IWA 17th SWWS AND 9th ROS
10th–14th November, 2024
Curitiba-Brazil

Removal of *Enterococcus*, *Staphylococcus*, total coliforms, and *E. coli* from sewage by tubular vertical column PBR under light and dark conditions

Pompei, C.M.E.*, Belasco, G. *, Mondim, G. **, Ruas, G. *, and Silva, G.H.R.*.

*Department of Civil and Environmental Engineering, São Paulo State University (UNESP), School of Engineering Bauru, Bauru, SP, Brazil, 17033-360.
**Department of Biology, São Paulo State University (UNESP), Bauru, SP, Brazil.

Highlights:

·The four pathogens evaluated were better removed by tubular-PBR during light phase;

·The total coliforms removals had statistical difference between dark and light samplings;

·*Escherichia coli* was the pathogen better removed by the tubular- PBR.

Keywords: pathogens removal; photoperiod; microalgae; sewage.

INTRODUCTION

In underdeveloped or developing countries, poor health infrastructure is a major issue. Infectious diseases remain a significant cause of morbidity and mortality. One possible solution to treat sanitary wastewater is microalgae-based systems. Among these, the tubular system is one of the most promising ones commercially. It has a large specific surface area, high continuous cultivation capacity, flexible system control, high photosynthetic efficiency, and low fluctuations in photosynthetic efficience during the day, according to a study by Cui et al. (2021).

Considering the increasing requirement to close production cycles and improve resource reuse, the sanitation model needs to shift to a circular one, in which wastewater treatment plants will become a more energy-efficient industry, able to generate marketable products rather than wastes (Silva et al., 2019).

This study aimed to evaluate the effect of different times of collections of samples into the photoperiod (light and dark phases) on the removal of four pathogens (*Enterococcus* sp., *Staphylococcus* sp., total coliforms, and *E. coli*) from a real sanitary sewage, in a pilot scale closed tubular vertical column photobioreactor.

METHODOLOGY

A vertical tubular photobioreactors (T-PBR) was operated for seven days reaching a steady-state. It was operated in semi-continuous mode, using real domestic sanitary sewage (collected after grating) from the Wastewater Treatment Plant Candeia, Bauru, Brazil. The T-PBR was constructed using acrylic tube, with a total capacity of 8.5 L and a total volume used of 5.5 L in each. The dimensions were: 5 mm thick, 104 mm internal diameter and 1000 mm high. It was operated in a controlled temperature room (24 °C), with a photoperiod of 12/12h. The artificial lighting was generated using cold light LED reflectors, with 900W of power and a luminous flux of 270 µm of photons m^{-2} s^{-1}. The samples (200 mL) were taken 2 times per day (light and dark periods).

the international water association · ABES · UFPR UNIVERSIDADE FEDERAL DO PARANÁ · inct ETEs Sustentáveis · SANEPAR

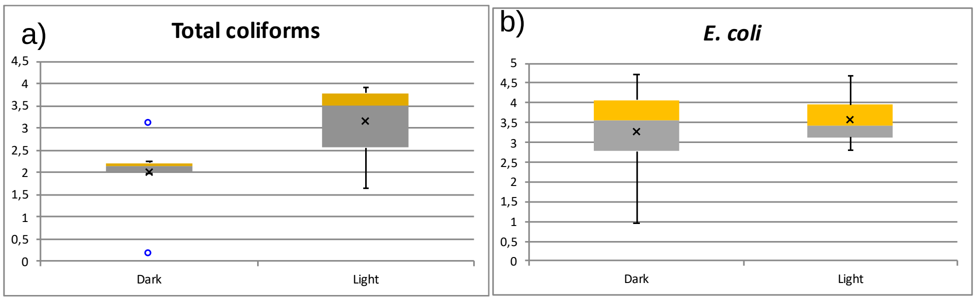
Two methods were used to quantify the colonies forming unit (CFU) of the pathogens present in the sanitary sewage samples. The pour plate technique was used for *Enterococcus* sp., and the filter membrane technique was used for *Staphylococcus* sp., total coliforms, and *E. coli*., all according to previous tests done for the pathogen's concentration in the sanitary sewage samples (APHA, 2022). The identification and quantification of *Enterococcus* sp. was done by using M-Enterococcus Agar Base (Himedia, India), while the Baird Parker Agar Base (Himedia, India) was used for *Staphylococcus* sp. Total coliforms and *E. coli* were identified using Chromocult®. The efficacy of the T-PBR and the impact of light and dark periods were compared using Student's T-Test (Microsoft Excel® 2021 with Real Statistics Resource Pack software (Release 7.6) (Zaiontz, 2020) and R® (R Core Team R, 2020). The data obtained were logarithmized. The study also considered the environmental operating conditions such as pH, dissolved oxygen and turbidity.

