



Microalgae-Bacteria System for Surfactant and Pathogen Removal: Assessing Potentials and Prospects for Domestic Sewage Treatment

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AND PROSPECTS FOR DOMESTIC SEWAGE TREATMENT**



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**UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL
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Thesis presented for obtaining a PhD degree in the Post-graduate Program in Environmental Technologies of the Federal University of Mato Grosso do Sul, area of concentration: Environmental Sanitation and Water Resources.

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DEDICATION

*To my daughter, Ana Clara,
You are my greatest inspiration, and the reason I never gave up.
This work is for you.*

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First and foremost, and always, I thank God. Even without deserving it, He has constantly shown His love and care, and this achievement is, without a doubt, yet another great blessing in my life.

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EPIGRAPH

“Que a importância de uma coisa não se mede com fita métrica nem com balanças nem barômetros etc. Que a importância de uma coisa há que ser medida pelo encantamento que a coisa produza em nós.”

— **Manoel de Barros**

“The importance of a thing cannot be measured with a measuring tape, scales, or barometers, etc. The importance of a thing must be measured by the enchantment it produces within us.”

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RESUMO

Farias, S.L. (2025). *Sistema de microalgas-bactérias para remoção de surfactantes e patógenos: avaliando potenciais e perspectivas para tratamento de esgoto doméstico*. Campo Grande, 2025, 115p. Tese (Doutorado) - Universidade Federal de Mato Grosso do Sul, Brasil.

A aplicação de sistemas microalgas-bactérias para o tratamento de esgoto doméstico representa uma estratégia promissora para aliar remoção de poluentes e produção de biomassa, alinhando-se aos princípios da economia circular e das soluções baseadas na natureza. Entretanto, a consolidação dessa tecnologia em escala real depende do entendimento e da otimização de parâmetros operacionais que garantam desempenho estável e previsível. A escolha de surfactantes aniônicos e patógenos como contaminantes modelos justifica-se pela sua relevância ambiental e sanitária. Assim, avaliar a remoção desses poluentes permite verificar a robustez da tecnologia frente aos desafios do saneamento. Nesta tese, investigaram-se variáveis críticas como regime de alimentação, tempo de detenção hidráulica, mistura, pH e suplementação de carbono em reatores do tipo High Rate Algal Pond (HRAP), operados em condições tropicais. Também foram avaliadas diferentes condições de cultivo, ausência e presença de luz; ajuste de pH; suplementação com CO₂; adição de fonte orgânica de carbono; sistemas combinados anóxico-aeróbios; e aeração atmosférica, bem como os mecanismos envolvidos na remoção de surfactantes e patógenos. Os resultados demonstraram que a associação de microalgas e bactérias alcançou as maiores eficiências na remoção de surfactantes aniônicos, atingindo até 97% sob condições com aeração atmosférica, pH controlado e suplementação de CO₂. Os regimes semi-contínuos favoreceram maior produtividade de biomassa e concentração de sólidos suspensos totais, enquanto os contínuos apresentaram maior estabilidade e remoção de nutrientes, mas com menor inativação de *E. coli*. A operação intermitente dos HRAPs manteve elevadas remoções de surfactantes e patógenos, com menor demanda energética e sem comprometer a qualidade da biomassa. Essas evidências reforçam a importância do ajuste integrado dos parâmetros operacionais para aprimorar a eficiência e a sustentabilidade dos HRAPs, oferecendo subsídios para seu dimensionamento e aplicação em larga escala em condições tropicais.

PALAVRAS-CHAVE: Desinfecção; Economia circular; HRAP; Surfactantes aniônicos; Tratamento de esgoto doméstico.

ABSTRACT

Farias, S.L. (2025). *Microalgae-bacteria system for surfactant and pathogen removal: assessing potentials and prospects for domestic sewage treatment. Campo Grande, 2025, 1115p. Thesis – Federal University of Mato Grosso do Sul, Brazil.*

The application of microalgae–bacteria systems for domestic wastewater treatment represents a promising strategy to simultaneously achieve pollutant removal and biomass production, aligning with the principles of the circular economy and nature-based solutions. However, consolidating this technology at full scale depends on understanding and optimizing operational parameters that ensure stable and predictable performance. The choice of anionic surfactants and pathogens as model contaminants is justified by their environmental and sanitary relevance. Thus, evaluating the removal of these pollutants allows assessing the robustness of the technology when addressing real sanitation challenges. In this thesis, critical variables were investigated, including feeding and mixing regime, hydraulic retention time, pH and carbon supplementation, in High Rate Algal Ponds (HRAPs) operated under tropical conditions. Additionally, different cultivation conditions were assessed; presence and absence of light; pH adjustment; CO₂ supplementation; addition of an organic carbon source; combined anoxic–aerobic systems; and atmospheric aeration, as well as the mechanisms involved in the removal of surfactants and pathogens. The results showed that the combination of microalgae and bacteria achieved the highest efficiencies in anionic surfactant removal, reaching up to 97% under conditions with atmospheric aeration, controlled pH, and CO₂ supplementation. Semi-continuous feeding regimes promoted higher biomass productivity and total suspended solids concentrations, whereas continuous regimes favored system stability and nutrient removal, albeit with lower *Escherichia coli* inactivation. Intermittent mixing in HRAPs maintained high surfactant and pathogen removal efficiencies, reduced energy demand, and did not compromise biomass quality. These findings reinforce the importance of integrated operational adjustments to improve the efficiency and sustainability of HRAPs, providing guidance for their design and large-scale application in tropical settings.

KEYWORDS: Disinfection; Circular economy; HRAP; Anionic surfactants; Domestic wastewater treatment

GENERAL INTRODUCTION

The increasing demand for environmentally sustainable and economically viable wastewater treatment has intensified the search for alternatives to conventional, energy-intensive technologies. Nature-based solutions have gained prominence in this context, as they integrate ecological processes to achieve pollutant removal while promoting resource recovery. Adequate sewage treatment is a key element in preventing waterborne diseases and protecting public health (Cohen-Shacham, Andrade, et al., 2019). Among these approaches, microalgae–bacteria systems stand out as a promising strategy for domestic sewage treatment, offering the dual benefit of contaminant mitigation and the generation of biomass that can be converted into bioenergy and value-added bioproducts (Vassalle, Ferrer, et al., 2023; Fernández, Reis, et al., 2021).

Microalgae-based processes have demonstrated remarkable versatility, with proven potential to remove nutrients, heavy metals, micropollutants, surfactants, and pathogenic microorganisms from wastewater (Torres-Franco, Passos, et al., 2021; Hasan, Muhamad, et al., 2023; Shahid, Malik, et al., 2020). Their implementation in open configurations, particularly High-Rate Algal Ponds (HRAPs), is especially advantageous in warm climates, where high solar irradiation and elevated temperatures enhance photosynthetic activity, enabling low-energy operation and alignment with circular economy principles (Mohsenpour, Hennige, et al., 2021; Barboza-Rodríguez, Rodríguez-Jasso, et al., 2024; Arashiro, Montero, et al., 2018).

Despite these advantages, the scaling-up of microalgae–bacteria systems faces significant challenges. Their performance is governed by a complex interplay of operational parameters—including hydraulic retention time (HRT), water depth, pH, mixing regime, and carbon source supplementation—that directly affect light distribution, nutrient availability, microbial community structure, and ultimately, pollutant removal efficiency and biomass productivity (Velásquez-Orta, Yáñez-Noguez, et al., 2024; Sutherland and Ralph, 2020). Variability in these parameters often leads to inconsistent treatment performance, limiting the predictability and reliability required for regulatory acceptance and full-scale implementation (Robles, Capson-Tojo, et al., 2020).

Among the pollutants of concern, surfactants and pathogenic microorganisms are particularly relevant due to their environmental persistence and public health risks. Global surfactant production exceeds 15 million tons per year, with linear alkylbenzene sulfonates (LAS) representing one of the most widely used anionic surfactants in household detergents

(Ramprasad and Philip, 2016). After use, surfactants and their by-products are mainly discharged into sewage systems and can reach surface waters through effluent or contaminate soils via sludge disposal. Their environmental behavior and toxicity depend on the final concentration in aquatic systems and the persistence or bioaccumulation of degradation by-products (Lechuga, Fernández-Serrano, et al., 2016; Macedo, Okada, et al., 2015).

Wastewater is a major reservoir for diverse human pathogens, particularly bacteria from the intestinal microbiota that are shed in feces, posing significant health risks when effluents are discharged into recreational waters or reused without adequate treatment (Chahal, Akker, Van Den, et al., 2016). Most waterborne pathogens reach drinking water sources and recreational waters due to human and animal waste, poor sanitation infrastructure, and insufficient treatment processes (Aw and Rose, 2012). According to the United Nations, in 2022, 43% of global population lacked safely managed sanitation, including 1.9 billion with basic services, 570 million with limited services, 545 million with unimproved services, contributing to the spread of waterborne diseases (WHO and UNICEF, 2023). In low- and middle-income countries, diarrheal diseases remain among the leading yet largely preventable causes of death in children, and expanding access to effective wastewater treatment is a key element for reducing pathogen exposure and preventing these avoidable deaths (Merid, Alem, et al., 2023).

Microalgae–bacteria systems offer a promising approach for the simultaneous removal of these contaminants, based on complex interactions between both groups. Microalgae can enhance surfactant degradation by supplying oxygen and organic exudates that stimulate bacterial activity (Chan, Khoo, et al., 2022; Serejo, Farias, et al., 2020), while mechanisms such as high pH, solar irradiation, and biological interactions contribute to pathogen inactivation (Berney et al., 2006; Arias et al., 2018). Among the various configurations that explore these synergies, high rate algal ponds (HRAPs) stand out for their low cost and operational simplicity. Investigating how operational parameters affect contaminant removal in HRAPs is essential to optimize performance and support their large-scale application as a sustainable wastewater treatment technology.

Nevertheless, there is still a lack of systematic knowledge about the optimal operational conditions that ensure both treatment efficiency and biomass productivity, which limits the consolidation of HRAPs as a reliable and scalable wastewater treatment technology. This thesis is based on the hypothesis that the performance of microalgae–bacteria systems can be significantly improved by optimizing parameters such as cultivation arrangement, feeding

mode, and mixing regime, thereby enhancing surfactant and pathogen removal while sustaining biomass productivity. By systematically evaluating these conditions, this work aims to provide evidence-based operational guidelines to support the large-scale implementation of High-Rate Algal Ponds (HRAPs) in tropical regions.

OBJECTIVES

General Objectives

Investigation of critical operational parameters in microalgae-bacteria systems for the treatment of domestic wastewater, aiming at optimizing system performance and substantiating the feasibility of large-scale application, using surfactant and pathogen removal as key indicators of treatment efficiencies.

Specific Objectives

- Evaluate different cultivation conditions (isolated microalgae, isolated bacteria, and microalgae-bacteria consortia), as well as operational factors (light, pH, CO₂ supplementation, addition of organic carbon, anoxic-aerobic systems, and atmospheric aeration) regarding the removal of anionic surfactants.
- Investigate the effect of feeding regimes (continuous and semi-continuous) on the removal of organic matter, nutrients, surfactants, and pathogens, as well as on biomass productivity and settleability.
- Analyze the impact of mixing regimes (continuous and intermittent) in HRAP reactors operated under tropical conditions, considering removal efficiency, biomass quality, and energy consumption.
- Identify the mechanisms involved in the removal of surfactants and pathogens under the different operational scenarios studied.
- Propose operational guidelines to optimize the performance of HRAPs at full scale, integrating treatment efficiency and biomass valorization potential.

Organization of this Thesis

This thesis is organized into five chapters, each structured to progressively advance from theoretical foundations to experimental investigations and, finally, to the integration of findings:

- **Chapter 1** reviews the state-of-the-art in microalgae cultivation systems for wastewater treatment. It presents key concepts related to photobioreactor types, use as secondary or tertiary treatment, and species selection. The chapter also outlines technical, economic, and regulatory barriers to scale-up and highlights recommendations for large-scale implementation. In this sense, it serves as the theoretical framework of the thesis, providing the necessary background for understanding the subsequent experimental chapters.
- **Chapter 2** presents comparative batch-scale experiments conducted under controlled conditions, aiming to guide the design of subsequent outdoor experiments. It investigates the best operational and cultivation configurations for surfactant removal, while also clarifying the specific role of each microorganism in the treatment process and the underlying removal mechanisms.
- **Chapter 3** focuses on the influence of feeding regimes (continuous and semi-continuous) on the removal of surfactants and pathogens. It also analyzes correlations between environmental variables (e.g., pH, light) and removal mechanisms, while assessing the implications for biomass productivity. This chapter builds on the findings of Chapter 2 by advancing from static to dynamic conditions, thereby providing insights into how operational mode influences treatment stability and efficiency.
- **Chapter 4** analyzes the effect of continuous and intermittent mixing regimes on treatment efficiency, biomass quality, and energy demand in HRAP reactors operated under tropical conditions. By linking hydraulic operation to performance and sustainability, this chapter further refines the evaluation of key parameters critical for large-scale feasibility.
- **Chapter 5** integrates the results obtained from all experimental chapters, discussing their implications for the design and operation of HRAPs in tropical scenarios. It consolidates the evidence gathered to propose operational guidelines, directly addressing the central hypothesis of the thesis and outlining perspectives for full-scale application.

Together, these chapters establish a logical progression from conceptual foundations to applied experimentation, ensuring that each stage contributes to testing the central hypothesis: that optimizing operational conditions in microalgae–bacteria systems

enhances contaminant removal while sustaining biomass productivity, thus supporting the consolidation of HRAPs as a scalable wastewater treatment technology.

REFERENCES

- Arashiro, L. T., Montero, N., Ferrer, I., Acién, F. G., Gómez, C., and Garfí, M. (2018) Life cycle assessment of high rate algal ponds for wastewater treatment and resource recovery. *Science of the Total Environment*, **622–623**, 1118–1130. [online] <https://doi.org/10.1016/j.scitotenv.2017.12.051>.
- Aw, T. G. and Rose, J. B. (2012) Detection of pathogens in water: From phylochips to qPCR to pyrosequencing. *Current Opinion in Biotechnology*, **23**(3), 422–430. [online] <http://dx.doi.org/10.1016/j.copbio.2011.11.016>.
- Barboza-Rodríguez, R., Rodríguez-Jasso, R. M., Rosero-Chasoy, G., Rosales Aguado, M. L., and Ruiz, H. A. (2024) Photobioreactor configurations in cultivating microalgae biomass for biorefinery. *Bioresource Technology*, **394**(December 2023).
- Chahal, C., Akker, Van Den, B., Young, F., Franco, C., Blackbeard, J., and Monis, P. (2016) “Pathogen and Particle Associations in Wastewater: Significance and Implications for Treatment and Disinfection Processes” in *Advances in Applied Microbiology*, 63–119.
- Chan, S. S., Khoo, K. S., Chew, K. W., Ling, T. C., and Show, P. L. (2022) Recent advances biodegradation and biosorption of organic compounds from wastewater: Microalgae-bacteria consortium - A review. *Bioresource Technology*, **344**(PA), 126159. [online] <https://doi.org/10.1016/j.biortech.2021.126159>.
- Cohen-Shacham, E., Andrade, A., Dalton, J., Dudley, N., Jones, M., Kumar, C., Maginnis, S., Maynard, S., Nelson, C. R., Renaud, F. G., Welling, R., and Walters, G. (2019) Core principles for successfully implementing and upscaling Nature-based Solutions. *Environmental Science and Policy*, **98**(June 2018), 20–29. [online] <https://doi.org/10.1016/j.envsci.2019.04.014>.
- Fernández, F. G. A., Reis, A., Wijffels, R. H., Barbosa, M., Verdelho, V., and Llamas, B. (2021) The role of microalgae in the bioeconomy. *New Biotechnology*, **61**, 99–107.
- Hasan, H. A., Muhamad, M. H., Ji, B., Nazairi, N. A., Jiat, K. W., Sim, S. I. S. W. A., and Poh, A. F. M. S. (2023) Revolutionizing wastewater treatment with microalgae: Unveiling resource recovery, mechanisms, challenges, and future possibilities. *Ecological Engineering*, **197**(June), 107117. [online] <https://doi.org/10.1016/j.ecoleng.2023.107117>.
- Lechuga, M., Fernández-Serrano, M., Jurado, E., Núñez-Olea, J., and Ríos, F. (2016) Acute toxicity of anionic and non-ionic surfactants to aquatic organisms. *Ecotoxicology and Environmental Safety*, **125**, 1–8. [online] <http://dx.doi.org/10.1016/j.ecoenv.2015.11.027>.
- Macedo, T. Z., Okada, D. Y., Delforno, T. P., Braga, J. K., Silva, E. L., and Varesche, M. B. A. (2015) The comparative advantages of ethanol and sucrose as co-substrates in the degradation of an anionic surfactant: microbial community selection. *Bioprocess and Biosystems Engineering*, **38**(10).
- Merid, M. W., Alem, A. Z., Chilot, D., Belay, D. G., Kibret, A. A., Asratie, M. H., Shibabaw, Y. Y., and Aragaw, F. M. (2023) Impact of access to improved water and sanitation on diarrhea reduction among rural under-five children in low and middle-income countries:

