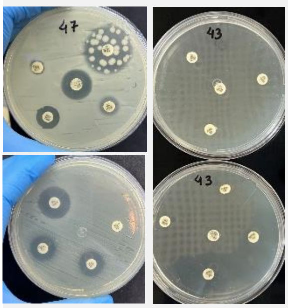
Coaction of Sulfate and Hydroxyl Radicals in Enhanced Solar Photo-Fenton: ARB Resistance Profile

ORAL
Ph.D. Aluno: N
Diário: NENHUM

V. Vilete[1], *P.B. Vilela*[1], *E.C. Machado*[2], *M.C.V.M. Starling*[1], *C.C. Amorim*[1]. *(1) Universidade Federal de Minas Gerais, Research Group on the Environmental Application of Advanced Oxidation Processes (GruPOA), Av. Pres. Antônio Carlos, 6627, Belo Horizonte - MG, Brazil, vilete@ufmg.br. (2) Universidade Federal de Minas Gerais, National Institute of Science and Technology in Sustainable Sewage Treatment Stations*



The pervasive presence of resistant microorganisms (*i.e.* antibiotic-resistant bacteria, ARB, which harbor antibiotic resistance genes, ARG) after conventional wastewater treatment may lead to environmental and public health risks. As a potential alternative for post-treatment, advanced oxidation processes are effective to remove and inactivate ARB. Nevertheless, some gaps in the mechanistic actions of these processes towards ARB and ARG removal remain unclear. This study aimed investigate the impact of enhanced solar photo-Fenton, using two oxidants (H_2O_2 and $S_2O_8^{2-}$) simultaneously, on the antibiotic-resistant bacteria susceptibility profile from a secondary effluent. To the best of our knowledge on this area of research, such investigations have not been previously explored or reported elsewhere.

Introduction

Solar photo-Fenton has undergone extensive investigation for the removal and inactivation of ARB and ARGs. However, investigations about the potential of using different oxidants/radicals simultaneously in homogeneous catalysts rarely occur [1]. Since solar photo-Fenton efficiency is influenced by the complex interplay between matrix constituents [2], this study aimed to investigate the impact of enhanced solar photo-Fenton (solar/Fe^{2+}/H_2O_2+$S_2O_8^{2-}$) as post-treatment of secondary WWTP effluent (WWTPE) on ARB profile.

Material and Methods

Wastewater was sampled from a conventional activated sludge system located in Belo Horizonte, Brazil. Enhanced solar photo-Fenton treatment (solar/Fe^{2+}/H_2O_2+$S_2O_8^{2-}$) was performed at circumneutral pH using intermittent iron additions (Table 1) and conducted under simulated solar spectrum with irradiance set at 268 W m^{-2} for 60 min. The spread plate method was used for the cultivation of total heterotrophic bacteria. Selected strains were isolated, replicated, and sub-cultured for identification by MALDI-TOF. For strains with a high secure species-level identification (≥2.000) antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion.

Table 1. Experimental Enhanced solar photo-Fenton set-up: Oxidant ratio and molar concentration of H_2O_2 and $S_2O_8^{2-}$; Time and molar concentration for Fe2+ intermittent additions

Test	Oxidant Ratio	H_2O_2	$S_2O_8^{2-}$	Fe^{2+}
1	1:1	0.750 mM	0.75 mM	t_0 → 0.25 mM
2	1.5:1	0.750 mM	0.5 mM	$t_{5,\ 10,\ 15\ min}$ → 0.08 mM
3	1:10	0.075 mM	0.75 mM	Total → 0.50 mM

The selection of target antibiotics (Table 2) was based on the EUCAST guideline [3] and the susceptibility profile was performed with nine antibiotics classes: aminoglycosides (AMK, GEN, and STR), β-lactams: carbapenems (ETP), cephalosporins (CAZ and LEX), and penicillins (AMC), fluoroquinolones and quinolones (CIP and NAL), macrolides (AZM and ERY), sulfonamides and trimethoprim (SXT), and tetracyclines (TET). The interpretation of results followed the CLSI guideline.

Table 2. Strains known to be resistant to target classes of antibiotics and antibiotics selected for susceptibility tests

Group	Expected resistance	Tested Antibiotics
Enterobacterales (i.e. Klebsiella spp., Enterobacter spp.)	*Benzylpenicillin, glycopeptides, lipoglycopeptides, fusidic acid, macrolides (with some exceptions - Azithromycin and erythromycin), lincosamides, streptogramins, rifampicin, and oxazolidinones*	*Amikacin (AMK, 30µg), Amoxillin-Clavulanic Acid (AMC, 30µg), Azitromycin (AZM, 15µg), Ceftazidime (CAZ, 30µg), Cephalexin (LEX, 30µg), Ciprofloxacin (CIP, 5µg), Ertapenem (ETP, 10µg), Erythromycin (ERY, 15µg), Gentamicin (GEN, 10µg), Nalidixic Acid (NAL, 30µg), Streptomycin (STR, 10µg), Tetracyclin (TET, 30µg), and Sulfazotrim (SXT, 25µg)*
Gram-positive bacteria (i.e. Bacillus spp.)	*Aztreonam, temocillin, polymyxin B, colistin and nalidixic acid*	*Erythromycin (ERY, 15µg), Gentamicin (GEN, 10µg), Tetracyclin (TET, 30µg), Vancomycin (VAN) and Sulfazotrim (SXT, 25µg)*

