

# Modeling dynamical removal of pathogens in sanitary wastewater treatment using microalgae in photobioreactors with support medium

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Highlights:

- Model accurately predicts removal of pathogens *Staphylococcus* spp. and *Enterococcus* spp.
- Support medium influence on removal rate was comparable to the main mechanisms
- pH was the primary removal mechanism for *Staphylococcus* spp., while light intensity for *Enterococcus* spp.

Keywords: Sanitary wastewater; Pathogen removal; Photobioreactor; Modeling; Microalgae

## **INTRODUCTION**

In recent years, there has been a growing focus on adopting circular economy principles and the concept of closing the loop in sanitary wastewater treatment (Hernández-Chover et al., 2023). Sanitary wastewater, in addition to containing various pollutants, has numerous pathogens, which can cause a number of diseases, if not properly treated (USEPA, 2002). Effectively removing these pathogens is essential to achieve the circular economy in the sanitation sector. The utilization of microalgae-based photobioreactors has emerged as a suitable alternative to meet this target (Pompei et al., 2024). Furthermore, incorporating a support medium facilitates the formation of biofilms, which can aid in biomass harvesting (Patwardhan et al. 2022).

One crucial step, when optimizing such processes, is the development of mechanistic models capable of quantifying the effects of operation parameters on the process output. Models can then be used to operate the reactor optimally, or to assess if the removal of pathogens will be adequate given the local conditions. Only recently, a model describing the removal of *E. coli* in a high rate algal pond has been developed (Chambonniere et al., 2023). In this work, we extend this model to include two significant genera of pathogenic bacteria, *Staphylococcus* and *Enterococcus*, while also assessing the influence of the support medium on their removal. Our calibrated model quantifies the principal mechanisms of removal for each pathogen, by leveraging experimental data obtained from two photobioreactors, with and without support medium.

## **METHODOLOGY**

Experimental data used to calibrate the model were obtained from experiments conducted using two vertical tubular photobioreactors operated in semi-continuous mode (5 mm thick, 104 mm internal diameter and 1000 mm height; total volume used of 5.5 L in each), one with ( $R_s$ ) and one without ( $R_0$ ) support medium. A support medium, mini Biobob®, filled one-third of the height of reactor  $R_s$ . This













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medium consists of a cylindrical-shaped support structure, with a diameter of 10 mm and a height of 18 mm, filled with polyurethane foam. Hydraulic retention time (HRT) was 5 days. The reactors were fed daily at 14h with sanitary wastewater (from a Wastewater Treatment Plant in Bauru-SP, collected after grating), operating with light-dark cycles (12h:12h), light intensity of  $260 \,\mu mol/(m^2 s)$  from 9 a.m. to 9 p.m.. Room temperature was controlled at 24 °C using an air conditioner. Measurements of pH, dissolved oxygen (DO), optical density, reactor temperature, and turbidity were performed for the reactor's effluent and for the wastewater used as inoculum at 8h, and 14h, before reactor feeding. Total suspended solids (TSS) was measured every 7 days. The concentrations of *Staphylococcus* spp. and *Enterococcus* spp. were also measured at 8h, and 14h. *Enterococcus* spp. was done by using M-Enterococcus Agar Base (Himedia, India) according to pour plate technique (APHA, 2022), while the Baird Parker Agar Base (Himedia, India) was used for *Staphylococcus* spp., using the membrane technique (APHA, 2022). We adapted a model for the removal of *Escherichia coli* in High Rate Algal Ponds (Chambonniere et al. , 2023) by including a term representing the removal of bacteria due to the presence of the support medium. The dynamics of pathogen removal are described by an ordinary differential equation:

$$\frac{dC}{dt} = -k_{removal}C(t)$$

where *C* is the concentration of pathogens.  $k_{\text{removal}}$  is given by the sum of four terms quantifying the removal of the support medium, pH, temperature (dark decay), and light intensity:

$$k_{removal} = k_{support} + k_{pH} f_{pH}(pH,T) + k_{T24} f_{T}(T) + \alpha f_{light}(I,TSS)$$

Here,  $k_{support}$ ,  $k_{pH}$ ,  $k_{T24}$  and  $\alpha$  are the model parameters calibrated using data collected over time of colony-forming units (CFU) for *Staphylococcus* spp. and *Enterococcus* spp. in both reactors, R<sub>0</sub> and R<sub>s</sub>.  $k_{support}$  represents the removal rate of pathogens due to their adhesion to the support structure. The functions  $f_{pH}$  and  $f_T$  increase exponentially with pH and temperature, while  $f_{light}$  is linearly dependent on the light intensity and decreases with TSS. These functions are described in detail in Chambonniere et al. (2023).

Parameter calibration was performed using the Differential Evolution algorithm, which minimized the absolute error between experimentally measured and model-predicted pathogen concentrations. Parameters for each pathogen were calibrated separately. Data from the first four days of pathogen measurements were used for parameter calibration, while the subsequent days were reserved for model validation.

#### **RESULTS AND CONCLUSIONS**

Figure 1 illustrates the results of model calibration and validation for both pathogens with and without the support medium. The model was able to well predict removal rates for all cases. The value of  $k_{\text{support}}$  calibrated for *Enterococcus* spp. was 1.37 day<sup>-1</sup>, while for *Staphylococcus* spp. it was 3.17 day<sup>-1</sup>. For both pathogens, the support medium had a considerable impact on removal rates. On average, the contribution of the support medium in removal rates was 77% ±10% for *Staphylococcus* spp., whereas for *Enterococcus* spp. it was 19%, during the light phase, and increased to 98% during the dark phase.













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It is expected that  $k_{support}$  would depend on the number of pathogens already attached to the support structure, reaching zero when the structure has no remaining surface area for new cells to attach. Representing this effect would require adding more parameters to the model. However, even without this adjustment, the model still performed well. The parameter could be transformed into a function to determine when the structure no longer impacts pathogen removal, signaling when it could be removed from the reactor for cleaning.

The model identified that the main removal mechanism for *Staphylococcus* spp. was pH (contribution up to 33%), while for *Enterococcus* spp., light intensity was the dominant factor (contributing up to 81%). The model successfully identified the primary removal mechanisms for *Staphylococcus* spp. and *Enterococcus* spp., highlighting the significant role of the support medium in pathogen removal. These insights have practical implications for optimizing wastewater treatment processes, allowing for the customization of system conditions based on the specific pathogens present in the wastewater.

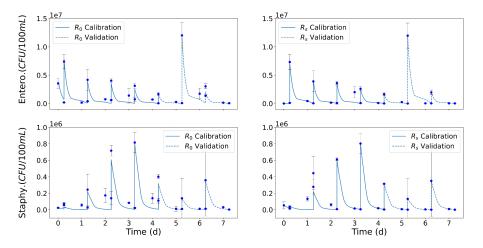


Figure 1 - Model results and experimental data of the dynamics of *Staphylococcus* spp. and *Enterococcus* spp. concentrations (CFU/100mL) in a photobioreactor with supported medium ( $R_s$ ) and without ( $R_0$ ). Solid points represent experimental data, with two standard deviations error bars. Solid and dashed lines show, respectively, the results of model calibration and validation.













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