- a propensity score matched analysis. *Tropical Medicine and Health*, **51**(1). [online] <https://doi.org/10.1186/s41182-023-00525-9>.
- Mohsenpour, S. F., Hennige, S., Willoughby, N., Adeloye, A., and Gutierrez, T. (2021) Integrating microalgae into wastewater treatment: A review. *Science of the Total Environment*, **752**(September 2020), 142168. [online] <https://doi.org/10.1016/j.scitotenv.2020.142168>.
- Ramprasad, C. and Philip, L. (2016) Surfactants and personal care products removal in pilot scale horizontal and vertical flow constructed wetlands while treating greywater. *Chemical Engineering Journal*, **284**, 458–468. [online] <http://dx.doi.org/10.1016/j.cej.2015.08.092>.
- Robles, Á., Capson-Tojo, G., Galès, A., Ruano, M. V., Sialve, B., Ferrer, J., and Steyer, J. P. (2020) Microalgae-bacteria consortia in high-rate ponds for treating urban wastewater: Elucidating the key state indicators under dynamic conditions. *Journal of Environmental Management*, **261**(January).
- Serejo, M. L., Farias, S. L., Ruas, G., Paulo, P. L., and Boncz, M. A. (2020) Surfactant removal and biomass production in a microalgal-bacterial process: effect of feeding regime. *Water Science and Technology*, **82**(6), 1176–1183.
- Shahid, A., Malik, S., Zhu, H., Xu, J., Nawaz, M. Z., Nawaz, S., Asraful Alam, M., and Mehmood, M. A. (2020) Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Science of the Total Environment*, **704**, 135303. [online] <https://doi.org/10.1016/j.scitotenv.2019.135303>.
- Sutherland, D. L. and Ralph, P. J. (2020) 15 years of research on wastewater treatment high rate algal ponds in New Zealand: discoveries and future directions. *New Zealand Journal of Botany*, **0**(0), 1–24. [online] <https://doi.org/10.1080/0028825X.2020.1756860>.
- Torres-Franco, A., Passos, F., Figueredo, C., Mota, C., and Muñoz, R. (2021) Current advances in microalgae-based treatment of high-strength wastewaters: challenges and opportunities to enhance wastewater treatment performance. *Reviews in Environmental Science and Biotechnology*, **20**(1), 209–235.
- Vassalle, L., Ferrer, I., Passos, F., Filho, C. R. M., and Garfí, M. (2023) Nature-based solutions for wastewater treatment and bioenergy recovery: A comparative Life Cycle Assessment. *Science of the Total Environment*, **880**(April).
- Velásquez-Orta, S. B., Yáñez-Noguez, I., Ramírez, I. M., and Ledesma, M. T. O. (2024) Pilot-scale microalgae cultivation and wastewater treatment using high-rate ponds: a meta-analysis. *Environmental Science and Pollution Research*, **31**(34), 46994–47021. [online] <https://doi.org/10.1007/s11356-024-34000-7>.
- WHO and UNICEF (2023) *Progress on household drinking water, sanitation and hygiene 2000-2022. Special focus on gender*, New York. [online] <https://washdata.org>.

CHAPTER 1 :

WASTEWATER TREATMENT IN MICROALGAE CULTIVATION SYSTEMS: RECOMMENDATIONS FOR LARGE-SCALE APPLICATION¹

ABSTRACT

The cultivation of microalgae, prized for their versatile applications, from biofuel to pharmaceuticals, involves selecting cultivation systems like open ponds or closed photobioreactors, considering factors such as application, space and scale. This chapter explores the cultivation of microalgae using (pretreated) domestic effluent as the culture medium in High-Rate Algal Pond (HRAP) reactors. Optimization of environmental and operational parameters is crucial for increasing the technology readiness level (TRL) of microalgae-based systems. Pilot systems tested variations in feeding regime, hydraulic retention time (HRT), light intensity and CO₂ addition. Under the conditions tested in a tropical climate with real primary and secondary wastewater, the main suggestions for process scalability are: operation in semi-continuous mode, HRT of 5 to 7 days, cultivation without shading, and addition of as much CO₂ as possible, always aiming for the highest biomass productivity while optimizing the removal of pathogens and other emerging pollutants. The system presents significant efficiency in reducing pollutant load and mitigating eutrophication with less need for treatment area, as well as the potential for nutrient recovery for agriculture. The conditions tested guide the applicability of using this technology on a large scale, positioning it as an important tool for bioeconomy advancement.

A short history of microalgae-based wastewater treatment

The use of microalgae for wastewater treatment goes back several decades; shortly after the first oil crisis in 1973, and based on earlier ideas of Oswald based on complex interactions between both groups, (1960) the National Renewable Energy Laboratory (NREL) started the “Aquatic Species” research project aimed at identifying biological processes capable of producing large amounts of vegetable oils, to be used as an alternative biofuel. One of the conclusions was that microalgae cultivation could provide a technically viable alternative source for transportation fuels, but in order to keep costs low, domestic wastewater, rather

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than fertilizers, should be used to supply the nutrients necessary for the biomass to grow. Experiments with High Rate Algal Ponds were initiated in California and Hawaii, and later on in Roswell-NM, showing very promising results (Sheehan, Dunahay, et al., 1998). In the following decades, in which very low petroleum prices inhibited economic viability of alternatives, these experiences were almost forgotten, but since the beginning of the 2000s, as a result of increasing oil prices, but also because of the increasing urgency to limit global warming, the cultivation of oil-rich microalgae using wastewater became a hot topic again (Wijffels and Barbosa, 2010; Chisti, 2007). Since those early days, a lot has been done, and because of cost concerns, currently the focus of research on microalgae cultivation in wastewater has shifted from optimizing production of triglycerides and biodiesel to actually treating the wastewater, and the production of interesting products from these algae, which can be much more than only algae-oil, has turned into a secondary, albeit very interesting, objective (Leite and Daniel, 2020). Following up on the results of the Aquatic Species program, the approach is often to run the algae-based processes close to a facility that produces CO₂, like a fermentor, an anaerobic digester, or even a gas-fired electric power plant: additional carbon is necessary for algal biomass production, as treated sewage has a carbon deficit, when considering its C, N and P contents and comparing these with the Redfield ratio (C:N:P = 106:16:1), a typical ratio for algal biomass composition often used as a starting point for studies and calculations (Rogers, Rosenberg, et al., 2014; Dammak, Fersi, et al., 2023). Such CO₂-supply is not essential though; in the absence of a supply of CO₂, the algae will use atmospheric CO₂ introduced by the mixing of the HRAP with a paddlewheel.

The growing importance of the use of microalgae in wastewater treatment can be explained when looking at various factors. In the first place the increasing necessity of nutrient removal. Biological wastewater treatment processes are designed to remove organic matter, in order to avoid receiving waters to become anaerobic and devoid of life. But activated sludge processes, and especially anaerobic digestion, generally do not remove significant amounts of nutrients (Chernicharo, 2007). Nutrient removal is becoming increasingly necessary, not only to avoid eutrophication of receiving water bodies, but even more so to move on to a circular economy, in which materials are not wasted (creating problems elsewhere), but recovered and reused (Manthos, Koutra, et al., 2022). In contrast to the bacteria normally used in wastewater treatment, microalgae need relatively large amounts of nutrients, which will accumulate in the produced biomass, and, when separating the algae, can be recovered, permitting reuse when using algae or derived products as fertilizers (Yang, van Lier, et al., 2022; Calicioglu and

Demirer, 2015). Also, microalgae are capable of absorbing heavy metals, and thus much higher removal efficiencies of heavy metals can be achieved then when only using bacterial processes (Muñoz and Guieysse, 2006). And in the third place, microalgae are capable of limiting the number of pathogens present in the effluent, for various reasons, like the variations in pH and oxygen level they induce, but also because of the release of some antibacterial compounds (Muñoz and Guieysse, 2006).

Actually, there are three main lines of research on the use of microalgae in wastewater treatment, which will be discussed in separate sections below. In the first line of work, an algae-based process is used for treatment of a secondary effluent, often domestic sewage that has been treated in a UASB reactor (anaerobic digester), but this may also be the secondary effluent of an activated sludge process. This way, the emphasis is on polishing the secondary effluent: removing nutrients, pathogens and emerging contaminants, and the biogas produced in the UASB reactor can be upgraded by the algae-based process, which benefits from the CO₂ present in the biogas (Rodero, Lebrero, et al., 2019; Scarcelli, Ruas, et al., 2021).

In a second, more recent line of work, an algae reactor, often a HRAP-type reactor, is fed with primary sewage, and the mixed culture present in the reactor contains a significant amount of activated sludge, whilst algae growth may be limited by the reduced light penetration in the medium. In this case, the emphasis is on removing organic matter, nutrients and pathogens (Ruas, Farias, et al., 2020a; Park, Craggs, et al., 2011; Farias, Ruas, et al., 2023). Depending on the situation, the general composition of the algae cultivation medium (the (pretreated) wastewater), will vary greatly, as shown in Table 1-1 below. As sewage characteristics depend heavily on the economic and cultural situation, but also on factors such as water availability and the use of kitchen grinders, the actual characteristics of a local domestic wastewater may be outside the ranges presented here (Metcalf & Eddy, Burton, et al., 2014).

Table 1-1- Characteristics of primary and secondary domestic sewage- Upflow anaerobic sludge blanket (UASB) and activated sludge reactors (Dammak, Fersi, et al., 2023; Metcalf & Eddy, Burton, et al., 2014; Von Sperling, 2007).

Parameter	Primary treatment	Secondary treatment	
		Effluent UASB	Effluent act. sludge
Total Suspended Solids (TSS) (mg L ⁻¹)	120–400	60-100	10-30
Chemical Oxygen Demand (COD) (mg L ⁻¹)	250–800	200-280	50-100
Total Organic Carbon (TOC) (mg L ⁻¹)	80-260	100-130	20-40
Ammonia (mg L ⁻¹)	12-45	>15	1-10
Total Kjeldahl Nitrogen (TNK) (mg L ⁻¹)	30-70	40-50	10-20
Total nitrogen (TN) (mg L ⁻¹)	20-70	>20	15-35
Total Phosphorus (TP) (mg L ⁻¹)	4-11	8-12	1-5

Important aspects of algae-based wastewater treatment processes

Types of photobioreactors

As stated in the introduction, microalgae can be cultivated in two fundamentally different types of systems: open and closed photobioreactors (Barboza-Rodríguez, Rodríguez-Jasso, et al., 2024), but nowadays there are also hybrid systems (Li, Liu, et al., 2019). The choice of the best reactor design for a specific situation is made on the basis of a cost-benefit analysis, taking into consideration the desired efficiency and treatment requirements.

Open photobioreactors are the most common design used for domestic wastewater treatment, because they are simple to install, operate and maintain, and provide an efficient removal of organic matter and nutrients (Muñoz, Teresa, et al., 2006). Among the open photobioreactor designs available, the High Rate Algal Pond (HRAP) stands out for operating in such a way that autotrophic and heterotrophic metabolisms coexist and support each other. The HRAP, originally developed by Oswald in the 1950s (Oswald and Gotaas, 1957; Oswald and Golueke, 1960; Nurdogan and Oswald, 1995; Sutherland and Ralph, 2020) is an obvious choice because of its simplicity and low operational costs (Barboza-Rodríguez, Rodríguez-Jasso, et al., 2024). This system comprises shallow and open ponds to ensure light penetration, and the use of paddlewheels to facilitate thorough mixing of the culture, prevent sedimentation of microalgae/bacteria flocs and enhance nutrient diffusion across the cellular boundary layer, without damaging the cells through excess shear forces (Sutherland and Ralph, 2020). Key features of these reactors include high efficiency, operational simplicity, scalability and an energy consumption below 10 W/m^3 , whilst there are already patented models with an even lower energy requirement of less than 2 W/m^3 , significantly outperforming other reactor technologies. Given the need for a low depth and a high hydraulic retention time to permit sufficient biomass growth and pollutant removal, the large land requirement for implementation is the most significant restriction for this technology (Torres-Franco, Passos, et al., 2021). However, it is crucial to note that while conventional technologies rely on mechanical and electrical equipment that depreciates over time, the main investment for HRAPs is the acquisition of land, of which the value may actually increase in the long term, while reducing capital costs (Alcántara, Posadas, et al., 2015).

In contrast, the closed photobioreactors, which eliminate the risk of contamination, are more commonly used for microalgae cultivation when the biomass has a higher valued destination, such as the production of nutraceuticals (supplements, antioxidants, etc. for human or animal nutrition), dyes, phytochemicals (polyphenols, polysaccharides), medicines and biochars (da

Rosa, Alves, et al., 2023), as the high costs of building, operating and maintaining closed reactors are only justified for biomass production systems when the sale of the product and its added value will support the viability of the system's operation. However, in these cases often more severe restrictions concerning the quality and cleanliness of the cultivation medium exist (Severo, de Lira, et al., 2024; Lorenz and Gerald R Cysewski, 2000). As a result of higher costs and the lack of need to avoid contamination, closed photobioreactors are less relevant for wastewater treatment processes.

Microalgae species

Many different species of microalgae can be used in photobioreactors, and some species have special characteristics and capabilities for producing certain specific products of interest. However, in the case of wastewater treatment, the cultivation medium is never sterile, and an invasion by another species is always possible. A complete change of the dominant biomass species can occur within a week (Sutherland, Turnbull, et al., 2017). Thus, relatively little importance should be paid to the selection of specific microorganisms, as over time natural selection will inevitably cause the biomass composition to evolve to a certain, case specific optimal composition anyway (Bohutskyi, Liu, et al., 2015), depending on the type of effluent, cultivation system and applied conditions (Abdelaziz, Leite, et al., 2014). What has been gaining momentum is prospecting mixed cultures in natural environments and effluents to arrive at a favorable population more quickly (Pompei, Ruas, et al., 2023; Pompei, Ruas, et al., 2024).

The use of native and bioprospected microalgae in the effluents to be treated has been a successful alternative for improving treatment, as it reduces the time needed to adapt the culture and shows better removal of target pollutants (Krustok, Truu, et al., 2015; Wilkie, Edmundson, et al., 2011). The more diverse the community of microalgae present, the more resilient and efficient the system, especially regarding the removal or inactivation of pathogens (Krustok, Truu, et al., 2015), since we know that different species of microalgae affect the pathogens in domestic effluents differently (Ruas, Farias, et al., 2023). In most cases we will see the presence and/or dominance of *Chlorella* spp. and *Scenedesmus* spp. (Al-Hammadi and Güngörmüşler, 2024; Silambarasan, Logeswari, et al., 2023; Msanne, Polle, et al., 2020). The Solids Residence Time (SRT) in the reactor is a critical process parameter, as longer SRTs create less diverse biomass, while shorter SRTs increase selective pressure on the microbial community structure and force a higher biomass diversity, thus affecting stability, efficiency and reliability of the process as a whole (Bradley, Sevillano-Rivera, et al., 2019).

Biomass and by-products

Even though microalgae can be used for the production of many different types of compounds, often with a higher aggregated value (da Rosa, Alves, et al., 2023; Hassan, Meenatchi, et al., 2022; Suganya, Varman, et al., 2016), when primary and secondary domestic effluents are used as a growing medium, the recommended applications are production of biofuels, bioplastics, biopolymers and biochars. Even though these are less valuable products, their production may still contribute to a circular economy and to a more cost effective operation of the wastewater treatment plant. Biomass productivity in HRAPs fed with anaerobically digested effluent can be around $0,30 \text{ g.L}^{-1}.\text{d}^{-1}$, at a final dry weight concentration of $\approx 2,2 \text{ g.L}^{-1}$ (Ziganshina, Bulynina, et al., 2022). For a city of 100 000 inhabitants producing 100 L of sewage per capita per day, treated in a HRAP with a HRT of 5 days this would come down to 15 tons of biomass per day. This biomass can then be further processed, depending on its composition; a detailed example of biomass composition as a result of different types of feed is given by Villaró-Cos (2024). In general, processes should be prioritized in which the biomass will be processed chemically and/or thermally, in order to minimize the risk of product contamination by pathogens, heavy metals and inorganic pollutants (Markou, Wang, et al., 2018). Another prominent use of the produced biomass is in its anaerobic digestion for biogas production. Several studies already combine effluent treatment, algal biomass production, and biogas production and purification (Oswald, Golueke, et al., 1956; Olguín, 2012; Scarcelli, Ruas, et al., 2021; Ruas, López-Serna, et al., 2022). Given the importance of natural gas in the world's energy matrix, and the possibility to upgrade biogas to compatibility with natural gas (Muñoz, Meier, et al., 2015), this will remain an important ongoing research topic in the foreseeable future.

