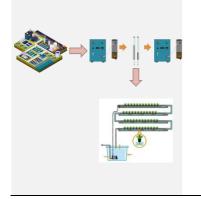
# Advanced Multiple Barrier System for urban wastewater reuse: ORAL disinfection and toxicity

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The study investigates the effectiveness of an innovative multiple barriers (MB) system: O<sub>3</sub>-BAC-O<sub>3</sub>, for secondary effluent reuse. By combining ozone (O<sub>3</sub>) treatment with biological activated carbon (BAC) filtration, the research aimed to enhance pathogen inactivationl, reduce toxicity, and improve disinfection efficiency. Results indicate significant reductions in total coliforms and Escherichia coli within a short treatment duration of 90 minutes, highlighting the efficacy of the applied protocols in reducing contamination. Additionally, microbiological the studv demonstrates negligible impact on seed germination and reduced toxicity to Artemia salina post-treatment, underscoring the system's benefits for both plant and aquatic life. These findings emphasize the importance of comprehensive treatment approaches in managing wastewater and safeguarding environmental health.

### Introduction

The advanced oxidation process (AOP) emerges as a promising solution to mitigate water contamination. This approach harnesses the hydroxyl radical's remarkable oxidative power to enhance ozone's oxidative action ( $O_3$ ). AOPs offer a range of significant advantages, including robust oxidizing power, complete mineralization of pollutants and oxidation of inorganic species, versatility, and operational efficiency, as well as the ability to decompose oxidizing reagents into final products with reduced environmental impact [1].

However, existing AOP methods have several disadvantages that need to be addressed. AOPs based on  $O_3$ +UV consume a lot of energy and installation is not economical when the UV source is used for large volumes of wastewater. On the other hand,  $O_3$ +H<sub>2</sub>O<sub>2</sub>-based AOPs require excessive injection of H<sub>2</sub>O<sub>2</sub> to generate the hydroxyl radical (•OH) and inhibit microbial growth. Additionally, photocatalytic AOPs using photocatalysts such as TiO<sub>2</sub> are inactive under visible light and exhibit low luminous efficiency [2].

In this scenario, a viable and promising strategy, which has been increasingly drawing attention in the scientific community for reuse purposes, is the concept of multiple barriers (MB). The MB system proposes the implementation of a series of barriers that will prevent the passage of microbial pathogens and reduce the levels of harmful compounds, utilizing the principle of incorporating redundant steps [3].

Thus, this study aimed to investigate the effectiveness of  $O_3$ -BAC- $O_3$  for polishing samples of secondary effluents. To achieve this,  $O_3$ , BAC,  $O_3$ /BAC, and  $O_3$ /BAC/ $O_3$  were tested in the MB system, evaluating germination rate, toxicity removal, and disinfection efficiency during treatment.

## Material and Methods

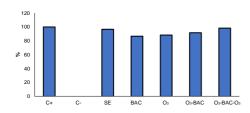
Samples of effluent were collected from the outlet of the secondary treatment unit of the Água da Serra wastewater treatment plant (WWTP) located in the SP City of Limeira (Brazil). The main physicochemical characteristics of the effluent were: The experiments were carried out in batch mode. using one liter of sample for the treatment consisting of O<sub>3</sub>/BAC/O<sub>3</sub>, being subjected to each stage of the treatment used. An ozone generator produced ozone with a maximum 12 g  $O_3$  h<sup>-1</sup> capacity. The reaction was initiated (stage 1), with 4 mgL<sup>-1</sup> of ozone. The ozone concentration in the aqueous phase was measured by indirect iodometry. Once exposed to the first dose of  $O_{3}$ , the effluent sample was removed from the reactor and filtered through BAC (stage 2) using a diaphragm pump. The sample was then returned to the reactor for a second dose (4 mgL<sup>-1</sup>) of  $O_3$  (stage 3), thus completing the  $O_3$ -BAC-O<sub>3</sub> treatment.

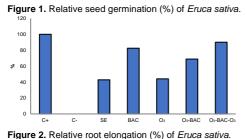
The effect of each step separately was evaluated: only ozonation, only filtration by BAC,  $O_3$ /BAC and  $O_3$ /BAC/ $O_3$ .

The disinfection of the samples was assessed by: bacterial inactivation, measured by the Colilert® test, which quantifies the presence of *Escherichia coli* (*E. coli*) and total Coliforms in the effluent with a chromogenic reagent. Helminth egg counts, according to zinc sulphate flotation technique [4]. The toxicity of the samples was assessed by the mortality rate of *Artemia salina* and tests with arugula seed.

### **Results and Discussion**

Figures 1 and 2 show the results of arugula seeds, figure 3 the mortality rate of *Artemia salina*, and figure 4 the disinfection of: (a) total coliforms and *E. coli* and (b): Helminth eggs.





100 90 70 60 40 30 20 0 Control SE BAC O<sub>3</sub> O<sub>2</sub>-BAC O<sub>2</sub>-BAC-O<sub>3</sub>

Figure 3. Mortality rates of Artemia salina.

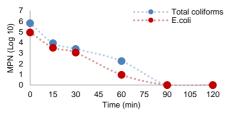


Figure 4. Inactivation of total coliforms and *E. coli* in secondary effluent sample.

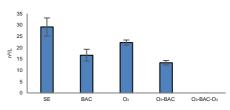


Figure 5. Number of helminth eggs/L in each stage of multibarrier treatment in secondary effluent.

Our analysis of the effluent indicates that it didn't harm seed germination. However, we did observe negative effects on root growth, although these effects decreased by the end of our treatment process. This suggests that while the initial exposure may have been harmful, our treatment methods were effective in reducing its impact.

Furthermore, we noticed a decrease in the mortality rate of Artemia salina after completing the treatment. This positive outcome suggests that our efforts reduced harm to plants and benefited aquatic organisms.

The significance of our findings is evident in "Figure 4 and 5," which illustrates the impressive efficacy of our disinfection process in reducing microbiological contamination, including total coliforms and *E. coli* and helmints eggs. The graph demonstrates that a short treatment duration of just 90 minutes significantly reduced the presence of these harmful pathogens, showcasing the efficiency of our treatment protocols.

In summary, our results suggest that while the effluent initially posed risks to both plant and aquatic life, our multi-barrier treatment approach effectively mitigated these risks, leading to improved environmental outcomes. These findings highlight the importance of comprehensive treatment processes in managing wastewater and protecting ecological health.

#### Conclusions

Regarding arugula seed germination, it can be concluded that pollutant concentrations were at levels that did not cause obvious toxic effects on seed germination. In the root's relative elongation, a growth decrease is observed when secondary effluent is used. In the analysis of A. salina, it was concluded that when samples were subjected to treatment with BAC and the combined treatments  $O_3$ -BAC and  $O_3$ -BAC- $O_3$ , the toxicity of the effluent was reduced, showing the importance of this stage of treatment. It is also concluded that the time of 90 minutes was sufficient to remove almost all microbiological contamination. Therefore, the treatment proved to be efficient in disinfecting the secondary effluent.

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2