

.	H_2O_2 Log (CFU/mL)				H_2O_2 + UV-A Log (CFU/mL)			
Time (min)	**0 mM**	**2.5 mM**	**5 mM**	**10 mM**	**0 mM**	**2.5 mM**	**5 mM**	**10 mM**
1	3.1 ± 2.0	3.0 ± 0.0	2.6 ± 2.5	2.6 ± 0.0	3.1 ± 0.5	2.8 ± 1.0	2.4 ± 1.5	2.8 ± 3.5
30	3.1 ± 0.5	2.7 ±1.5	2.4 ± 0.5	2.6 ± 0.0	2.9 ± 5.5	2.6 ± 2.0	2.0 ± 0.0	2.7 ± 0.5
60	2.9 ± 1.0	2.7 ± 1.5	2.6 ± 2.0	2.4 ± 1.5	3.0 ± 1.5	2.6 ± 0.0	2.0 ± 0.0	n.d.
120	3.0 ± 0.5	2.2 ± 0.5	1.7 ± 0.5	2.6 ± 0.0	3.1 ± 0.5	2.7 ± 3.0	1.7 ± 0.5	n.d.
180	3.2 ± 0.0	n.d.	n.d.	n.d.	3.0 ± 0.0	1.7 ± 0.5	n.d.	n.d.
240	3.2 ± 1.0	n.d.	n.d.	1.7 ± 0.5	3.0 ± 3.5	n.d.	n.d.	n.d.
300	3.1 ± 2.5	n.d.	1.7 ± 0.5	n.d.	2.7 ± 0.5	n.d.	n.d.	n.d.
360	3.0 ± 2.0	n.d.	n.d.	n.d.	2.9 ± 2.0	n.d.	n.d.	n.d.

In general, the combination of H_2O_2 with UV-A LED radiation was more efficacious against *C. albicans* at higher H_2O_2 concentrations (5 and 10 mM $p < 0.05$). The highest microbial inactivation rate was achieved in 60 min using 10 mM of H_2O_2 combined with UV-A LED radiation, with a 3-log reduction and no detectable (re)growth afterwards. Contrary, the total inactivation with H_2O_2 alone was achieved much later (3-log at 300 min). Also, for lower H_2O_2 concentrations (2.5 and 5.0 mM) combined with UV-A, no regrowth or recovery was detected after 240 and 180 min, respectively. Therefore, the combination of H_2O_2 and UV-A radiation induced higher and faster *C. albicans* inactivation rates. Some authors claim that, when H_2O_2 is introduced into the process, the degree of inactivation of *C. albicans* with the effect of UV radiation tends to increase [19], a statement that is in line with the results obtained. The rate of photodecomposition of H_2O_2 determines the efficiency of the process depending on the intensity of UV radiation, as well as the nature of the impurities and concentrations [20–22]. These responses may explain the effect of H_2O_2 and the combined effect of H_2O_2 and UV-A on the inactivation rate of *C. albicans*.

Comparing the results obtained in both treatments with those of the respective controls, without the addition of H_2O_2, the results were satisfactory, as it appears that the inactivation of *C. albicans* cells occurred throughout the process.

4. Conclusions

The data obtained in this study draw attention to the importance of finding an effective procedure for disinfecting water and inactivating pathogenic microorganisms such as *C. albicans*. Based in these results, it can be concluded that (1) UV-A radiation enhances the conversion of H_2O_2, leading to higher production of hydroxyl radicals responsible for the inactivation of *C. albicans* cells; (2) H_2O_2/UV-A process can reduce this species in an aqueous matrix, avoiding potential hazard to human and animal health; (3) UV-A LED radiation is an attractive alternative to the use of conventional UV lamps in microbial inactivation processes, since LEDs are environmental friendly, have a low operating cost and high energy efficiency.