Microalgae-based systems and Nature-based Solutions

The concept of Nature-based Solutions (NbS) appeared in scientific literature in the 2000s and is used to define the sustainable use of nature, or systems mimicking nature, to solve society's problems and challenges (IUCN, 2013; Eggermont, Balian, et al., 2015). NbS are different from ecosystem-based approaches in that they must follow three principles: i. application alone or integrated with other solutions; ii. application on a landscape scale; iii. integrating a management policy with the participation of all stakeholders, with the aim of solving a problem/challenge (Cohen-Shacham, Andrade, et al., 2019). The famous HRAP system, detailed in the previous section, as well as other types of microalgae-based systems (Faraloni, Touloupakis, et al., 2023; Malyan, Kumar, et al., 2024), has already been identified as an NbS

(Vassalle, Ferrer, et al., 2023). The use of microalgae-based NbS for effluent treatment is still growing, and the establishment of guidelines and regulations is essential to expand their use. A great example can be seen in South Australia, where the use and application of HRAP systems is already regulated, and where guidelines are drafted to ensure that the characteristics and efficiency of the system are complied with (LGA, 2020). The publication of government documents, standards and guidelines strengthens the use of NbS with microalgae, since this integration of technology, management and users is imperative for any ecological solution to be considered a NbS (Cohen-Shacham, Andrade, et al., 2019).

Algae-based processes for secondary wastewater treatment

Microalgae-bacteria systems, and especially the High Rate Algae Ponds, have traditionally been developed as an option for the tertiary treatment of wastewater, removing remaining nutrients and other pollutants and promoting disinfection. However, it is also possible, and increasingly common, to use HRAPs immediately after primary sedimentation, acting as a secondary treatment of an effluent that contains both soluble organic and inorganic compounds, thus enabling the removal of both nutrients and organic carbon in one step (Acién, Gómez-Serrano, et al., 2016; Posadas, Alcantara, et al., 2017). Utilizing primary treated wastewater for microalgae cultivation presents benefits such as a higher microalgal biomass concentration and a more efficient nutrient recovery. This approach can yield biomass concentrations of up to 0.65 g L^{-1} and enable the production of up to 1 kg of microalgae biomass per m^3 of processed wastewater (Acién Fernández, Gómez-Serrano, et al., 2018). Considering that this matrix only goes through the physical process to remove coarse materials, with substantial removal of settleable solids of $\approx 70\%$, and a slight reduction of organic matter of $\approx 40\%$ COD, there is no effective change in the chemical composition of the effluent (Posadas, Muñoz, et al., 2017).

The proportion of macronutrients carbon, nitrogen and phosphorus in the effluent stands out as one of the main factors that influence biomass productivity. The C/N ratio impacts the equilibrium among heterotrophic, nitrifying bacteria, and microalgae. A higher ratio tends to promote the predominance of heterotrophic bacteria and a higher lipid content in the biomass, whereas a lower ratio supports nutrient treatment and recovery (Zhu, Qin, et al., 2019; Torres-Franco, Passos, et al., 2021). The composition of primary treated wastewater can be closer to the ideal proportion of traditional cultivation mediums for microalgae production. Nevertheless, carbon limitation is a common problem when using sewage as a cultivation medium, which can be solved with supplementation of inorganic carbon. If no source of CO_2

or carbonates can be found, more intense mixing with a paddlewheel permits increased CO₂ absorption from the air.

The N/P ratio affects the elemental composition of the biomass, leading to varying degrees of accumulation of these nutrients, and influencing the biomass growth (Gonçalves, Pires, et al., 2016). Wang (2010) noted that, when assessing the growth of the green alga *Chlorella* sp. at four distinct points in a wastewater treatment plant's treatment process flow, the most favorable proportions of inorganic N/P were found at the points before and after primary sedimentation, close to the ideal range of 6.8-10.

Among the microalgae reported in the literature, the most representative genera present in primary sewage are *Chlorella* sp. and *Scenedesmus* sp, whose growth is also favorable in secondary and tertiary effluents (de Cassia Soares Brandão, Oliveira, et al., 2023). The application of *Chlorella* in treatment of primary effluent stands out for its ability to recover nutrients combined with the production of biomass, and presents great potential for replacing treatment by means of an activated sludge process, for example (Wang, Min, et al., 2010). Likewise, *Scenedesmus*, commonly grouped in colonies, is notable for nitrogen and phosphorus removal efficiencies ranging from 30% to 100%, and for its resilience in adverse environmental conditions (Cai, Park, et al., 2013; Sánchez Zurano, Garrido Cárdenas, et al., 2020).

The microalgae-bacteria system employed in HRAPs fosters the removal of nutrients and pollutants through a complex, synergistic interaction between these microorganisms. In order to achieve maximum removal efficiencies of both organic matter and nutrients, it is crucial to understand the removal mechanisms, investigating in detail the effect of environmental and operational parameters such as pH, temperature, light intensities, hydraulic retention time (HRT) and CO₂ supplementation. Pilot studies, conducted under real-world environmental conditions, prove more effective than controlled laboratory experiments, offering valuable insights that can guide large-scale system applications (Forruque, Mofijur, et al., 2022). In this section, we will explore studies of parameters applied to HRAPs fed with primary domestic sewage, which are summarized in Table 1-2. These investigations sought to improve reactor performance, with greater nutrient recovery and biomass productivity, guiding the adoption of full scale technology.

Hydraulic retention time (HRT) is an important operational parameter that affects the proportion and composition of biomass and determines the nutrient loading rate. Controlling this parameter makes it possible to overcome system limitations such as long-term operating

stability and biomass production limitations (Rani, Gunjyal, et al., 2021). It is also an important parameter in relation to costs, as the final energy consumed per cubic meter of treated wastewater is a function of the specific energy consumption in the reactor and the hydraulic retention time of the system (Acién, Gómez-Serrano, et al., 2016). The determination of the HRT must consider characteristics of wastewater and climatic factors, considering that solar irradiance impacts light penetration and temperature affecting respiration and decomposition (Posadas, Alcantara, et al., 2017). A shorter HRT can be applied at higher temperatures, intensifying productivity, however greater dilution can impact the quality of the final effluent (Sutherland and Ralph, 2021; Donna L Sutherland, Turnbull, et al., 2014). Long HRTs, on the other hand, are more suitable in winter, considering that microalgae growth is slower during this period.

García (2000), observed that for nitrogen removal from primary effluent with low organic matter, an HRT of 4 days is adequate in the spring and summer seasons, while for autumn/winter the HRT of 10 days was more appropriate. (Ruas, Farias, et al., 2020a) obtained similar results of biomass gain with the application of HRT of 5 and 7 days, but the higher HRT improved the removals of COD, TOC, TN and TP.

Table 1-2 - Reported operational parameters with percentage nutrient removal and biomass production in primary domestic wastewater treatment used as the influent in High Rate Algal Pond studies on a pilot scale.

Reference	Operational parameters						Results				
	HRT (d)	Temp. (°C)	Water depth (m)	CO ₂ addition (L min ⁻¹)	Feeding regime	Shading	Representative species	Biomass Productivity (g m ⁻² d ⁻¹)	Removal efficiencies (%)		
									TN	TP	COD
(García, Mujeriego, et al., 2000)*	4 10	21.7 11.8	0.3	-	Continuous	-	<i>D. pulchellum</i> , <i>Chlorella</i> sp., <i>M. pusillum</i> , <i>S. armatus</i> , <i>S. acutus</i>	N/R	57 73	N/R	N/R
(Craggs, Sutherland, et al., 2012)*	N/R	15.4 ± 3	0.35	N/R	N/R	-	<i>Micractinium</i> sp., <i>Desmodesmus</i> sp	11.5	64–67% (only NH ₄ -N)	14–24% (DRP)	82–91% (fBOD ₅)
(Donna L. Sutherland, Turnbull, et al., 2014)*	4	19.4 ± 4	0.3	-	Continuous	-	<i>Mucidosphaerium pulchellum</i>	13.8 ± 2.6	75 ± 21 (NH ₄ -N)	58 ± 29 (DRP)	N/R
(Donna L Sutherland, Turnbull, et al., 2014)*	4	16 ± 2.4 16.1 ± 2	0.2 0.4	1.6	Continuous	-	<i>Mucidosphaerium pulchellum</i>	150 ± 36 201 ± 61 (Chl- α mg m ⁻² d ⁻¹)	70.3 ± 6.5 (NH ₄ -N)	23.4 ± 18 (DRP)	N/R
(Kim, Kang, et al., 2014)*	2	25.8	0.3	-	Semi-continuous:	-	<i>Chlorella</i> sp., <i>Scenedesmus</i> sp., <i>Stigeoclonium</i> sp.	0.5 ± 0.03 (g L ⁻¹ d ⁻¹)	92.68	82.65	85.44
(Matamoros, Gutiérrez, et al., 2015)*	4 8	25 ± 1	0.3	-	Continuous	-	<i>Chlorella</i> sp, <i>Stigeoclonium</i> sp, <i>Monoraphidium</i> sp., <i>Stigeoclonium</i> sp	316 ± 50 346 ± 38	99 99	N/R	75 84
(Posadas, Morales, et al., 2015)*	2.7 ± 0.1	23 ± 1	0.3	20	Continuous	-	<i>Scenedesmus</i> sp	17 ± 1	73 ± 1	3 ± 0	88 ± 0
(Ruas, Serejo, et al., 2018)*	5	35 ± 4.5	0.15	0.005 of 30% CO ₂	Continuous	-	<i>Chlorella vulgaris</i> , <i>Microspora</i> sp	4.1 ± 1.3	27 ± 11	8 ± 1	88 ± 9
(Arashiro, Ferrer, et al., 2019)*	4.5	N/R (cold// warm seasons)	0.3	-	Continuous	-	<i>Chlorella</i> sp	15 ± 6	49 ± 17	37 ± 52 (PO ₄ ³⁻)	62 ± 22
(Serejo, Farias, et al., 2020; Farias, Ruas, et al., 2023)*	7	27.4 ± 2 27.3 ± 2	0.16	-	Continuous Semi-continuous: 12 h ⁻¹ 0.1h d	-	<i>Scenedesmus</i> sp.	1.5 3.6 3.3	65 ± 8 74 ± 6 76 ± 8	65 ± 8 86 ± 1 80 ± 7	74 ± 8 73 ± 8 70 ± 13
(Ruas, Farias, et al., 2020a)	5 5 7	21.9 ± 3 22.3 ± 3 22.3 ± 3	0.16	0.025 ± 0.4	Continuous	-	<i>Scenedesmus</i> sp.	3.2 ± 0.2 2.0 ± 0.2 2.6 ± 0.2	78 ± 4 80 ± 6 90 ± 1	58 ± 9 58 ± 5 87 ± 7	41 ± 5 34 ± 4 67 ± 6
(Pham, Laurent, et al., 2021)*	4	18.2 ± 3	0.3	-	Continuous	-	<i>Chlorella</i> sp., <i>Ulothrix</i> sp., <i>Scenedesmus</i> sp	4.1 ± 0.2	77 ± 11.4	47.2 ± 25	57.7 ± 189
(Ruas, Farias, et al., 2022)	5	21.9 ± 3 21.6 ± 4 21.7 ± 3	0.16	0.025 ± 0.4 - -	24 h d ⁻¹ 24 h d ⁻¹ Semi-continuous 0.1 h d ⁻¹	50%	<i>Scenedesmus acutus</i> <i>Scenedesmus obliquus</i>	3.94 ± 1.00 2.83 ± 0.51 5.93 ± 2.0	73 ± 3 67 ± 2 60 ± 18	29 ± 7 26 ± 12 35 ± 14	63 ± 8 66 ± 13 59 ± 16

Abbreviations: Hydraulic retention time (HRT); Temperature\\(Temp); Total Nitrogen (TN); Total Phosphorus (TP); Chemical Oxygen Demand (COD); Not reported (N/R).

* Data from the best conditions tested in relation to biomass productivity, considering the different stages developed.

Temperature and light are critical environmental factors influencing microalgae growth. Given the diverse environmental conditions in open cultivation systems, microalgae strains must exhibit tolerance to variables like temperature fluctuations and changes in illumination. Temperature plays an essential role in the metabolic development of microalgae, as well as influencing the composition of biomass, cell physiology and can affect the solubility of CO₂ in the cultivation medium (Dammak, Fersi, et al., 2023). The optimal growth temperatures for most microalgae species, including the genera *Chlorella* sp and *Scenedesmus* sp, commonly found in primary wastewater, typically range from 15°C to 35°C (Posadas, Alcantara, et al., 2017).

While higher temperatures are advantageous for microalgae production, it's crucial to assess whether they surpass the ideal range, as excessive heat can have more detrimental effects than low temperatures, diminishing both photosynthesis and respiration (Ras, Steyer, et al., 2013). Echenique-Subiabre et al. (2023), comparing predicted biomass productivity based on local climatic conditions revealed significant variability across sites, not solely explicable by these conditions. It emphasizes the need to differentiate and quantify the influence of local climatic conditions versus experimental conditions on productivity to increase confidence in scaling up experimental results.

Linked directly to light, the ratio of illuminated surface to volume (depth) is an important parameter affecting both volumetric productivity and pollutant removal rates (Muñoz and Guieysse, 2006). The depths applied in cultivation with primary sewage reported in the literature vary from 0.1 to 0.4 m (Table 1-2). Correlated with HRT, shallower depths may enhance productivity; however, microalgae become more vulnerable to temperature fluctuations and the risk of overheating (Béchet, Shilton, et al., 2016). Sutherland et al. (2014b), when comparing HRAPs with depths of 0.2 and 0.4 m, noted that doubling the depth resulted in up to a 200% increase in productivity, in which despite the reduction in the euphotic zone, microalgae proved to be more photosynthetically efficient, and suffered less carbon limitation during certain parts of the day.

Low light intensity restricts the metabolic energy of algae, whereas high intensity induces photoinhibition or photooxidation, which occurs when the cellular capacity of algae to utilize energy is exceeded (Assemany, Calijuri, et al., 2014). In this context, there are studies reported in the literature that evaluated the effect of light intensity on productivity, using shading screens. (Ruas, Farias, et al., 2022), conducted an experiment in a tropical climate using primary wastewater as a cultivation medium, with a 50% shade screen. The productivity

results closely resembled values obtained in HRAPs operated with 5–7 days of HRT and light intensities ranging between $\approx 156 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $\approx 725 \mu\text{mol m}^{-2} \text{s}^{-1}$. Consequently, it was observed that under these conditions, light intensity was not a limiting factor for microalgae growth.

The external addition of CO_2 is an operational arrangement that makes it possible to overcome the common carbon limitation in sewage due to unbalanced nutrient ratios (Torres-Franco, Passos, et al., 2021). CO_2 addition also contributes to reducing pH, thus mitigating the environmental impact of ammonia volatilization and preventing phosphorus precipitation, which typically occurs at pH levels above 9 (Sutherland and Ralph, 2020). Life Cycle Assessment (LCA) studies identify that one of the potential highest investment cost points in the cultivation of microalgae in sewage is CO_2 supplementation, which demonstrates that it is a gap that is still under constant investigation for better economic viability aimed at applications on a large scale (Magalhães, Ferreira, et al., 2021). A cost-effective and environmentally sustainable solution for CO_2 supplementation involves utilizing biogas produced during anaerobic wastewater treatment. This process requires scrubbing to eliminate H_2S and harness the CO_2 , presenting the algal-bacterial process as a promising option for upgrading this biogas (Chaudhary, Tong, et al., 2017).

CO_2 supplementation in primary wastewater in HRAPs, reported in the literature, proves the efficiency in the growth of microalgae, but depending on the flow rate applied and other factors, it may not significantly influence the removal of nutrients and other pollutants. (Ruas, Farias, et al., 2022), observed that the addition of CO_2 did not impact the removal of pathogens, but improved the productivity and sedimentability of the microalgal biomass. Similar findings were observed in a study of Sutherland et al. (2015), where the addition of CO_2 enhanced the photophysiology of microalgae, resulting in increased microalgal biomass.

