KPC-producing *Klebsiella pneumoniae* and *bla*$_{KPC}$ gene inactivation by heterogeneous photo-Fenton process mediated by mining residue

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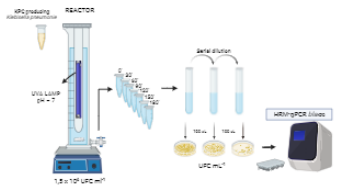

The Wastewaters Treatment Plants (WWTPs) are considered reservoirs of these pathogens, contributing to their dissemination into the environment. Therefore, to remove multi-resistant bacteria and, consequently, reduce environmental risks, it is necessary to implement alternative disinfection treatments. The objective of this study was to investigate the potential of mining residue in a heterogeneous photo-Fenton process with hydrogen peroxide and the presence of UVA light in removing multidrug-resistant *Klebsiella pneumoniae* and the *bla*$_{KPC}$ gene in synthetic and treated effluents. The process proved to be efficient in eliminating *K. pneumoniae* and reducing the number of *bla*$_{KPC}$ gene copies in treated effluents. The results of this study highlight the need to implement alternative technologies for disinfecting effluents, in order to contribute to reducing the spread of bacterial resistance in the environment.

Introduction

The Wastewaters Treatment Plants (WWTPs) play an important role in the spread of antimicrobial resistance in the environment (1). Conventional treatments at WWTPs are not efficient in disinfecting resistant bacteria in effluents, since after these treatments, pathogenic microorganisms can remain in the treated effluents and be disseminated into the environment (2). Because of this, it is necessary to implement advanced disinfection technologies in order to improve the quality of treated effluents and interrupt the cycle of bacterial resistance spreading throughout the environment (3). Advanced Oxidative Processes (AOPs) are efficient water and wastewater treatment technologies and may be a promising approach for pathogen inactivation (4). Different types of AOPs can be used to increase efficiency in the disinfection of effluents, and the use of iron minerals has been used as catalysts and source of iron ions in heterogeneous photo-Fenton processes (5). Although some studies have reported the efficiency of using iron ores as catalysts in the inactivation of bacteria (6), more studies are needed to evaluate the efficiency and economic viability of this method in different multidrug-resistant bacteria and their resistance genes. In this context, the present study aims to evaluate the efficiency of heterogeneous photo-Fenton processes using mining waste in removing multidrug-resistant *Klebsiella pneumoniae* and its resistance *bla*$_{KPC}$ gene in different aqueous matrices.

Material and Methods

To evaluate the efficiency in the degradation of *K. pneumoniae* XDR and the *bla*$_{KPC}$ gene, 20 mg.L^{-1} of hydrogen peroxide (H_2O_2) concentration and 277 mg.L^{-1} (7) of the catalyst were used in 0.85% saline solution, synthetic and treated effluent. The bacterial strain was inoculated in 500 mL of matrices at a final density of 10^4-10^6 CFU mL^{-1}, and were magnetically stirred during the test by constant temperature around 25 °C and at neutral pH (approximately 7). For each experiment time, 0, 30, 60, 90, 120, 150 and 180 min, aliquots were removed, diluted and plated on BHI agar for subsequent quantification of colonies in CFU mL^{-1} and also for monitoring the concentration of hydrogen peroxide based on colorimetric methods. Physicochemical analyzes of the effluents were carried out using colorimetric analysis using spectrophotometer measurements. After counting the colonies, the DNA of the isolates was extracted, and the presence of the *bla*$_{KPC}$ gene in the samples was detected by qPCR on Applied Biosystems QuantStudio 7 Flex Real-Time PCR System.

