Poultry slaughterhouse wastewater treatment using UV/H_2O_2 process: Effects on metaplasmidome and removal of antibiotic resistance genes

SHORT-ORAL

Ph.D. Student: Y

Journal: Chemical Engineering Journal

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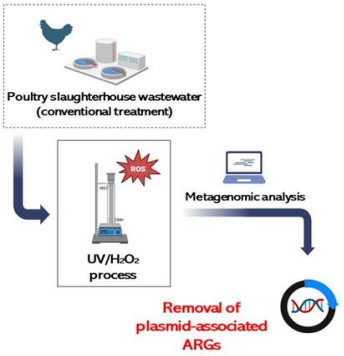

This study investigated the impact of UV/H_2O_2 treatment on poultry slaughterhouse wastewater, focusing on its effects on the bacterial community and plasmid-linked antibiotic resistance genes (ARGs) through a metagenomic approach. Results revealed a dominant presence of Enterobacterales in the metaplasmidome of both the influent and effluent from the wastewater treatment plant (WWTP), whereas UV/H_2O_2 treatment resulted in Burkholderiales prevalence in treated samples. The influent presented 102 GRAs and conventional WWTP treatment exhibited limited efficacy in ARGs mitigation (reduction of 7.8%). The UV/H_2O_2 process (H_2O2 concentration of 0.01 mol/L), at pH 3 and 7, led to a reduction of ARGs by 91.5% and 90.4% to the effluent, respectively. Results demonstrates UV/H_2O_2 treatment as a potent strategy for reduction of pathogen-associated plasmids and for substantially reducing ARGs within the effluent metaplasmidome.

Introduction

Slaughterhouse Wastewater Treatment Plants (WWTPS) are pointed as hotspots for dissemination of ARGs, mainly mediated by plasmids [1]. This poses significant risks to public health and the environment, as treated effluents are discharged into the aquatic ecosystems of receiving water bodies, which can be used for anthropogenic and agricultural activities [2].

Advanced Oxidation Processes (AOPs), such as UV/H_2O_2, show promise in degrading pollutants and inactivating ARGs in wastewater [2, 3]. Despite this, data on the use of AOPs in treating slaughterhouse wastewater to eliminate ARGs is limited. Therefore, this study conducted an unprecedented investigation into the impact of the UV/H_2O_2 process on the mobile resistome of poultry slaughterhouse effluents using a metagenomic sequencing approach.

Material and Methods

Samples were collected from both the influent and effluent of a poultry slaughterhouse's WWTP located in São José do Vale do Rio Preto, Rio de Janeiro, Brazil. Detection of antimicrobials was performed using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). The effluent samples underwent treatment with UV/H_2O_2 in a benchtop reactor equipped with a 6W lamp emitting UV-A radiation. Reactions were carried out for 60 minutes using UV/H_2O_2 and only H_2O_2 (in the dark) at varied oxidizing agent concentrations (0.005, 0.01, 0.05, and 0.15 mol/L) and different pH levels (3, 5, 7, 9). Treated samples were concentrated by filtration through 0.22 µm membranes for the extraction of total RNA and DNA, and plasmid DNA (pDNA). The RNA and DNA were used to quantification of the markers *rrs* 16S rRNA, *uidA*, *sul1*, and *int1*, performed using RT-qPCR and qPCR, respectively. Shotgun sequencing of pDNA from selected samples was carried out on MiSeq Illumina platform. Sequences were assembled and trimmed using the ATLAS and compared against the PLSDB, RGI/CARD, and Kaiju databases.

Results and Discussion

Residues of sulfonamides (sulfamethoxazole), macrolides (erythromycin), and fluoroquinolones (enrofloxacin, ciprofloxacin, and ofloxacin) were detected in WWTP influent. Sulfamethoxazole, enrofloxacin, and ofloxacin residues were still present in the treated effluent.