An additional critical factor that can enhance productivity and lower costs is the feeding regimen. This cultivation method has the potential to surpass the efficiency constraints of batch regimes and overcome operational control and cost limitations of continuous systems, making it a highly suitable option for large-scale cultivation (Yadav, Dubey, et al., 2020). The comparative performance of removal of pollutants such as surfactants, heavy metals and pathogens and microalgae growth of semi-continuous and continuous regimes in HRAPs treating primary sewage was explored by (Serejo, Farias, et al., 2020; Ruas, Farias, et al., 2022; Farias, Ruas, et al., 2023). Ruas et al. (2022) observed that the semicontinuous feed regime improved the biomass productivity and TSS concentration while reducing *Escherichia*

E. coli removal. Serejo et al. (2020) also considered the condition with continuous feeding at 0.1 h d^{-1} to be more advantageous, with respect to both the microalgal biomass productivity and the nutrient removal efficiencies. However, under these conditions, the removal of *E. coli* was lower, likely attributed to the high dilution over a short timeframe, which might have compromised the treatment efficiency (Farias, Ruas, et al., 2023).

Combining anaerobic digestion and microalgae-based processes for tertiary wastewater treatment

When combining microalgae with activated sludge processes, probably a secondary treatment process is more efficient (*vide ante*), but microalgae processes can also be very well combined with anaerobic digestion (AD). Anaerobic Digestion is a biological process without oxygen, resulting in the production of biogas ($\approx 60\%$ methane, $\approx 35\%$ carbon dioxide, $\approx 5\%$ nitrogen, and trace gasses, like NH_3 , H_2S and VOCs), a source of renewable energy, and a digestate rich in nutrients (Guan, Zhao, et al., 2024). The use of anaerobic processes to treat domestic wastewater is more attractive in tropical- and subtropical regions, due the necessity of temperatures above 20°C for the microbial metabolism (Chernicharo, 2007), and also because at lower temperatures increased methane solubility will cause significant loss of methane, a potent greenhouse gas, to the environment with the treated effluent (Stazi and Tomei, 2021). In spite of advantages like easy operation, and low installation and operating costs, there are also drawbacks, as AD requires post-treatment, since removal of organic carbon is limited to around 80%, and AD does not remove nutrients (nitrogen and phosphorus) and pathogens (Bressani-Ribeiro, Chamhum-Silva, et al., 2019; Guan, Zhao, et al., 2024). In addition, when not used *in-situ*, the biogas produced needs to be purified (“upgraded”) in order to meet standards for distribution and use (Vasan, Sridharan, et al., 2024), removing especially the large amount of CO_2 present, as well as the traces of H_2S , which is corrosive.

The use of microalgae-based processes to treat secondary domestic wastewater (digestates) is a good option for recovering macro- (nitrogen (N), phosphorus (P), and potassium (K)) and micronutrients (sulfur (S), magnesium (Mg), cobalt (Co), iron (Fe), molybdenum (Mo), and nickel (Ni)) and thus promoting the circular economy (Chojnacka and Chojnacki, 2024; Guan, Zhao, et al., 2024). Depending on the predominant species of microalgae produced, these can be rich in proteins, sugars, and/or fats (Villaró-Cos, Cuaresma Franco, et al., 2024), and thus can be used as a source of animal feed, or as a feedstock for fuels, chemicals, cosmetics and/or fertilizer production (Stiles, Styles, et al., 2018). In addition, microalgae-based systems

can be used for biogas upgrading, as their growth is often carbon limited, and biogas upgrading requires exactly removing the excess of CO₂ present in it (*vide ante*) (Serejo et al. 2015; Posadas et al. 2017b). Biogas upgrading was seen to be more efficient when cultivating microalgae in secondary wastewater than in primary wastewater, as secondary wastewater has a lower C/N ratio than primary wastewater and thus a higher demand for CO₂ (Rodero, Lebrero, et al., 2019).

Dimensioning

When operating a full scale HRAP for treatment of secondary effluent, a number of parameters needs to be taken into account. The first is the HRT to be applied. The process depends on both bacteria and microalgae, of which the latter depend on sunlight for photosynthesis, producing the oxygen the bacteria will need for degradation of organic matter and nitrification, amongst others. In order to guarantee exposure to sunlight for all wastewater that enters the process, the HRT needs to be at least 24 hours, but generally HRAPs tend to be operated at longer HRTs of between 3 and 8 days (Arbib, Ruiz, et al., 2013; Posadas, Morales, et al., 2015), with a recommended minimum of 4 days (Ortiz, Díez-Montero, et al., 2022; Craggs, Park, et al., 2014). Second, attention should be paid to light penetration. Illumination matters, and as a result, the reactor depth is limited by the need to permit light penetration. The darker or more turbid the suspension is, the shallower the reactor should be, with a proportionally larger footprint. Typical depth of a HRAP for tertiary treatment is between 30 and 70 cm (Sutherland and Ralph, 2020; Ortiz, Díez-Montero, et al., 2022). A shallower pond does not always result in better performance though. In a shallower pond, biomass concentration will increase more, and this may cause light shielding, and the effect may be that a deeper pond, in spite of lower biomass concentration, offers a higher volumetric and especially areal rate of nutrient removal (Sutherland and Ralph, 2020). HRT and depth being defined, the necessary surface area can be calculated, defining reactor length and width. CFD calculations show that HRAP performance tends to be optimized at length to width ratios higher than 10, as this gives a better velocity uniformity and reduces shear stress (Hadiyanto, Elmore, et al., 2013). A paddlewheel is used for mixing the liquid. Mixing is required for introducing CO₂, for keeping the cells in suspension and avoiding precipitation and also for maintaining a flow of nutrients to the biomass, and maintaining liquid homogeneity. Paddlewheel speed should be kept low to avoid shear stress on the cells and reduce turbulence, and the number of paddles in the paddlewheel should be high, in order to avoid backflow and improve paddlewheel efficiency (Ortiz, Díez-Montero, et al., 2022).

As in other suspended cell processes, without intervention, the Solids Retention Time (SRT) in the reactor would be the same as the HRT, and the amount of solids in the effluent would be the same as in the reactor. To avoid this situation, solids (the macroalgae/bacteria suspension) need to be separated and partially recycled, just as is done in the activated sludge process. In order to do so, microalgae/bacterial flocs can be separated in a settler, kept in the system using membrane filtration, or dissolved air flotation can be used. Generally, a SRT of around 7 days is applied, but a higher SRT, and the resulting higher biomass concentration, results in a higher process efficiency (Xu, Li, et al., 2015). Settleable biomass recycling also exerts selective pressure on the biomass, leading to a higher proportion of better settleable biomass, facilitating process operation (Sutherland and Ralph, 2020). The biomass that is not going to be recycled to the process can be separated from the effluent using coagulants. In this case, the possibilities depend on the pretended use of the biomass. The best results are usually obtained with FeCl_3 or alum ($\text{Al}_2(\text{SO}_4)_3 \cdot 12\text{H}_2\text{O}$), although at \$130 and \$65 per megaton of algae harvested these are also the most expensive options (Udom, Zaribaf, et al., 2013). However, when the microalgae will be used as fertilizer, for instance, the use of such metal salt flocculants should be avoided, as these salts will accumulate in the fertilized soil, and a cationic polymer, also cheaper with \$50 per Mton, might be considered. Given the problems arising from the use of traditional coagulants, a shift towards natural (and biodegradable) coagulants, like those derived from *Moringa Oleifera* seeds (Cassini, Francisco, et al., 2017), but more recently also those based on vegetable tannins, like the commercial Tanfloc SG, can now be observed (Dammak, Fersi, et al., 2023).

Operation

Operation of a large-scale HRAP is straightforward and does not require much interference. The reactor contents are to be kept mixed using the paddlewheel, which should maintain the reactor contents at a horizontal speed of 15 – 30 cm/s (Craggs, Park, et al., 2014). During operation, the pH in the reactor will vary, as during the day bicarbonate is consumed through photosynthesis, while during the night aerobic respiration produces bicarbonate. Usually, the pH will not drop below 6,5. If the pH tends to rise to values above 10,0, this indicates carbon limitation, and nutrient removal will be affected. To make matters worse, at $\text{pH} > 9,0$, ammonia may be lost to the environment by volatilization, as a result of the shift of the ammonia - ammonium ion equilibrium. In such cases, the pH can be controlled (lowered) by admitting CO_2 gas; in this case the pH will vary between 6,5 and the set upper value. Good results were obtained limiting the pH at a value of 8,0 using CO_2 sparging (Park and Craggs,

2010). Biomass concentration can be optimized controlling the sludge recirculation rate, as is the case in activated sludge plants; a low biomass concentration will result in reduced removal efficiency, but a too high biomass concentration will result in internal shading, reduced photosynthesis, reduced algal activity and an increase of the bacteria/algae ratio in the biomass.

Results

Given the operational parameters (HRT, SRT, pH, feed) as stated before, the use of microalgae/bacterial combined biomass for tertiary treatment tends to present good results. Removal efficiencies of N and of P of > 90% or even close to 100% are frequently observed (Yu, Ko, et al., 2023; Alcántara, Fernández, et al., 2015; Romero Villegas, Fiamengo, et al., 2017), and pathogen removal, depending on the species tested, can be of 1-3 units log (Ruas, Serejo, et al., 2021; Bhatt, Arora, et al., 2023). Factors that may be responsible for pathogen removal in algae-based processes may be pH, Dissolved Oxygen, and sunlight mediated processes; microalgae-based processes tend to result in fluctuating values for all these parameters, with the bacteria lacking capabilities to respond to rapidly changing environmental conditions. Entrapment of the pathogens in the microalgal/bacterial flocs may also play a role (Bhatt, Arora, et al., 2023). Pathogen removal is not always complete though, and pending better results, chlorine disinfection after this process may still be necessary, depending on discharge requirements. Data on removal of heavy metals, of which especially lead, mercury and cadmium are reasons for concern (Wilde and Benemann, 1993), show that heavy metal removal by microalgae can reach from tens to a few hundred mg per gram of dry biomass (Kumar, Dahms, et al., 2015), but heavy metal fixation is favored at lower pH, around 5, which is below typical operating range. Metal removal may be desired, but especially when the intended final destination of the biomass is agriculture, avoiding metal removal by the microalgae, for instance by maintaining a certain pH, may be preferred.

Biomass productivity in laboratory and small-scale experiments has been good, but biomass productivity on treated wastewater when using a full-scale system is significantly less, with productivities of around $6 \text{ g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ being recorded for such cases. Considering 40 cm reactor depth and a 5-day HRT, this corresponds to $15 \text{ g} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$.

Current limitations in advancing large-scale application of microalgae-based wastewater treatment

Despite the high potential presented here in this chapter and the advancement in this scientific field, the large-scale algal biomass production technologies are not popular – are not part of

the daily operation units of conventional Wastewater Treatment Plants (WWTP), not even in decision-making, particularly in Latin America. In other countries, mainly Europe and the United States, they already have larger plants, financing coming from large scientific collaboration and innovation projects.

Most of the studies presented in the literature have been carried out on a small-laboratory scale. There are limits about the possibility of obtaining reliable data for a comprehensive assessment of the efficiency of such solutions. Therefore, there is a need to verify the results in pilot-scale and the full technical-scale studies (Dębowski, Zieliński, et al., 2020). This chapter summarizes information of microalgal technologies for wastewater treatment applications, based on bringing criteria to full-scale projects, including specificities that must be considered on a real scale.

Limitation exists because the systems for algal biomass production, separation, and conversion into energy carriers and other benefits still are difficult to bring economic feasibility in the face of usual simplified processes. Where the sector view is only to (wastewater) treat and meet environmental conditions, without incorporating the context of circular economy, water reuse, and the nexus approach (water, energy, and food) as part of a holistic, systemic, and sustainable management sight of sanitation based on the concepts of resource-oriented sanitation.

The road to large-scale implementation—production and use of microalgal biomass—is not only fraught with economic difficulties, but also with technological, normative, and legal challenges. Unsuitable climate conditions are also an impediment; proper thermal and light conditions are crucial factors in microalgal growth and improved performance. Sites of anaerobic reactor exploitation can serve as technologically and commercially viable locations. However, it is reasonable to assume that microalgae, with the multitude of commercial applications, possess properties that grant them a competitive advantage over conventional WWTP in terms of commercial applicability (Leu and Boussiba, 2014; Dębowski, Zieliński, et al., 2020). This will require local public policies and state strategies (climate change, nature-based solutions, urban resilience) to encourage the local productive sector in the implementation of a WWTP-biorefinery approach—including economic benefits— and facilitate the bureaucratic legal and regulatory process. The concept of recovering resources affords an opportunity for the development of technologies based on the use of microalgal biomass.

Some important limitations were pointed out by Yap, Sankaran, et al. (2021), based on a review carried out with several studies in the area. Therefore, this chapter was based on bringing information to full-scale projects, based on specificities that must be considered on a real scale. Some relevant and highlighted points are as follows: (a) high cost in cultivation and harvesting process; and (b) criteria to design based on studies that are not comparable because data on yields and costs relate to different cultural systems, different stresses, and different environmental and social conditions

For large-scale application open raceway ponds appear to be more favorable— with the potential for CO₂ sequestration. With biomass harvesting being one of the major challenges at full-scale application, some of the recovery methods being considered include filtration, centrifugation, gravity sedimentation, chemical flocculation, and dissolved air flotation. However, the method of choice has to be selected based on a positive techno-economic and environmental evaluation, also taking the energy balance into consideration. Open pond systems and photobioreactors for large-scale commercial production are limited to culturing microalgae for high value products and not biodiesel. Some criteria that need to be optimized for large-scale application include (Rawat, Ranjith Kumar, et al., 2013):

- strain selection and seed culture preparation;
- biomass and lipid yield optimization;
- bioreactor configuration;
- physic-chemical parameters; and most importantly
- harvesting and extraction of the lipid from the biomass.

Major breakthroughs are still needed toward design and development of technologies (Amaro, Guedes, et al., 2011) at full scale that can reduce costs while increasing yields. Integrated studies, following coherent and long-run, well-funded R&D program, with social, governance, and environmental management goals, are necessary. The processes should be integrated in the existing biofuels industry to accelerate full-scale implementation, especially in Third World countries. Developing this sector within an integrated social and economic policy agenda and not just through disjointed environmental actions will speed things up: not only meant for profit making and benefiting the environment, but also to help local communities in terms of food and energy security (Lam and Lee, 2012).

The scale-up process is often expensive and frequently carries high risks. It will be important that projects adapting technologies to local contexts, as they are not the same criteria and

operational practices for all cases, clearly indicate: (i) practical recommendations and discuss common challenges of large-scale systems associated with; (ii) cost-effective design; (iii) operation and maintenance; (iv) shortage of experienced staff (especially phycologists); and (v) social barriers, such as wider acceptance of algae as a food source and regulatory gaps (Novoveská, Nielsen, et al., 2023).

In addition to the issues inherent to the specific innovation process itself and the challenges to disseminate the technology on a real scale, it is important to highlight that transforming urban society from a linear to a circular metabolism is a trend in many developed countries. In low-income countries, green values and opportunities are less developed and have a generally lower priority among many key stake-holders, and public budgets are too constrained to make the right investments. With the development of microalgae-based wastewater treatment, many innovative components should be developed to meet the requirement of wastewater treatment and result in the appearance of the new related technologies. The cost of some components (e.g., solid-liquid separators) may decrease with manufacturing development and mass production, which could improve the high-cost dilemma. Actions are needed that go beyond process optimization, but public policies that integrate social and economic-environmental dimensions (Magalhães Filho, Moreira, et al., 2022).

REFERENCES

- Abdelaziz, A. E. M., Leite, G. B., Belhaj, M. A., and Hallenbeck, P. C. (2014) Screening microalgae native to Quebec for wastewater treatment and biodiesel production. *Bioresource Technology*, **157**, 140–148. [online] <http://dx.doi.org/10.1016/j.biortech.2014.01.114>.
- Acién, F. G., Gómez-Serrano, C., Morales-Amaral, M. M., Fernández-Sevilla, J. M., and Molina-Grima, E. (2016) Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? *Applied Microbiology and Biotechnology*, **100**(21).
- Acién Fernández, F. G., Gómez-Serrano, C., and Fernández-Sevilla, J. M. (2018) Recovery of Nutrients From Wastewaters Using Microalgae. *Frontiers in Sustainable Food Systems*, **2**(September), 1–13.
- Al-Hammadi, M. and Güngörmüşler, M. (2024) New insights into *Chlorella vulgaris* applications. *Biotechnology and Bioengineering*, **121**(5), 1486–1502.
- Alcántara, C., Fernández, C., García-Encina, P. A., and Muñoz, R. (2015) Mixotrophic metabolism of *Chlorella sorokiniana* and algal-bacterial consortia under extended dark-light periods and nutrient starvation. *Applied Microbiology and Biotechnology*, **99**(5), 2393–2404.
- Alcántara, C., Posadas, E., Guieysse, B., and Muñoz, R. (2015) “Microalgae-based Wastewater Treatment” in *Handbook of Marine Microalgae: Biotechnology Advances.*, 439–455.