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References

1. Kainz, K.; Bauer, M.A.; Madeo, F.; Carmona-Gutierrez. D. Fungal infections in humans: The silent crisis. *Microb Cell* **2020**, *7*, 143–145.
2. Lewis, R.E.; Viale, P.; Kontoyiannis, D.P. The potential impact of antifungal drug resistance mechanisms on the host immune response to *Candida*. *Virulence* **2012**, *3*, 368–376. https://doi.org/10.4161/viru.20746.
3. Chin, V.K.; Lee, T.Y.; Rusliza, B.; Chong. P.P. Dissecting *Candida albicans* infection from the perspective of *C. albicans* virulence and omics approaches on host–pathogen interaction: A review. *Int. J. Mol. Sci.* **2016**, *18*, 1643.
4. *NP 4434:2005*; Norma Portuguesa sobre reutilização de águas residuais urbanas tratadas na rega. Instituto Português da Qualidade, Caparica, Portugal, 2005.
5. WHO. *Guidelines for the Safe Use of Wastewater, Excreta and Greywater*; World Health Organization: Geneve, Switzerland, 2006.
6. USEPA. *Guidelines for Water Reuse*; United States Environmental Protection Agency: Washington, DC, USA, 2012.
7. Rodríguez-Chueca, J.; Moreira, S.I.; Lucas, M.S.; Fernandes, J.R.; Tavares, T.B.; Sampaio, A.; Peres, J.A. Disinfection of simulated and real winery wastewater using sulphate radicals: Peroxymonosulphate/transition metal/UV-A LED oxidation. *J. Clean Prod*. **2017**, *149*, 805–817.
8. Ganiyu, S.O.; Sable, S.; El-din, M.G. Advanced oxidation processes for the degradation of dissolved organics in produced water: A review of process performance, degradation kinetics and pathway. *Chem. Eng. J.* **2022**, *429*, 132492. https://doi.org/10.1016/j.cej.2021.132492.
9. Jorge, N.; Teixeira, R.A.; Lucas, S.M.; Peres, A.J. Combined organic coagulants and photocatalytic processes for winery wastewater treatment. *J. Environ. Manag.* **2023**, *326*, 116819. https://doi.org/10.1016/j.jenvman.2022.116819.
10. Wang, J.; Chen, H. Catalytic ozonation for water and wastewater treatment: Recent advances and perspective. *Sci. Total Environ*. **2020**, *704*, 135249.
11. Dowd, K.O.; Pillai, S.C. Photo-Fenton disinfection at near neutral pH: Process, parameter optimization and recent advances. *J. Environ. Chem. Eng*. **2020**, *8*, 104063.
12. Baxendale, J.H.; Wilson, J.A. The photolysis of hydrogen peroxide at light intensities. *Trans. Faraday Soc*. **1957**, *57*, 344.
13. Eaton, A.D.; Clesceri, L.S.; Rice, E.W.; AE Greenberg, A.E.; Franson, M.A.H. *Standard Methods for Examining Water and Wastewater*; APA-AWWA-WEF: Washington, DC, USA, 2005.
14. Nasution, O.; Srinivasa, K.; Kim, M.; Kim, Y.J.; Kim, W.; Jeong, W.; Choi, W. Hydrogen peroxide induces hyphal differentiation in *Candida albicans*. *Eukaryot Cell*. **2008**, *7*, 2008–2011. https://doi.org/10.1128/EC.00105-08.
15. Miramon, P.; Dunker, C.; Kasper, L.; Jacobsen, I.D.; Barz, D.; Kurzai, O.; Hube, B. A family of glutathione peroxidases contributes to oxidative stress resistance in *Candida albicans*. *Med. Mycol*. **2014**, *52*, 223–239.
16. Li, Y.; Du, J.; Huang, S.; Wang, S.; Wang, Y.; Cai, Z.; Lei, L.; Huang, X. Hydrogen peroxide potentiates antimicrobial photodynamic therapy in eliminating *Candida albicans* and *Streptococcus mutans* dual-species biofilm from denture base. *Photodiagnosis Photodyn. Ther*. **2022**, *37*, 6. https://doi.org/10.1016/j.pdpdt.2021.102691.
17. Rodríguez-Chueca, J.; Silva, T.; Fernandes, J.R.; Lucas, M.S.; Puma, G.L.; Peres, J.A.; Sampaio, A. Inactivation of pathogenic microorganisms in freshwater using HSO_5^-/UV-A LED and HSO_5^-/M^{n+}/UV-A LED oxidation processes. *Water Res*. **2017**, *123*, 113–123.
18. Zatorska, B.; Moser, D.; Diab-Elschahawi, M.; Ebner, J.; Lusignani, L.S.; Presterl, E. The effectiveness of surface disinfectants and a micellic H_2O_2 based water disinfectant on *Candida auris*. *J. Mycol. Med.* **2021**, *31*, 5. https://doi.org/10.1016/j.mycmed.2021.101178.
19. Soboleva, N.M.; Saprykina, M.N.; Kosinova, V.N.; Nosonovich, A.A.; Goncharuk, V.V. Inactivation of *Candida albicans* in the Photo-Fenton System. *J. Water Chem Technol*. **2012**, *34*, 96–102.

20. Feurstein, O.; Moreinos, D.; Steinberg, D.J. Synergic antibacterial effect between visible light and hydrogen peroxide on *Streptococcus mutans*. *J. Antimicrob. Chemother*. **2006**, *57*, 872–876.
21. Rajala-Mustonen, R.L.; Toivola, P.S. Heinonen-Tanski, H. Effects of peracetic acid and UV irradiation on the inactivation of coliphages in wastewater. *Water Sci. Technol*. **1997**, *35*, 237–241.
22. Parkinson, A.; Barry, M.J.; Roddick, F.A.; Hobday, M.D. Preliminary toxicity assessment of water after treatment with UV-irradiation and UVC/H_2O_2. *Water Res.* **2001**, *35*, 3656–3664.

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