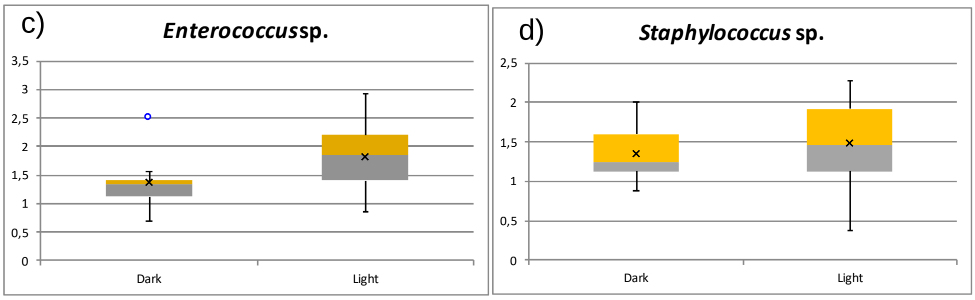
RESULTS AND CONCLUSIONS

During the dark phase, the average pH value was 9.42±0.24, temperature was 24.7±0.35, dissolved oxygen (DO) was 6.94±0.50, turbidity was 248.25±21.92; whereas during the light phase, the pH value was 11.00±0.05, temperature was 28.4±0.87, DO was 6.4±0.65, and turbidity was 223.5±29.69. These results show that the vertical tubular PBR has an increase in pH value and DO during the light phase showing the photosynthetic activity of microalgae (Posadas et al., 2015).

The four pathogens evaluated in this study was better removed by the T-PBR during the light phase. During this study, corroborating with other studies that evaluated the removal efficiency of total coliforms and *E. coli* in PBR, but in a natural environment without parameters control, the *E. coli* had better removal (light phase = 3.57 Log-Re, dark phase = 3.29 Log-Re) than total coliforms (light phase = 3.16 Log-Re, dark phase = 2.00 Log-Re) (Pompei et al., 2024). However, compared with a PBR in natural conditions (Pompei et al., 2024), the T-PBR operated inside the lab with controlled conditions had better results.Considering the removals of *Staphylococcus* sp. (light phase = 1.48 Log-Re, dark phase = 1.34 Log-Re), the *Staphylococcus* sp. had similar removal reported by Ruas et al., (2022a): 1.8 to 2.0 log units. The *Enterococcus* sp. had removals during the light phase = 1.82 Log-Re, and dark phase = 1.37 Log-Re. Some authors describe that the removal of *Enterococcus faecalis* is strongly linked to the DO concentration (Davies-Colley, 2000; Ruas et al., 2022b). The total coliforms removal presented a significant statistical difference (p = 0.013) between dark and light phases, whereas for the other three pathogens, the difference was not significant statistically (p > 0.05).

Figure 1: Box plot of the log-removals at T-PBR for: a) Total coliforms, b) *E. coli*, c) *Enterococcus* sp., d) *Staphylococcus* sp.





Acknowledgments: The authors would like to acknowledge the support obtained from the following Brazilian institutions: PROEC- São Paulo State University (2023 /1693 - Projeto Minerva), Fundação de Amparo à Pesquisa do Estado de São Paulo (Proc. FAPESP n°: 2022/07475-3), National Council for Scientific and Technological Development (CNPq processes 308663/2021-7), and Coordination for the Improvement of Higher Education Personnel (CAPES - 88887.716731/2022-00).

References

APHA, AWWA, WEF. Standard methods for the examination of water and wastewater, 22nd ed. American Public Health Association, Washington, DC, 2022.

Cui, X., Yang, J., Cui, M., Zhang, W., & Zhao, J. (2021). Comparative experiments of two novel tubular photobioreactors with an inner aerated tube for microalgal cultivation: Enhanced mass transfer and improved biomass yield. *Algal Research*, *58*, 102364.

Davis-Colley, R. J., Donnison, A. M., & Speed, D. J. (2000). Towards a mechanistic understanding of pond disinfection. Water Science and Technology, 42(10-11), 149-158.

Pompei, C. M. E., Ruas, G., Bolzani, H. R., Fernandes, L. M., & da Silva, G. H. R. (2024). The influence of light intensities and micropollutants on the removal of total coliforms and E. coli from wastewater in a flat-panel photobioreactor. Environmental Pollution, 123935.

Posadas, E., del Mar Morales, M., Gomez, C., Acién, F. G., & Muñoz, R. (2015). Influence of pH and CO2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chemical Engineering Journal, 265, 239-248.

Ruas, G., Farias, S. L., dos Reis, B. A., Serejo, M. L., da Silva, G. H. R., & Boncz, M. Á. (2022a). Removal of Clostridium perfringens and Staphylococcus spp. in Microalgal–Bacterial Systems: Influence of Microalgal Inoculum and CO2/O2 Addition. Water, 15(1), 5.

Ruas, G., Serejo, M. L., Farias, S. L., Scarcelli, P., & Boncz, M. Á. (2022b). Removal of pathogens from domestic wastewater by microalgal-bacterial systems under different cultivation conditions. International Journal of Environmental Science and Technology, 19(10), 10177-10188.

Silva, Gustavo HR, et al. "Feasibility of closing nutrient cycles from black water by microalgae-based technology." *Algal Research* 44 (2019): 101715.

Zaiontz, 2020 C. Zaiontz The Data Analysis for This Paper Was Generated Using the Real Statistics Resource Pack Software (Release 7.6) (2020).