- Amaro, H. M., Guedes, A. C., and Malcata, F. X. (2011) Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy*, **88**(10), 3402–3410. [online] <http://dx.doi.org/10.1016/j.apenergy.2010.12.014>.
- Arashiro, L. T., Ferrer, I., Rousseau, D. P. L., Van Hulle, S. W. H., and Garfí, M. (2019) The effect of primary treatment of wastewater in high rate algal pond systems: Biomass and bioenergy recovery. *Bioresource Technology*, **280**(November 2018), 27–36. [online] <https://doi.org/10.1016/j.biortech.2019.01.096>.
- Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C., Barragan, J., and Perales, J. A. (2013) Effect of pH control by means of flue gas addition on three different photo-bioreactors treating urban wastewater in long-term operation. *Ecological Engineering*, **57**, 226–235. [online] <http://dx.doi.org/10.1016/j.ecoleng.2013.04.040>.
- Assemany, P. P., Calijuri, M. L., Santiago, A. D. F., Couto, E. D. A. Do, Leite, M. D. O., and Bermudez Sierra, J. J. (2014) Effect of solar radiation on the lipid characterization of biomass cultivated in high-rate algal ponds using domestic sewage. *Environmental Technology (United Kingdom)*, **35**(18), 2296–2305.
- Barboza-Rodríguez, R., Rodríguez-Jasso, R. M., Rosero-Chasoy, G., Rosales Aguado, M. L., and Ruiz, H. A. (2024) Photobioreactor configurations in cultivating microalgae biomass for biorefinery. *Bioresource Technology*, **394**(December 2023).
- Béchet, Q., Shilton, A., and Guieysse, B. (2016) Maximizing Productivity and Reducing Environmental Impacts of Full-Scale Algal Production through Optimization of Open Pond Depth and Hydraulic Retention Time. *Environmental Science and Technology*, **50**(7), 4102–4110.
- Bhatt, A., Arora, P., and Prajapati, S. K. (2023) *Chlorella pyrenoidosa*-mediated removal of pathogenic bacteria from municipal wastewater – Multivariate process optimization and application in the real sewage. *Journal of Environmental Chemical Engineering*, **11**(2), 109494. [online] <https://doi.org/10.1016/j.jece.2023.109494>.
- Bohutskyi, P., Liu, K., Nasr, L. K., Byers, N., Rosenberg, J. N., Oyler, G. A., Betenbaugh, M. J., and Bouwer, E. J. (2015) Bioprospecting of microalgae for integrated biomass production and phytoremediation of unsterilized wastewater and anaerobic digestion centrate. *Applied Microbiology and Biotechnology*, **99**(14), 6139–6154.
- Bradley, I. M., Sevillano-Rivera, M. C., Pinto, A. J., and Guest, J. S. (2019) Impact of solids residence time on community structure and nutrient dynamics of mixed phototrophic wastewater treatment systems. *Water Research*, **150**, 271–282. [online] <https://doi.org/10.1016/j.watres.2018.11.065>.
- Bressani-Ribeiro, T., Chamhum-Silva, L. A., and Chernicharo, C. A. L. (2019) Constraints, performance and perspectives of anaerobic sewage treatment: Lessons from full-scale sewage treatment plants in Brazil. *Water Science and Technology*, **80**(3), 418–425.
- Cai, T., Park, S. Y., and Li, Y. (2013) Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews*, **19**, 360–369. [online] <http://dx.doi.org/10.1016/j.rser.2012.11.030>.
- Calicioglu, O. and Demirer, G. N. (2015) Integrated nutrient removal and biogas production by *Chlorella vulgaris* cultures. *Journal of Renewable and Sustainable Energy*, **7**(3). [online] <http://dx.doi.org/10.1063/1.4922231>.
- de Cassia Soares Brandão, B., Oliveira, C. Y. B., dos Santos, E. P., de Abreu, J. L., Oliveira, D. W. S., da Silva, S. M. B. C., and Gálvez, A. O. (2023) Microalgae-based domestic

- wastewater treatment: a review of biological aspects, bioremediation potential, and biomass production with biotechnological high-value. *Environmental Monitoring and Assessment*, **195**(11). [online] <https://doi.org/10.1007/s10661-023-12031-w>.
- Cassini, S. T., Francisco, S. A., Antunes, P. W. P., Oss, R. N., and Keller, R. (2017) Harvesting microalgal biomass grown in anaerobic sewage treatment effluent by the coagulation-flocculation method: Effect of pH. *Brazilian Archives of Biology and Technology*, **60**(December), 1–12.
- Chaudhary, R., Tong, Y. W., and Dikshit, A. K. (2017) CO₂-assisted removal of nutrients from municipal wastewater by microalgae *Chlorella vulgaris* and *Scenedesmus obliquus*. *International Journal of Environmental Science and Technology*, **15**(10), 2183–2192.
- Chernicharo, C. A. L. (2007) *Anaerobic Reactors*, IWA Publishing.
- Chisti, Y. (2007) Biodiesel from microalgae. *Biotechnology advances*, **25**(3), 294–306.
- Chojnacka, K. and Chojnacki, M. (2024) Nutrient recovery from anaerobic digestate: Fertilizer informatics for circular economy. *Environmental Research*, **245**(October 2023).
- Cohen-Shacham, E., Andrade, A., Dalton, J., Dudley, N., Jones, M., Kumar, C., Maginnis, S., Maynard, S., Nelson, C. R., Renaud, F. G., Welling, R., and Walters, G. (2019) Core principles for successfully implementing and upscaling Nature-based Solutions. *Environmental Science and Policy*, **98**(June 2018), 20–29. [online] <https://doi.org/10.1016/j.envsci.2019.04.014>.
- Craggs, R., Park, J., Heubeck, S., and Sutherland, D. (2014) High rate algal pond systems for low-energy wastewater treatment, nutrient recovery and energy production. *New Zealand Journal of Botany*, **52**(1), 60–73.
- Craggs, R., Sutherland, D., and Campbell, H. (2012) Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *Journal of Applied Phycology*, **24**(3), 329–337.
- Dammak, I., Fersi, M., Hachicha, R., and Abdelkafi, S. (2023) Current Insights into Growing Microalgae for Municipal Wastewater Treatment and Biomass Generation. *Resources*, **12**(10).
- Dębowski, M., Zieliński, M., Kazimierowicz, J., Kujawska, N., and Talbierz, S. (2020) Microalgae cultivation technologies as an opportunity for bioenergetic system development—advantages and limitations. *Sustainability (Switzerland)*, **12**(23), 1–37.
- Echenique-Subiabre, I., Greene, J. M., Ryan, A., Martinez, H., Balleza, M., Gerber, J., Jebali, A., Getto, S., O’Kelly, C. J., Mandal, S., Quinn, J. C., Starkenburg, S. R., Corcoran, A. A., and Shurin, J. B. (2023) Site-specific factors override local climatic conditions in determining microalgae productivity in open raceway ponds. *Algal Research*, **74**(March), 103235. [online] <https://doi.org/10.1016/j.algal.2023.103235>.
- Eggermont, H., Balian, E., Azevedo, J. M. N., Beumer, V., Brodin, T., Claudet, J., Fady, B., Grube, M., Keune, H., Lamarque, P., Reuter, K., Smith, M., Van Ham, C., Weisser, W. W., and Le Roux, X. (2015) Nature-based solutions: New influence for environmental management and research in Europe. *GAIA - Ecological Perspectives for Science and Society*, **24**(4), 243–248.

- Faraloni, C., Touloupakis, E., and Santos, E. (2023) Enhancing Nature-Based Solutions: Efficient Removal of Hydroxytyrosol in Olive Mill Wastewater Treatment for Value Creation. *Water (Switzerland)*, **15**(12).
- Farias, S. L., Ruas, G., Serejo, M. L., and Boncz, M. Á. (2023) Evaluation of the effect of the feeding regime on the removal of metals and pathogens in microalgae – bacterial systems. *Water Science & Technology*, **88**(1), 11–22.
- Forruque, S., Mofijur, M., Ahmed, T., Islam, N., Kusumo, F., Inayat, A., Le, V. G., Anjum, I., Khan, T. M. Y., and Chyuan, H. (2022) Progress and challenges of contaminate removal from wastewater using microalgae biomass. *Chemosphere*, **286**, 131656. [online] <https://doi.org/10.1016/j.chemosphere.2021.131656>.
- García, J., Mujeriego, R., and Hernández-Mariné, M. (2000) High rate algal pond operating strategies for urban wastewater nitrogen removal. *Applied Phycology*, **12**, 331–339.
- Gonçalves, A. L., Pires, J. C. M., and Simões, M. (2016) A review on the use of microalgal consortia for wastewater treatment. *Algal Research*, **24**, 403–415. [online] <http://dx.doi.org/10.1016/j.algal.2016.11.008>.
- Guan, D., Zhao, J., Wang, Y., Fu, Z., Zhang, D., Zhang, H., Xie, J., Sun, Y., Zhu, J., and Wang, D. (2024) A critical review on sustainable management and resource utilization of digestate. *Process Safety and Environmental Protection*, **183**(January), 339–354. [online] <https://doi.org/10.1016/j.psep.2024.01.029>.
- Hadiyanto, H., Elmore, S., Van Gerven, T., and Stankiewicz, A. (2013) Hydrodynamic evaluations in high rate algae pond (HRAP) design. *Chemical Engineering Journal*, **217**, 231–239. [online] <http://dx.doi.org/10.1016/j.cej.2012.12.015>.
- Hassan, S., Meenatchi, R., Pachillu, K., Bansal, S., Brindangnanam, P., Arockiaraj, J., Kiran, G. S., and Selvin, J. (2022) Identification and characterization of the novel bioactive compounds from microalgae and cyanobacteria for pharmaceutical and nutraceutical applications. *Journal of Basic Microbiology*, **62**(9), 999–1029.
- IUCN (2013) *The IUCN Programme 2013–2016*, Gland. [online] https://cmsdata.iucn.org/downloads/iucn_programme_2013_2016.pdf.
- Kim, B. H., Kang, Z., Ramanan, R., Choi, J. E., Cho, D. H., Oh, H. M., and Kim, H. S. (2014) Nutrient removal and biofuel production in high rate algal pond using real municipal wastewater. *Journal of Microbiology and Biotechnology*, **24**(8), 1123–1132.
- Krustok, I., Truu, J., Odlare, M., Truu, M., Ligi, T., Tiirik, K., and Nehrenheim, E. (2015) Effect of lake water on algal biomass and microbial community structure in municipal wastewater-based lab-scale photobioreactors. *Applied Microbiology and Biotechnology*, **99**(15), 6537–6549.
- Kumar, K. S., Dahms, H., Won, E., Lee, J., and Shin, K. (2015) Microalgae – A promising tool for heavy metal remediation. *Ecotoxicology and Environmental Safety*, **113**, 329–352. [online] <http://dx.doi.org/10.1016/j.ecoenv.2014.12.019>.
- Lam, M. K. and Lee, K. T. (2012) Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnology Advances*, **30**(3), 673–690. [online] <http://dx.doi.org/10.1016/j.biotechadv.2011.11.008>.
- Leite, L. de S. and Daniel, L. A. (2020) Produção de microalgas acopladas ao tratamento de esgoto: panorama e desafios. *Revista Ibero-Americana de Ciências Ambientais*, **11**(1), 184–200.

- Leu, S. and Boussiba, S. (2014) Advances in the Production of High-Value Products by Microalgae. *Industrial Biotechnology*, **10**(3), 169–183.
- LGA, S. (2020) *High Rate Algal Pond (HRAP) Design Guideline - an element in CWMS Wastewater Treatment Trains*,
- Li, K., Liu, Q., Fang, F., Luo, R., Lu, Q., Zhou, W., Huo, S., Cheng, P., Liu, J., Addy, M., Chen, P., Chen, D., and Ruan, R. (2019) Microalgae-based wastewater treatment for nutrients recovery: A review. *Bioresource Technology*, **291**(July), 121934. [online] <https://doi.org/10.1016/j.biortech.2019.121934>.
- Lorenz, R. T. and Gerald R Cysewski (2000) Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends in Biotechnology*, **18**(4), 160–167.
- Magalhães Filho, F., Moreira, A., Pinto, V., and Paulo, P. (2022) “Relationship between resource-oriented sanitation and the Nexus approach: water, energy and food perspectives on management and technologies” in F. de A. Moreira;, M. Dalla, F. Giulio., T. F. Malheiros;, and G. M. Di Giulio (eds.), *The Water-energy-food nexus: what the Brazilian research has to say*. São Paulo, USP, 259–279.
- Magalhães, I. B., Ferreira, J., de Siqueira Castro, J., Assis, L. R. de, and Calijuri, M. L. (2021) Technologies for improving microalgae biomass production coupled to effluent treatment: A life cycle approach. *Algal Research*, **57**(March).
- Malyan, S. K., Kumar, S., Singh, R., Singh, S., Anand, G., Upadhyay, S., Saini, K., and Kumar, S. S. (2024) “Algal intervention as nature-based solution for treatment of landfill leachate” in *Algae Based Bioelectrochemical Systems for Carbon Sequestration, Carbon Storage, Bioremediation and Bioproduct Generation*. Academic Press, 2–3.
- Manthos, G., Koutra, E., Mastropetros, S. G., Zagklis, D., and Kornaros, M. (2022) Mathematical Modeling of Microalgal Growth during Anaerobic Digestion Effluent Bioremediation. *Water*, **14**(23), 3938.
- Markou, G., Wang, L., Ye, J., and Unc, A. (2018) Using agro-industrial wastes for the cultivation of microalgae and duckweeds: Contamination risks and biomass safety concerns. *Biotechnology Advances*, **36**(4), 1238–1254. [online] <https://doi.org/10.1016/j.biotechadv.2018.04.003>.
- Matamoras, V., Gutiérrez, R., Ferrer, I., García, J., and Bayona, J. M. (2015) Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. *Journal of Hazardous Materials*, **288**, 34–42. [online] <http://dx.doi.org/10.1016/j.jhazmat.2015.02.002>.
- Metcalf & Eddy, Burton, F. L., Tchobanoglous, G., and Stensel, H. D. (2014) *Wastewater Engineering: Treatment and Resource Recovery*, Porto Alegre, McGraw Hill Education.
- Msanne, J., Polle, J., and Starkenburg, S. (2020) An assessment of heterotrophy and mixotrophy in *Scenedesmus* and its utilization in wastewater treatment. *Algal Research*, **48**(March), 101911. [online] <https://doi.org/10.1016/j.algal.2020.101911>.
- Muñoz, R. and Guieysse, B. (2006) Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Research*, **40**(15), 2799–2815.
- Muñoz, R., Meier, L., Diaz, I., and Jeison, D. (2015) A review on the state-of-the-art of physical / chemical and biological technologies for biogas upgrading. *Rev Environ Sci Biotechnol*, **14**.