Enhanced efficiency in the UV/H_2O_2 process for the removal of bacterial community (*rrs* 16S rRNA) and viable *Escherichia coli* (*uidA*) was achieved using a H_2O_2 concentration of 0.001 mol/L at pH 3, in which 2.60 and 1.57 logs of target genes were removed, respectively. Reduction of 1.31-0.82 logs for *sul1* and *int1* genes were also observed. At pH 7, reductions of less than one log unit were observed for both *rrs* 16S rRNA and *uidA*. Nevertheless, this pH value was selected for metaplasmidome sequencing, as well as pH 3, considering its relevance to the applicability of the process.

Enterobacterales were predominant in the metaplasmidome of both WWTP influent and effluent (Figure 1). This order hosts various human and animal pathogens, positioning AMR as a significant factor that identifies Enterobacteriales as priority

pathogens according to the WHO [4]. Samples treated with UV/H_2O_2 exhibited a prevalence of Burkhoderiales, a bacterial order linked to phytopathogens and emerging pathogens that has been reported as resistant to disinfection treatments due to its biofilm-forming capability [5].

The influent displayed considerable diversity, with 102 ARGs associated with the metaplasmidome, conferring resistance to 14 clinically relevant pharmacological classes in both human and veterinary medicine. Prevalence quinolone resistance genes were observed (Figure 2). Quinolones are utilized to treat infections caused by *Salmonella* spp., significant avian pathogens identified in the influent. Resistance to this class is notably relevant in poultry farming contexts [6]. Following WWTP treatment, reduction of 7.8% in ARGs quantities was observed, predominantly associated with efflux pumps (resistance to multiple drugs). This fact may be linked to increased environmental oxidative stress [7].

UV/H_2O_2 treatments ([H_2O_2]= 0.01 mol/L), at pH 3 and 7, achieved a reduction in ARGs by 91.5% and 90.4%, respectively, compared to the effluent.

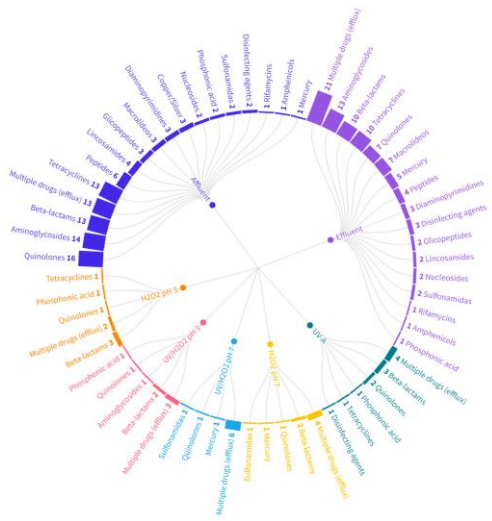

Figure 2. Absolute abundance of antimicrobial resistance genes detected in each sample.

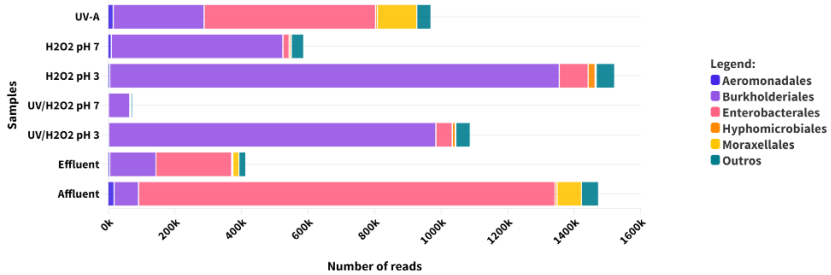

Figure 1. Absolute abundance of antimicrobial resistance genes detected in each sample.

Conclusions

Slaughterhouse wastewater harbor plasmid-linked ARGs associated with clinically relevant bacterial order, such as Enterobacteriales. WWTP exhibited inefficient removal of these elements, emphasizing the role of slaughterhouse effluents as agents in environment AMR dissemination. UV/H_2O_2 treatment, at pH 3 e 7, stands out in removing plasmids and reducing ARGs in the effluent metaplasmidome.

Acknowledgments

This study is supported by Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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