Results and Discussion

The results of the blanks and the experiment on exposure to UVA light are presented in Figure 1, with figure (A) representing bacterial quantification and (B) the consumption of H_2O_2 over the experiment times, which was represented by final concentration (C) by the initial concentration (C_0). The experiment carried out in the saline demonstrates that in 90 min there was a drop in bacterial growth of 1.1 log and in 120 min the bacteria was completely inactivated, showing a reduction of 4.4 logs (fig. 1A) and an H_2O_2 consumption of 24% (fig. 1B). For the synthetic effluent, there is a 1.1 log drop in the initial population in 180 min (fig. 1A), with a consumption of 12% (fig. 1B), and in the treated effluent there was bacterial inactivation in 150 min with a drop in 5.1 logs (fig. 1A), with a consumption of 20% H_2O_2 (fig. 1B). The UVA/H_2O_2/catalyst process was harmed in the synthetic effluent, due to the presence of ions present, such as phosphate (PO_4^{-3}) (4.7 mg.L^{-1}), which were absent in the treated effluent, making the efficiency degradation in the

synthetic effluent was lower than in the treated effluent and in the saline solution. The complexity of treated effluents from WWTPs reduces the efficiency of the photo-Fenton process in water disinfection. The hydroxyl radicals generated in the process are not selective, they can also react with organic compounds and inorganic anions in the matrix, which function as scavengers of these radicals, being able to form less reactive species and affecting the degradation efficiency of bacteria (8). In the photo-Fenton reaction, inorganic compounds present in water, such as sulfates, nitrates and chlorides, can react with iron, or with H_2O_2, limiting the amount of hydroxyl radical generated by the photo-Fenton reaction and reducing its ability to oxidize bacteria and matter. organic (9). In particular, the presence of phosphates can further impair the efficiency of the process, as these compounds sequester iron more efficiently, forming an insoluble complex and precipitating it, in addition to eliminating hydroxyl radicals, thus slowing down the speed of the reaction. Therefore, it is expected that secondary effluents do not contain phosphates, however when the effluent contains phosphate in its composition, even in a few $mg.L^{-1}$, it is necessary to add more iron to the solution (9). In the analysis of the quantification of the bla_{KPC} gene throughout the experiment in saline, there was an absence of cycle threshold (CT) value at 90 min, demonstrating that there was a possible degradation of the gene in saline solution before inactivation of the bacteria in 120 min. For the treated effluent, there was an absence of CT within 150 min, the moment of bacterial inactivation, that is, the gene was not degraded before the bacteria was inactivated, with the possibility of the gene still being present in the effluent after bacterial inactivation. No gene absence was observed in the synthetic effluent. The results of this experiment show that the UVA/H_2O_2/catalyst process showed better efficiency in removing the bla_{KPC} gene in saline solution. A study evaluated the impact on the bla_{KPC} gene, present in a *K. pneumoniae* strain, using a homogeneous photo-Fenton process at pH 7, during and after inactivation of the microorganism at concentrations of 5 $mg.L^{-1}$ Fe^{2+} and 50 $mg.L^{-1}$ of H_2O_2. It was demonstrated that the gene remained present in the total sample even after complete inactivation of *K. pneumoniae*, obtained in 120 min. However, at longer times, there was a reduction in the number of copies of the gene, indicating the need for more treatment time to ensure complete degradation of bla_{KPC}. Unlike the present study, in which the same gene was degraded in 90 min and the bacteria in 120 min in the process with catalyst at a lower concentration (10).

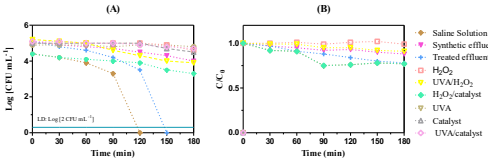

Figure 1. (A) Quantification of *Klebsiella pneumoniae* under UVA/H_2O_2/catalyst treatment in 0.85% saline solution, synthetic effluent and treated effluent and blanks: H_2O_2; UVA/H_2O_2; H_2O_2/catalyst; UVA; Catalyst and UVA/catalyst over times 0, 30, 60, 90, 120, 150 and 180 min. (B) H_2O_2 consumption. LD: Limit of detection. [H_2O_2] = 20 $mg.L^{-1}$; [catalyst] = 277 $mg.L^{-1}$; accumulated radiation = 6.80 mW cm^{-2}; pH: 7.

Conclusions

The results of this study indicate the effectiveness of mining residue in removing multi-resistant *K. pneumoniae* in a heterogeneous photo-Fenton process in treated effluent and an indication of bla_{KPC} gene degradation, under the experimental conditions tested. These results highlight the need to implement alternative technologies for disinfecting effluents, in order to contribute to reducing the spread of bacterial resistance in the environment.

Acknowledgments

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