- Muñoz, R., Teresa, M., Muñoz, A., Terrazas, E., Guieysse, B., and Mattiasson, B. (2006) Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. , **63**, 903–911.
- Novoveská, L., Nielsen, S. L., Tufan, O., Haznedaroglu, B. Z., Rinkevich, B., Fazi, S., Robbens, J., Vasquez, M., and Einarsson, H. (2023) Overview and Challenges of Large-Scale Cultivation of Photosynthetic Microalgae and Cyanobacteria. *Marine Drugs*, **21**(445), 1–23.
- Nurdogan, Y. and Oswald, W. J. (1995) Enhanced Nutrients Removal in High Rate Ponds. *Water Science & Technology*, **31**(12), 33–43.
- Olguín, E. J. (2012) Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery. *Biotechnology Advances*, **30**(5), 1031–1046. [online] <http://dx.doi.org/10.1016/j.biotechadv.2012.05.001>.
- Ortiz, A., Díez-Montero, R., García, J., Khalil, N., and Uggetti, E. (2022) Advanced biokinetic and hydrodynamic modelling to support and optimize the design of full-scale high rate algal ponds. *Computational and Structural Biotechnology Journal*, **20**, 386–398. [online] <https://doi.org/10.1016/j.csbj.2021.12.034>.
- Oswald, W. J. and Golueke, C. G. (1960) Biological transformation of solar energy. *Advances in applied microbiology*, **2**, 223–262.
- Oswald, W. J., Golueke, C. G., and Gotaas, H. B. (1956) Anaerobic Digestion of Algae. *Applied Microbiology*, **5**(1), 47–55.
- Oswald, W. J. and Gotaas, H. B. (1957) Photosynthesis in Sewage Treatment. *Transactions of the American Society of Civil Engineers*, **1**, 73–97.
- Park, J. B. K. and Craggs, R. J. (2010) Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Science & Technology*, **5**, 633–640.
- Park, J. B. K., Craggs, R. J., and Shilton, A. N. (2011) Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology*, **102**(1), 35–42. [online] <http://dx.doi.org/10.1016/j.biortech.2010.06.158>.
- Pham, L. A., Laurent, J., Bois, P., Teshome, T. M., and Wanko, A. (2021) Operating a semi-continuous raceway pond allows to link pH and oxygen dynamics to the interaction between microalgae and bacteria. *Desalination and Water Treatment*, **211**, 105–116.
- Pompei, C. M. E., Ruas, G., Bolzani, H. R., Fernandes, L. M., and Silva, G. H. R. da (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*, **349**(January), 123935. [online] <https://doi.org/10.1016/j.envpol.2024.123935>.
- Pompei, C. M. E., Ruas, G., Bolzani, H. R., and Silva, G. H. R. da (2023) Assessment of total coliforms and *E. coli* removal in algae-based pond under tropical temperature in addition of carbon dioxide (CO₂) and shading. *Ecological Engineering*, **196**(August), 107102. [online] <https://doi.org/10.1016/j.ecoleng.2023.107102>.
- Posadas, E., Alcantara, P. A., L.Gouveia, L. G.-E., Guieysse, B., Norvill, Z., Acién, F. G., Markou, G., Congestri, R., Koreiviene, J., and Muñoz, R. (2017) “Microalgae cultivation in wastewater” in C. Gonzalez-Fernandez and Raúl Muñoz (eds.), *Microalgae-Based Biofuels and Bioproduct. From Feedstock Cultivation to End-Products*. Woodhead Publishing, 67–91.

- Posadas, E., Marín, D., Blanco, S., Lebrero, R., and Muñoz, R. (2017) Simultaneous biogas upgrading and centrate treatment in an outdoors pilot scale high rate algal pond. *Bioresource Technology*, **232**, 133–141. [online] <http://dx.doi.org/10.1016/j.biortech.2017.01.071>.
- Posadas, E., Morales, M. M., Gomez, C., Acién, F. G., and 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. [online] <http://dx.doi.org/10.1016/j.cej.2014.12.059>.
- Posadas, E., Muñoz, R., and Guieysse, B. (2017) Integrating nutrient removal and solid management restricts the feasibility of algal biofuel generation via wastewater treatment. *Algal Research*, **22**, 39–46. [online] <http://dx.doi.org/10.1016/j.algal.2016.11.019>.
- Rani, S., Gunjyal, N., Ojha, C. S. P., and Singh, R. P. (2021) Review of Challenges for Algae-Based Wastewater Treatment: Strain Selection, Wastewater Characteristics, Abiotic, and Biotic Factors. *Journal of Hazardous, Toxic, and Radioactive Waste*, **25**(2), 03120004.
- Ras, M., Steyer, J. P., and Bernard, O. (2013) Temperature effect on microalgae: A crucial factor for outdoor production. *Reviews in Environmental Science and Biotechnology*, **12**(2), 153–164.
- Rawat, I., Ranjith Kumar, R., Mutanda, T., and Bux, F. (2013) Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, **103**, 444–467. [online] <http://dx.doi.org/10.1016/j.apenergy.2012.10.004>.
- Rodero, M. del R., Lebrero, R., Serrano, E., Lara, E., Arbib, Z., García-Encina, P. A., and Muñoz, R. (2019) Technology validation of photosynthetic biogas upgrading in a semi-industrial scale algal-bacterial photobioreactor. *Bioresource Technology*, **279**(January), 43–49. [online] <https://doi.org/10.1016/j.biortech.2019.01.110>.
- Rogers, J. N., Rosenberg, J. N., Guzman, B. J., Oh, V. H., Mimbela, L. E., Ghassemi, A., Betenbaugh, M. J., Oyler, G. A., and Donohue, M. D. (2014) A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. *Algal Research*, **4**(1), 76–88. [online] <http://dx.doi.org/10.1016/j.algal.2013.11.007>.
- Romero Villegas, G. I., Fiamengo, M., Acién Fernández, F. G., and Molina Grima, E. (2017) Outdoor production of microalgae biomass at pilot-scale in seawater using centrate as the nutrient source. *Algal Research*, **25**(April), 538–548. [online] <http://dx.doi.org/10.1016/j.algal.2017.06.016>.
- da Rosa, M. D. H., Alves, C. J., dos Santos, F. N., de Souza, A. O., Zavareze, E. da R., Pinto, E., Nosedá, M. D., Ramos, D., and de Pereira, C. M. P. (2023) Macroalgae and Microalgae Biomass as Feedstock for Products Applied to Bioenergy and Food Industry: A Brief Review. *Energies*, **16**(4), 1–14.
- Ruas, G., Farias, S. L., Nantes, M. A., Serejo, M. L., Henrique, G., and Boncz, M. Á. (2022) CO₂ Addition and Semicontinuous Feed Regime in Shaded HRAP — Pathogen Removal Performance. *Water*, **14**(24), 4047.
- Ruas, G., Farias, S. L., Reis, B. A. B., Serejo, M. L., Henrique, G., and Boncz, M. Á. (2023) 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., Farias, S. L., Scarcelli, P. G., Serejo, M. L., and Boncz, M. A. (2020) The effect of CO₂ addition and hydraulic retention time on pathogens removal in HRAPs. *Water Science and Technology*, **82**(6), 1184–1192.
- Ruas, G., López-Serna, R., Scarcelli, P. G., Serejo, M. L., Boncz, M. Á., and Muñoz, R. (2022) Influence of the hydraulic retention time on the removal of emerging contaminants in an anoxic-aerobic algal-bacterial photobioreactor coupled with anaerobic digestion. *Science of the Total Environment*, **827**, 154262.
- Ruas, G., Serejo, M. L., Farias, S. L., Scarcelli, P., and Boncz, M. Á. (2021) Removal of pathogens from domestic wastewater by microalgal - bacterial systems under different cultivation conditions. *International Journal of Environmental Science and Technology*, (0123456789). [online] <https://doi.org/10.1007/s13762-021-03820-2>.
- Ruas, G., Serejo, M. L., Paulo, P. L., and Boncz, M. Á. (2018) Evaluation of domestic wastewater treatment using microalgal-bacterial processes: effect of CO₂ addition on pathogen removal. *Journal of Applied Phycology*, 1–9.
- Sánchez Zurano, A., Garrido Cárdenas, J. A., Gómez Serrano, C., Morales Amaral, M., Acién-Fernández, F. G., Fernández Sevilla, J. M., and Molina Grima, E. (2020) Year-long assessment of a pilot-scale thin-layer reactor for microalgae wastewater treatment. Variation in the microalgae-bacteria consortium and the impact of environmental conditions. *Algal Research*, **50**(July), 101983. [online] <https://doi.org/10.1016/j.algal.2020.101983>.
- Scarcelli, P. G., Ruas, G., Lopez-sera, R., Serejo, M. L., Blanco, S., Boncz, M. Á., and Muñoz, R. (2021) Integration of algae-based sewage treatment with anaerobic digestion of the bacterial-algal biomass and biogas upgrading. *Bioresource Technology*, **340**(July), 125552.
- Serejo, M. L., Farias, S. L., Ruas, G., Paulo, P. L., and Boncz, M. A. (2020) Surfactant removal and biomass production in a microalgal-bacterial process: effect of feeding regime. *Water Science and Technology*, **82**(6), 1176–1183.
- Serejo, M. L., Posadas, E., Boncz, M. A., Blanco, S., García-Encina, P., and Muñoz, R. (2015) Influence of biogas flow rate on biomass composition during the optimization of biogas upgrading in microalgal-bacterial processes. *Environmental Science and Technology*, **49**(5), 3228–3236.
- Severo, I. A., de Lira, G. S., Ambati, R. R., Gokare, R. A., Vargas, J. V. C., Ordonez, J., and Mariano, A. B. (2024) Disruptive potential of microalgae proteins: Shaping the future of the food industry. *Future Foods*, **9**(February).
- Sheehan, J. D., Dunahay, T., Benemann, J. R., and Roessler, P. (1998) *A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae*, [online] <http://www.springerlink.com/index/10.1140/epjc/s10052-012-2043-9%5Cnhttp://arxiv.org/abs/1203.5015>.
- Silambarasan, S., Logeswari, P., Sivaramakrishnan, R., Incharoensakdi, A., Kamaraj, B., and Cornejo, P. (2023) *Scenedesmus* sp. strain SD07 cultivation in municipal wastewater for pollutant removal and production of lipid and exopolysaccharides. *Environmental Research*, **218**(September 2022), 115051. [online] <https://doi.org/10.1016/j.envres.2022.115051>.
- Silveira, E. O., Lutterbeck, C. A., Machado, Ê. L., Rodrigues, L. R., Rieger, A., Beckenkamp, F., and Lobo, E. A. (2020) Biomonitoring of urban wastewaters treated by an integrated

- system combining microalgae and constructed wetlands. *Science of the Total Environment*, **705**(xxxx), 135864. [online] <https://doi.org/10.1016/j.scitotenv.2019.135864>.
- Stazi, V. and Tomei, M. C. (2021) Dissolved methane in anaerobic effluents: A review on sustainable strategies for optimization of energy recovery or internal process reuse. *Journal of Cleaner Production*, **317**(April), 128359. [online] <https://doi.org/10.1016/j.jclepro.2021.128359>.
- Stiles, W. A. V., Styles, D., Chapman, S. P., Esteves, S., Bywater, A., Melville, L., Silkina, A., Lupatsch, I., Fuentes Grünewald, C., Lovitt, R., Chaloner, T., Bull, A., Morris, C., and Llewellyn, C. A. (2018) Using microalgae in the circular economy to valorise anaerobic digestate: challenges and opportunities. *Bioresource Technology*, **267**(June), 732–742. [online] <https://doi.org/10.1016/j.biortech.2018.07.100>.
- Suganya, T., Varman, M., Masjuki, H. H., and Renganathan, S. (2016) Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach. *Renewable and Sustainable Energy Reviews*, **55**, 909–941. [online] <http://dx.doi.org/10.1016/j.rser.2015.11.026>.
- Sutherland, D. L., Howard-Williams, C., Turnbull, M. H., Broady, P. A., and Craggs, R. J. (2015) The effects of CO₂ addition along a pH gradient on wastewater microalgal photo-physiology, biomass production and nutrient removal. *Water Research*, **70**, 9–26. [online] <http://dx.doi.org/10.1016/j.watres.2014.10.064>.
- Sutherland, D. L. and Ralph, P. J. (2020) 15 years of research on wastewater treatment high rate algal ponds in New Zealand: discoveries and future directions. *New Zealand Journal of Botany*, **0**(0), 1–24. [online] <https://doi.org/10.1080/0028825X.2020.1756860>.
- Sutherland, D. L. and Ralph, P. J. (2021) Shortening hydraulic retention time through effluent recycling: impacts on wastewater treatment and biomass production in microalgal treatment systems. *Journal of Applied Phycology*, **33**(6), 3873–3884. [online] <https://doi.org/10.1007/s10811-021-02573-2>.
- Sutherland, D. L., Turnbull, M. H., Broady, P. A., and Craggs, R. J. (2014) Effects of two different nutrient loads on microalgal production, nutrient removal and photosynthetic efficiency in pilot-scale wastewater high rate algal ponds. *Water Research*, **66**, 53–62. [online] <http://dx.doi.org/10.1016/j.watres.2014.08.010>.
- Sutherland, D. L., Turnbull, M. H., and Craggs, R. J. (2017) Environmental drivers that influence microalgal species in fullscale wastewater treatment high rate algal ponds. *Water Research*, **124**, 504–512. [online] <http://dx.doi.org/10.1016/j.watres.2017.08.012>.
- Sutherland, D. L., Turnbull, M. H., and Craggs, R. J. (2014) Increased pond depth improves algal productivity and nutrient removal in wastewater treatment high rate algal ponds. *Water Research*, **53**, 271–281.
- Torres-Franco, A., Passos, F., Figueredo, C., Mota, C., and Muñoz, R. (2021) Current advances in microalgae-based treatment of high-strength wastewaters: challenges and opportunities to enhance wastewater treatment performance. *Reviews in Environmental Science and Biotechnology*, **20**(1), 209–235.
- Udom, I., Zaribaf, B. H., Halfhide, T., Gillie, B., Dalrymple, O., Zhang, Q., and Ergas, S. J. (2013) Harvesting microalgae grown on wastewater. *Bioresource Technology*, **139**, 101–106. [online] <http://dx.doi.org/10.1016/j.biortech.2013.04.002>.

- Vasan, V., Sridharan, N. V., Feroskhan, M., Vaithiyanathan, S., Subramanian, B., Tsai, P. C., Lin, Y. C., Lay, C. H., Wang, C. T., and Ponnusamy, V. K. (2024) Biogas production and its utilization in internal combustion engines - A review. *Process Safety and Environmental Protection*, **186**(November 2023), 518–539. [online] <https://doi.org/10.1016/j.psep.2024.04.014>.
- Vassalle, L., Ferrer, I., Passos, F., Filho, C. R. M., and Garfí, M. (2023) Nature-based solutions for wastewater treatment and bioenergy recovery: A comparative Life Cycle Assessment. *Science of the Total Environment*, **880**(April).
- Villarro-Cos, S., Cuaresma Franco, M., García-Vaquero, M., Morán, L., Alarcón, F. J., and Lafarga, T. (2024) Composition of microalgae produced using different types of water and nutrient sources. *Algal Research*, **78**(January), 0–7.
- Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., and Ruan, R. (2010) Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology*, **162**(4), 1174–1186.
- Wijffels, R. H. and Barbosa, M. J. (2010) Perspective. An Outlook on Microalgal Biofuels. *Science*, **329.5993**(August), 796–799.
- Wilde, E. W. and Benemann, J. R. (1993) Bioremoval of heavy metals by the use of microalgae. *Biotechnology advances*, **11**(4), 781–812.
- Wilkie, A. C., Edmundson, S. J., and Duncan, J. G. (2011) Indigenous algae for local bioresource production: Phycoprospecting. *Energy for Sustainable Development*, **15**(4), 365–371. [online] <http://dx.doi.org/10.1016/j.esd.2011.07.010>.
- Xu, M., Li, P., Tang, T., and Hu, Z. (2015) Roles of SRT and HRT of an algal membrane bioreactor system with a tanks-in-series configuration for secondary wastewater effluent polishing. *Ecological Engineering*, **85**, 257–264. [online] <http://dx.doi.org/10.1016/j.ecoleng.2015.09.064>.
- Yadav, G., Dubey, B. K., and Sen, R. (2020) A comparative life cycle assessment of microalgae production by CO₂ sequestration from flue gas in outdoor raceway ponds under batch and semi-continuous regime. *Journal of Cleaner Production*, **258**, 120703. [online] <https://doi.org/10.1016/j.jclepro.2020.120703>.
- Yang, J., van Lier, J. B., Li, J., Guo, J., and Fang, F. (2022) Integrated anaerobic and algal bioreactors: A promising conceptual alternative approach for conventional sewage treatment. *Bioresource Technology*, **343**(August 2021), 126115. [online] <https://doi.org/10.1016/j.biortech.2021.126115>.
- Yap, J. K., Sankaran, R., Chew, K. W., Halimatul Munawaroh, H. S., Ho, S.-H., Rajesh Banu, J., and Show, P. L. (2021) Advancement of green technologies: A comprehensive review on the potential application of microalgae biomass. *Chemosphere*, **281**(April), 130886. [online] <https://doi.org/10.1016/j.chemosphere.2021.130886>.
- Yu, H., Ko, D., and Lee, C. (2023) Continuous cultivation of mixed-culture microalgae using anaerobic digestion effluent in photobioreactors with different strategies for adjusting nitrogen loading rate. *Bioresource Technology*, **387**(August), 129650. [online] <https://doi.org/10.1016/j.biortech.2023.129650>.
- Zhu, S., Qin, L., Feng, P., Shang, C., Wang, Z., and Yuan, Z. (2019) Treatment of low C/N ratio wastewater and biomass production using co-culture of *Chlorella vulgaris* and

activated sludge in a batch photobioreactor. *Bioresource Technology*, **274**(August 2018), 313–320. [online] <https://doi.org/10.1016/j.biortech.2018.10.034>.