Results and Discussion

In enhanced solar photo-Fenton processes, the activation of the persulfate anion ($S_2O_8^{-2}$) simultaneously with the solar photo-Fenton components (*i.e.* solar radiation, Fe^{2+}, and H_2O_2) allows for the simultaneous formation of distinct oxidative radicals [4]. However, the observed synergistic effect relies significantly on the concentrations of oxidants. Unsatisfactory outcomes were reported to occur due to a potential mechanism of radical scavenging attributed to excessive persulfate concentration [5]. Therefore, this study explored different combinations of molar concentrations. Despite variations in the concentrations of oxidants, no significant variations were observed in pH, a critical factor for the application of photo-Fenton as a post-treatment for secondary effluents [6]. In addition, the activation of $S_2O_8^{2-}$ by Fe^{3+} may improve the degradation process by limiting $SO_4^{\bullet -}$ scavenging [5,7]. As result, ARB log removal was above 1.6. Regarding microbiome profile, MWWTPE showed a predominance of *Proteobacteria* species, mainly *Klebsiella* and *Escherichia* ssp. (Table 3). This profile changed after enhanced solar photo-Fenton treatments as *Bacillus* spp predominated. No bacterial growth was observed after experiments carried out at a molar ratio of 1:10, only after an incubation period of 24/48h for which *Bacillus* spp. was observed (Table 4)

Table 3. Antibiotic resistance profile of *Enterobacterales* isolates in MWWTPE and samples taken within 60 minutes (1.6 KJ L^{-1}) of solar/Fe^{2+}/H_2O_2+$S_2O_8^{2-}$, and after 24/48h of incubation. Color intensity indicates the resistance profile.

		Species	Enterobacterales Resistance Phenotype												
			ETP	LEX	CAZ	AMC	ERY	AZM	STR	AMK	GEN	CIP	NAL	TET	SXT
MWWTPE		*Klebsiella variicola*			3										
		Klebsiella pneumoniae					X	X	X					X	
		Escherichia coli													
Oxidant ratio (H_2O_2:$S_2O_8^{2-}$)	1:1	*Escherichia coli*													
	1.5:1	*Klebsiella pneumoniae*					X	X	X					X	
		Escherichia coli													
	1:10	*Klebsiella pneumoniae*					X	X	X					X	
		Escherichia coli													
		Enterobacter cloacae													

[a]"X" indicates strains with intrinsic resistance to target antibiotics.
[b] Resistant Intermediate Susceptible Resistant Parental|Susceptible Oral Intermediate Parental|Susceptible Oral

Among the strains isolated from MWWTPE, a notable degree of resistance to different antibiotics was observed including an *E. coli* showing multi-resistance (MDR) to seven different antibiotics (Table 3). Notably, *K. pneumoniae*, recognized for the propensity to MDR development, was identified with resistance to parental applications of cephalexin. Despite the high efficacy of enhanced solar photo-Fenton in eliminating ARB, results show that the MDR profile persisted in the identified strains. Furthermore, the identification of species exhibiting resistance profile to multiple antibiotics, previously absent in MWWTPE samples, including gram (+) bacteria (Table 4), underscores the importance of continued surveillance and targeted interventions in wastewater treatment processes.

Table 4. Profile of gram (+) ARB in treated samples

Oxidant ratio (H_2O_2:$S_2O_8^{2-}$)			Species	ERY	GEN	TET	VAN
	1:1	60'	*B. cereus*				
			B. subtilis				
		24h	*B. cereus*				
		48h	*B. cereus*				
	1.5:1	60'	*B. subtilis*				
			B. cereus				
		24h	*B. cereus*				
			B. cereus				
		48h	*B.cereus*				
			B. subtilis				
	1:10	24h	*B. cereus*				
			B. subtilis				
		48h	*B. cereus*				
			B. subtilis				

Noteworthy, bacterial regrowth was observed for all tested treatments, with prevalence of *Bacillus* spp. These strains exhibited a consistent resistance profile, particularly to erythromycin. Additionally, all identified strains demonstrated some level of resistance, even at an intermediate degree, to tetracycline. Despite the efficiency to eliminate ARB, the regrowth of bacteria, including MDR strains, indicates the partial removal and/or inactivation of these organisms. Persistence of MDR strains poses significant concerns for public health and environmental safety, addressing the need for different strategies to ensure the removal and the inactivation of antibiotic-resistant organisms [8,9].

Conclusions

This pioneering research provides valuable insights into the complex dynamics of enhanced solar photo-Fenton for wastewater treatment, shedding light on its effectiveness in mitigating AMR spread. Despite the fact that $S_2O_8^{2-}$ addition showed a great synergistic effect, other H_2O_2:$S_2O_8^{2-}$ molar ratios should be tested to improve the understanding of the mechanistic action of the radicals upon ARB.

Acknowledgments
The authors would like to thank the Brazilian institutions CAPES, CNPq, and FAPEMIG for their financial support.

References

[1] L. Rizzo, et al. *Sci. Total Environ.*, 447 (2013) 345.
[2] A. Lado Ribeiro, et al. *Chem. Eng. J.,* 363 (2019) 155.
[3] *Definitions of "Expected Phenotypes" Expected Phenotypes*, EUCAST, 2021.
[4] S. Wacławek, et al. *Chem. Eng. J.,* 330 (2015) 44.
[5] P.B. Vilela, et al. *Chem. Eng. J.,* 441 (2022) 136060.
[6] L. Rizzo, *Sci. Total Environ.*, 655 (2019) 986.
[7] Y. Wu, et al. *Chem. Eng. J.,* 451 (2023).
[8] C. Manaia, et al. *Environ. Int.*, 115 (2017) 312.
[9] D. Fatta-Kassinos, et al. *Environ. Sci. Pollut. Res.*, 22 (2015) 7183.