Ziganshina, E. E., Bulynina, S. S., Yureva, K. A., and Ziganshin, A. M. (2022) Growth Parameters of Various Green Microalgae Species in Effluent from Biogas Reactors: The Importance of Effluent Concentration. *Plants*, **11**(24)

CHAPTER 2 :

COMPARATIVE STUDY OF DIFFERENT CULTIVATION CONDITIONS IN THE REMOVAL OF SURFACTANTS FROM SEWAGE BY MICROALGAE-BACTERIA SYSTEMS²

ABSTRACT

In this work, the influence of different cultivation arrangements on the removal of anionic surfactant in a microalgae-bacteria system was evaluated. Batch experiments using synthetic sewage and addition of 20 mg L⁻¹ Linear Alkylbenzene Sulfonate were carried out varying inoculum conditions (microalgae and/or bacteria and without inoculum, light and dark) and operating parameters (pH adjustment, addition of carbon by means of aeration, supply of carbon dioxide carbon and of organic carbon source, and use of a combined anoxic-aerobic system). The best result was obtained under the condition where microalgae and bacteria were cultivated with aeration, with a removal efficiency of $97 \pm 1\%$. Slightly lower removal efficiencies were achieved under the conditions with pH adjustment and CO₂ supplementation, with 87 ± 11 and $77 \pm 10\%$, respectively.

INTRODUCTION

The global production and use of chemical compounds, such as surfactants, has increased significantly, with anionic surfactants being the most prevalent, followed by nonionic and cationic types (Aloui, Kchaou, et al., 2009). Among the surfactants, linear alkylbenzene sulfonate (LAS) stands out as one of the most widely used, accounting for 25–30% of global surfactant production (Askari, Vahabzadeh, et al., 2021). Its widespread application is driven by its high detergent efficiency and cost-effectiveness, making it a fundamental ingredient in both industrial and household cleaning formulations (Pirsaheb, Khamutian, et al., 2014).

Due to their widespread use in household products, surfactants, classified as emerging pollutants, inevitably enter wastewater. The COVID-19 pandemic further increased the use of cleaning and disinfecting agents, resulting in even higher concentrations in wastewater (Wang, Liu, et al., 2023). In wastewater treatment plants, surfactants can compromise system performance by inducing foam formation, exerting biological toxicity, causing competitive inhibition in activated sludge, reducing substrate affinity, and increasing effluent chemical oxygen demand (Macedo, Silva, et al., 2019). Their persistence, particularly under anaerobic

² Short version presented at the IWA 13th Wastewater Pond and Algal Technology Conference, July 2022, Melbourne, Australia.

conditions, poses a challenge for wastewater treatment (McDonough, Casteel, et al., 2016). While biodegradable under aerobic conditions, they can still disrupt microbial activity and impair treatment efficiency (Zhang, Zhang, et al., 2021).

The microalgae-bacteria system is a sustainable and cost-effective technology with proven efficiency in removing various pollutants, including surfactants (Serejo, Farias, et al., 2020). In addition to wastewater treatment, these systems contribute to carbon sequestration and bioresource recovery, which make them a promising alternative for environmental management (Fernández, Reis, et al., 2021). Their effectiveness is attributed to the synergistic interactions between microalgae and bacteria, which enhance pollutant degradation, nutrient removal, and biomass production. However, enhancing system performance requires understanding the removal mechanisms and refining operational parameters, which are explored in this work.

LAS biodegradation in wastewater treatment systems is driven by microorganisms, with aerobic processes leading to mineralization into CO_2 and H_2O , and anaerobic conditions relying on electron acceptors like sulfate, nitrate, and carbonate to produce H_2S , N_2 , CH_4 , and NH_3 (Askari, Vahabzadeh, et al., 2021; Zhu, Ma, et al., 2018). Studying these microbial mechanisms is essential for optimizing surfactant removal. Furthermore, the removal of surfactants in microalgae-bacteria systems, and understanding the roles of both microorganisms, requires further exploration.

Among the operational parameters tested in this study, CO_2 supplementation stands out for enhancing carbon availability, optimizing pH for microalgae growth, and preventing nutrient loss processes such as ammonia volatilization and phosphate precipitation at high pH levels (Uggetti, Sialve, et al., 2018). pH control, whether through CO_2 supplementation or direct acid addition, is a critical parameter, as it not only regulates nutrient availability and CO_2 solubility for algal growth, but also influences microbial enzymatic activity and the solubility of environmental micropollutants, thereby enhancing overall treatment efficiency (Shahid, Malik, et al., 2020). Additionally, atmospheric air injection supports aerobic microbial processes, promoting microalgae development, organic matter degradation, and improving overall treatment efficiency (Ruas, Farias, et al., 2023; Dammak, Fersi, et al., 2023).

Additionally, the study evaluated the addition of a carbon source to promote cometabolism, which can improve the degradation of recalcitrant compounds (Macedo, Okada, et al., 2015; Motteran, Nadai, et al., 2018). Other key parameters included the integration of an anoxic system to facilitate denitrification and anaerobic degradation pathways, as denitrifying

bacteria can degrade xenobiotic compounds by coupling their metabolism to nitrogen reduction (Andrade, Sakamoto, et al., 2017). Integrated anoxic-aerobic photobioreactor systems have proven to be effective in removing emerging pollutants, enhancing denitrification processes and photosynthetic oxygenation, which in turn support biodegradation as a key mechanism (López-Serna, Posadas, et al., 2019).

These parameters are critical for optimizing system performance, ensuring stability, efficiency, and scalability for large-scale applications. Thus, the objective of this work was to determine the influence of culture type (microalgae and/or bacteria), organic carbon addition, anoxic-aerobic operation, pH control, and CO₂ and atmospheric air supplementation to identify the optimal conditions for anionic surfactant removal in a microalgae-bacteria system.

MATERIAL AND METHODS

Synthetic wastewater and microalgal-bacterial inoculum

To ensure consistency in composition, synthetic sewage was utilized as the matrix. It was prepared using tap water and had the following composition (in g L⁻¹): peptone (0.16), meat extract (0.11), urea (0.03), NaCl (0.007), CaCl₂·2H₂O (0.004), K₂HPO₄ (0.028), MgSO₄·7H₂O (0.002), CuCl₂·2H₂O (0.000005), glucose (0.25), and NaHCO₃ (1.1) (Frutos, Quijano, et al., 2016). The surfactant Linear Alkylbenzene Sulfonate (LAS) was added at a concentration of 20 mg L⁻¹, determined based on the average levels quantified in the literature for domestic wastewater (Pirsaheb, Khamutian, et al., 2014; Serejo, Farias, et al., 2020). This concentration was preliminarily tested to compare degradation performance with other concentrations and was prepared using a 70% Dodecylbenzene Sulfonic Acid reagent. (Aldrich). The average composition of synthetic sewage is presented in Table 2-1 ((Frutos, Quijano, et al., 2016; Ruas, López-Serna, et al., 2022).

Table 2-1 - Physical-chemical characterization of the synthetic wastewater.

Parameter	Unit	Value
Surfactant	mg L ⁻¹	20 ± 0.5
COD	mg L ⁻¹	529± 22
TOC	mg L ⁻¹	256 ± 23
IC	mg L ⁻¹	179 ± 16
TN	mg L ⁻¹	54 ± 3
N-NH ₄ ⁺	mg L ⁻¹	4.7 ± 4.3
P-PO ₄ ³⁻	mg L ⁻¹	12 ± 3
pH	-	8.2 ± 0.1

The microalgae used in this experiment were sourced from an outdoor High Rate Algal Pond (HRAP) treating domestic wastewater, with a total suspended solids (TSS) concentration of 7.1 g L^{-1} . They were then adapted in a glass bottle containing synthetic sewage. They were subsequently acclimated in a glass flask containing synthetic sewage, aerated with compressed air, and magnetically stirred at 200 rpm under a 16:8 h light-dark cycle. Illumination was provided by a LED reflector (100 W cold white), emitting $\approx 597 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At the end of the acclimation period, *Scenedesmus* sp. was identified as the predominant species, with an initial TSS concentration of 6.3 g L^{-1} . The nitrifying-denitrifying bacteria (TSS = 7.7 g L^{-1}) were obtained from aerobic activated sludge collected from a municipal wastewater treatment plant (WWTP).

Experimental conditions

Initially, the system consisted of cylindrical continuous stirred-tank (CSTR) photobioreactors (PBRs), each constructed from acrylic, with a working volume of 3.0 L. Illumination was provided by a 100 W cold white LED reflector, delivering an average light intensity of $\approx 597 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a photoperiod of 16:8 hours light:dark, as shown in Figure 2-1. The PBRs were inoculated with a consortium of pre-adapted microalgae and nitrifying-denitrifying activated sludge. The system operated in batch mode, with continuous agitation maintained by a mechanical mixer at 200 rpm, promoting optimal contact between the microorganisms and wastewater.

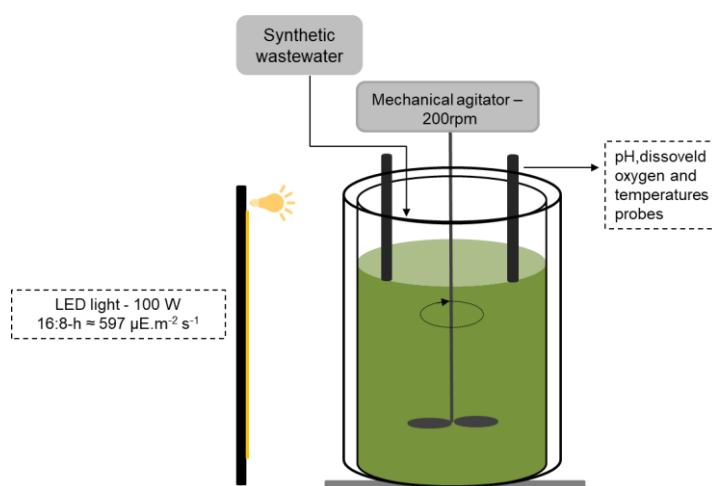


Figure 2-1 - Initial experimental configuration of CSTR type reactor operated in batch and fed with synthetic sewage.

To identify the optimal conditions for reactor performance in surfactant removal, four bench-scale tests were conducted, as detailed in Figure 2-2. These tests were specifically designed to evaluate the impact of different operating parameters and cultivation conditions on the efficiency of surfactant removal from domestic wastewater. Each experimental setup aimed to

isolate and assess a key factor, such as microbial composition, carbon source addition, pH control, and aeration strategy, providing a comprehensive understanding of their individual and combined effects on treatment performance.

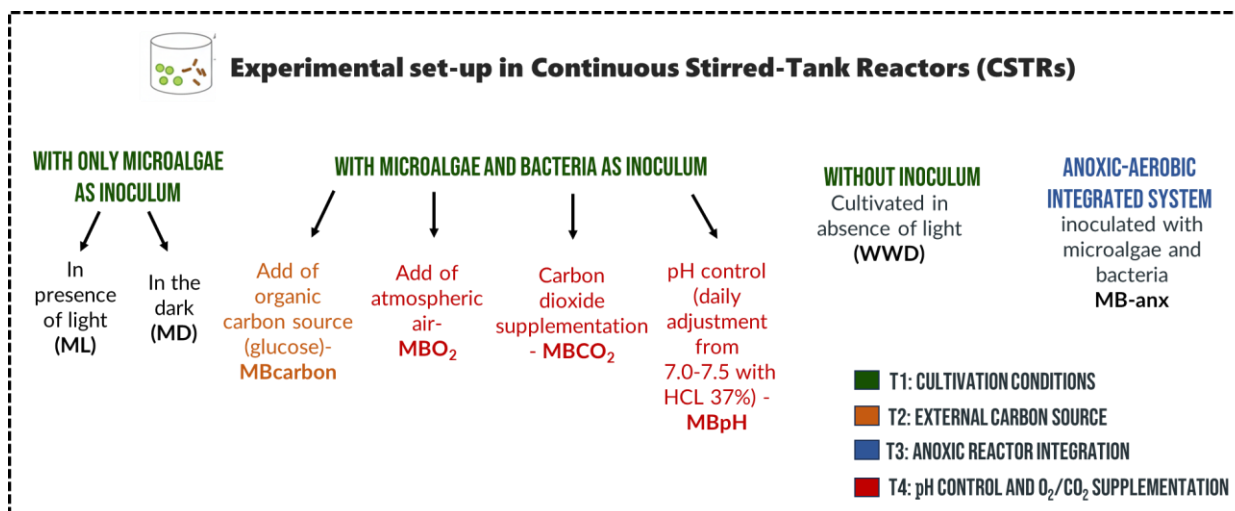


Figure 2-2 - Description of the four tests that were performed under different operating conditions and cultivation regimes in continuous stirred-tank reactors (CSTR) within a microalgae–bacteria system.

Test 1 - Variation of Cultivation conditions

In this section, we investigated how varying cultivation conditions influence the performance of the microalgae-bacteria system in the removal of anionic surfactants, with the aim of better understanding the roles of microalgae and bacteria under different conditions. Each PBR was inoculated with distinct combinations: microalgae only (exposed to both light and dark conditions), microalgae and bacteria, bacteria only (also under both light and dark conditions), and a control group without inoculum (subjected to light and dark conditions as well), as presented in Figure 2-2.

Operating Conditions Tests

Test 2 - Addition of carbon source for analysis of microalgae cometabolism

This test was designed based on studies highlighting the potential of microalgae cometabolism in the removal of micropollutants, facilitated by the excretion of extracellular polymeric substances and other enzymes (Phong Vo, Ngo, et al., 2020; Tran, Urase, et al., 2013). To evaluate this phenomenon, glucose (C₆H₁₂O₆) was added as a carbon source at a concentration of 300 mg L⁻¹, given its prominence as a cosubstrate for this purpose (Phong Vo, Ngo, et al., 2020).

Test 3- Integration of an anoxic reactor into the system

In this phase, an anoxic reactor was connected to the CSTR reactor to evaluate its influence on surfactant removal, as well as to assist in nitrogen removal through denitrification and promote the development of a microalgae population with higher sedimentation rates (García, Alcántara, et al., 2017). The experimental setup is illustrated in Figure 2-3. The anoxic reactor, with an effective volume of 1 L, was kept in complete darkness. The culture broth from the CSTR was continuously recirculated to the anoxic reactor at a flow rate of 0.48 L d^{-1} (Watson Marlow 505U peristaltic pump, UK), returning to the CSTR by gravity.

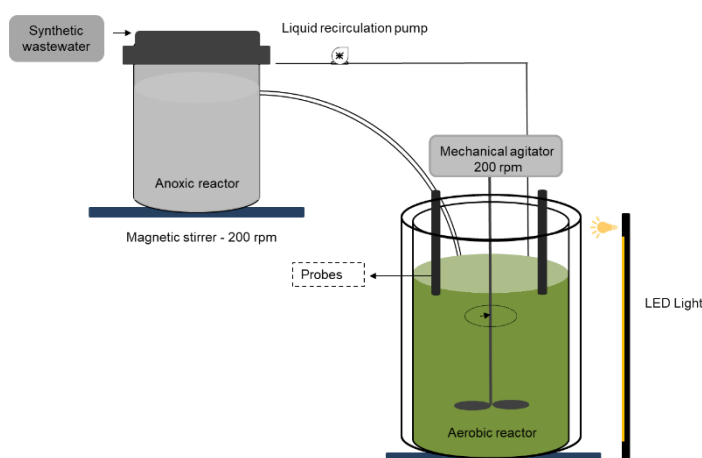


Figure 2-3 - A schematic diagram of the test phase for the integrated anoxic-aerobic system.

Test 4 - Assessment of pH control and CO₂ and O₂ supplementation

In this test, the system's performance was assessed under controlled pH conditions: the pH was maintained within the range of 7.0-7.5. In reactor MBpH, this was done by adding 37% hydrochloric acid. In reactor MBCO₂, the treatment efficiency was evaluated using CO₂ supplementation. CO₂ was supplied using a synthetic gas mixture composed of N₂ (70%) and CO₂ (30%) (White Martins, Brazil), injected at a flow rate of $11.27 \text{ ml min}^{-1}$, corresponding to 2.24 L d^{-1} of carbon dioxide (Watson Marlow 505U feed pump, UK). A third reactor, MBO₂ was kept aerated, with a continuous supply of atmospheric air at a rate of 20.7 L d^{-1} (Sarlobetter pump, Brazil).

Sampling and Analytical Procedures

The pH was monitored continuously throughout the experiment, while dissolved oxygen (DO), temperature, and turbidity were measured daily to ensure stable operating conditions. Surfactant analyses were performed daily during the stationary phase of microalgae growth, as this stage represents the period of maximum metabolic activity and potential

biodegradation. Consistent monitoring of these parameters was essential for assessing system performance and identifying correlations between environmental conditions and surfactant removal efficiency.

All analytical procedures followed the guidelines set forth in the Standard Methods for the Examination of Water and Wastewater (APHA, 2017). Light intensity was measured using a PAR MQ-200 radiation meter (Apogee Instruments, USA). DO and pH measurements were taken with Hanna HI2004-01 and HI2001-01 bench meters, respectively (Hanna Instruments, USA). Turbidity was assessed using a Hanna HI 98703-01 turbidimeter (Hanna Instruments, USA). Microalgae identification was performed through microscopic examination (Olympus BX41, USA), with samples fixed in 5% Lugol's solution and stored at 4 °C prior to analysis, following the methodology of Sournia (1978).

Analysis of anionic surfactants was carried out using a simplified procedure developed by Jurado, Fernández-Serrano, et al. (2006). The method involves adding 5 mL of the sample to a glass tube, followed by the sequential addition of the following reagents: 2 drops of phenolphthalein, 200 μL of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ buffer solution, 100 μL of methylene blue, and 4 mL of chloroform. The mixture is then vigorously shaken for 1 minute and refrigerated for 1 hour. The final step involved measuring the absorbance at $\lambda = 652 \text{ nm}$ using a DR 3900 spectrophotometer (Hach, Germany), with results interpreted based on a pre-established calibration curve. Biomass growth rates ($\text{mg TSS L}^{-1} \text{ d}^{-1}$) were determined by calculating the slope of total suspended solids (TSS) plotted against time.

Statistical analysis

Statistical analysis of the data was performed using the R project software (<https://www.r-project.org/>). The normality of all the data sets was checked using the Shapiro–Wilk test, and the comparison of means was conducted by analysis of variance (ANOVA) followed by the Tuckey hypothesis test, at a 95% confidence level. In. Pearson correlation analysis was also employed to assess the influence of environmental parameters on the observed removal efficiencies.

RESULTS AND DISCUSSION

Surfactant removal efficiencies

Cultivation Conditions

The highest removal efficiencies were achieved under the MB and BD conditions, reaching $76 \pm 7\%$ and $76 \pm 13\%$, respectively, as shown in Figure 2-4 (left). These values, particularly

for the BD condition—where only bacteria were inoculated under dark conditions—underscore the significant role of bacteria in surfactant degradation. The bacterial removal mechanism involves breaking down the surfactant's molecular properties, utilizing its degradation products as a source of carbon and energy for microbial metabolism (Aloui, Kchaou, et al., 2009).

The efficiency observed under the MB condition, where both microalgae and bacteria were present, suggests a synergistic effect likely driven by the complementary metabolic activities of these microorganisms (Pandey, Katam, et al., 2020). In this combined setup, microalgae may have enhanced bacterial activity by providing oxygen and organic substrates as byproducts of photosynthesis, fostering an environment that promotes bacterial breakdown of surfactants. This symbiotic interaction between microalgae and bacteria appears to be a key factor, amplifying the overall removal efficiency compared to conditions where each microorganism acted independently. Katam, Shimizu, et al. (2020), when evaluating the performance of trickling filters, compared LAS and caffeine removal under light and dark conditions, emphasizing the benefits of algal-bacterial symbiosis, where microalgal activity enhanced bacterial processes, achieving up to 99% LAS removal under light conditions with these inocula.

Reinforcing these findings, Figure 2-4 (right) illustrates faster degradation in the MB condition, showing a marked decline after the third day, contrasting with observed in the BD condition, which exhibited a longer degradation time. This behavior aligns with findings from Serejo, Farias, et al. (2020), who observed a significant reduction in surfactant concentrations, from $1.5 \pm 0.3 \text{ mg L}^{-1}$ to $0.3 \pm 0.1 \text{ mg L}^{-1}$, after 24 hours of treatment using a microalgal-bacterial process in domestic wastewater. Wang, Liu, et al. (2023), reported comparable differences in the speed of surfactant removal when comparing the performance of algal-bacterial aerobic granular sludge with bacterial aerobic granular sludge for treating wastewater containing surfactant (SDS). Their study demonstrated significant removals of SDS at both low and high concentrations, achieving approximately 98% removal within 90 minutes.

Under MD conditions, removal efficiency of 55 ± 4 demonstrated the ability of microalgae to perform heterotrophic degradation. Although typically associated with photosynthetic activity, microalgae can adapt to mixotrophic or heterotrophic pathways in the absence of light, utilizing organic carbon sources present in wastewater (Nirmalakhandan, Selvaratnam, et al., 2019). This metabolic flexibility allows them to directly degrade surfactants or enhance

their removal by assimilating intermediate byproducts generated during biodegradation. Supporting this, Hena, Abida, et al. (2015), demonstrated the high efficiency of facultative heterotrophic microalgae in removing surfactants and nutrients from municipal wastewater, with strains such as *Scenedesmus* sp., *Chlamydomonas* sp., and *Chlorella* sp. achieving removal rates exceeding 90%.

The results observed under the microalgae condition with light exposure were no longer significant, showing a removal efficiency of $46 \pm 8\%$. This suggests that the efficiency is higher in the absence of light when microalgae are used as the sole inoculum. In a study by Pandey, Katam, et al. (2020), higher removal efficiencies were achieved, reaching up to 94% of LAS removal in green microalgae cultivated in three different system types while treating laundry wastewater, similar to the 95% removal observed under the condition with a combined inoculum of green microalgae and activated sludge.

The significant LAS removal observed under anaerobic conditions (MD and BD) underscores the potential of anaerobic processes to degrade surfactant, addressing gaps in the understanding of its environmental fate under such conditions, as documented in the literature (Camacho-Muñoz, Martín, et al., 2014; Mungray and Kumar, 2008; Palmer and Hatley, 2018). Under anaerobic conditions, LAS degradation depends on alternative electron acceptors such as sulfate, nitrate, or carbonate and can occur under specific conditions, such as sulfur limitation, thermophilic environments, or the use of nitrate as an electron acceptor, but it is often limited by adsorption in anaerobic sludges rather than true biodegradation. (Zhu, Ma, et al., 2018; Mungray and Kumar, 2008)

Motteran, Nadai, et al., (2018), valuated LAS degradation in a fluidized bed reactor with a diverse microbial consortium, using ethanol and nonionic surfactants as co-substrates, highlighting metabolic interdependence where byproducts like sulfonate groups supported versatile microorganisms. Similarly, Askari, Vahabzadeh, et al. (2021), observed that LAS degradation under anaerobic conditions likely follows a distinct pathway starting with a hydrolytic phase, followed by beta-oxidation, desulfonation, and benzene ring cleavage, resembling anaerobic digestion stages.

In the condition with only wastewater and no inoculum of bacteria or algae, the system was unable to remove surfactants, with $7 \pm 7\%$. This result underscores the critical role of microbial inocula in the degradation process. In wastewater treatment, bacteria and algae serve as key agents in breaking down organic pollutants, including surfactants. A condition consisting of only wastewater under light exposure was also tested to evaluate whether

surfactant removal could occur via photodegradation. However, on the 6th day of the experiment, microalgae growth began to occur, compromising the maintenance of this controlled condition. It is noteworthy that, up to the day prior to the contamination, surfactant removal was insignificant ($3 \pm 4\%$), indicating that photodegradation was not a relevant mechanism for surfactant removal in this system. Similar results were observed by Hua, Li, et al. (2012), who investigated LAS degradation in laboratory batch experiments with light and natural biofilms, revealing that direct photolysis played a negligible role in its removal.

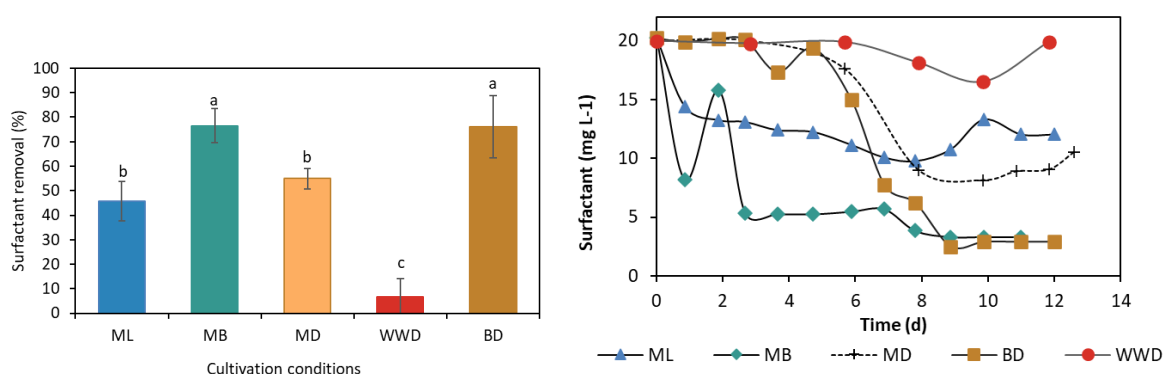


Figure 2-4 - Left: Surfactant removal efficiency percentages in the cultivation variation stage represented by their mean values and standard deviations ($n=4$). Means followed by the same letter do not differ statistically; Right: Temporal evolution of surfactant concentrations (LAS) in the different culture conditions studied ($n=12$).

Operational Conditions

Results highlighted the MBO₂ condition, characterized by the combination of microalgae and bacterial inoculation with an atmospheric air supply, which achieved the highest removal efficiency of $96 \pm 1\%$, as illustrated in Figure 2-5 (left). This condition also demonstrated faster degradation, with LAS concentrations significantly decreasing by the fourth day of cultivation and stabilizing at 0.6 mg L^{-1} from the tenth day onward (Figure 2-5, right). While this performance was outstanding, similar removal efficiencies were observed in the MBpH condition ($85 \pm 12\%$) and the MBCO₂ condition ($80 \pm 10\%$), with no statistically significant difference between them ($p > 0.05$).

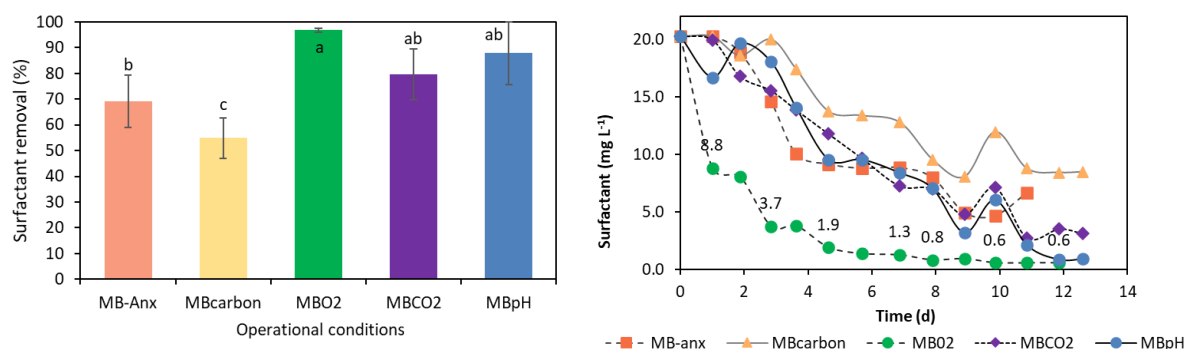


Figure 2-5 - Left: Surfactant removal efficiency in percentage at varying operating conditions stage represented by their mean values and standard deviations (n=6). Means followed by the same letter do not statistically differ; Right: Time courses of the concentrations of surfactant (LAS) in the different culture conditions studied (n=12).

CO₂ supplementation and atmospheric air injection is commonly used in microalgae-based systems treating domestic wastewater to supply carbon, maintain neutral pH, and prevent ammonia toxicity and phosphorus precipitation (Park and Craggs, 2010). It has also been explored to enhance the removal of pollutants such as nutrients, pathogens, and micropollutants (Ruas, Farias, et al., 2022; Ruas, Farias, et al., 2023; Posadas, Morales, et al., 2015; Matamoros, Uggetti, et al., 2016). In this study, CO₂/O₂ supplementation had a significant impact, but the similarity with the MBpH condition indicates that it was related to pH control in the medium.

The pH was identified as the parameter most strongly correlated with surfactant removal (Spearman's correlation coefficient $\rho = -0.7905$, p-value = 0.1114), showing a negative association. This indicates that surfactant removal was more effective under lower pH conditions, as observed in treatments such as MBO₂, MBCO₂, and MBpH (Table 2-2). Acidic conditions are known to enhance the metabolic activity of microorganisms, promoting pathways that facilitate surfactant degradation. The surfactant degradation process itself contributes to pH reduction through the generation of protons (Askari, Vahabzadeh, et al., 2021). Despite this, the pH in these conditions did not drop excessively due to the autotrophic activity of microalgae, which tends to increase the pH of the medium (Wang, Liu, et al., 2023).

In the condition with the carbon source treatment yielded a lower removal efficiency of $55 \pm 7\%$, highlighting that, for our study, the addition of an organic carbon source was not the most effective condition tested. In contrast, Phong Vo, Ngo, et al. (2020), observed an increase in efficiency, demonstrating that the addition of selective sole carbon sources in the

presence of micropollutants enhanced extracellular polymeric substances (EPS) and enzyme (superoxide dismutase and peroxidase) production by 2 to 100 times compared to carbon sources alone, resulting in significantly improved micropollutant removal by microalgae. Additionally, (Macedo, Silva, et al. (2019), investigated the use of ethanol as an organic carbon source for LAS degradation and found that its addition favored a smaller decrease in the specific substrate utilization rate, even at LAS concentrations typically considered inhibitory ($>14.4 \text{ mg L}^{-1}$), achieving up to 98.8% LAS removal efficiency.

Finally, the results obtained for the combined anoxic-aerobic algal-bacterial system demonstrate that, although initially designed to enhance nutrient removal, this reactor configuration also shows significant potential for removing other pollutants, including surfactants. The system achieved an overall removal efficiency of $66 \pm 9\%$, highlighting its effectiveness in pollutant degradation. Comparable removal efficiencies were observed under the MBCO₂ and MBpH conditions, with no statistically significant differences between them ($p > 0.05$), indicating that both aerobic and anoxic phases contribute effectively to the overall removal process. This dual-phase setup facilitates diverse microbial metabolic pathways, enhancing the system's versatility for broader pollutant removal beyond nutrients alone. Andrade, Sakamoto, et al. (2017), further demonstrated that an anoxic system utilizing ethanol as a co-substrate and nitrate as an electron acceptor achieved 99.9% LAS removal in batch reactors, underscoring the critical role of these factors in promoting degradation by denitrifying microorganisms.

Environmental and operational conditions, COD removal efficiency and Productivity

The varying cultivation and operational conditions resulted in distinct pH ranges across the photobioreactors (PBRs), as illustrated in Table 2-2. As anticipated, the highest pH values were recorded in reactors where microalgae were exposed to light, attributable to CO₂ uptake during photosynthesis. Under the tested operational conditions, the introduction of CO₂ (MBCO₂) and oxygen (MBO₂) did not yield significant differences in pH regulation. In contrast, the MBpH condition, which involved the direct addition of acid, successfully maintained the pH within the optimal range of 7.0 to 8.0, demonstrating its effectiveness in pH control.

Regarding dissolved oxygen, it was observed that under the ML condition, inoculated solely with algae and without bacteria to consume oxygen, concentrations were comparable to those in the MB condition, which included both microorganisms. Generally, conditions with both algae and bacteria exhibited higher average dissolved oxygen levels due to the combined

effects of algal photosynthesis and bacterial metabolic activity influencing oxygen dynamics in the medium. However, the MBO₂ condition displayed lower concentrations, maintaining oxygen levels at $7.0 \pm 1.0 \text{ mg L}^{-1}$. Similarly, Ruas, Farias, et al. (2023), observed reduced dissolved oxygen levels under cultivation conditions supplemented with atmospheric air and CO₂, attributing this decrease to the stripping of O₂ from the growth medium.

The results for organic matter removal under the tested cultivation conditions highlight the significant role of bacterial activity in oxidation processes. Notably, the best performance was observed under light-absent conditions, emphasizing the contribution of bacteria in these scenarios. The strong positive and linear correlation between surfactant removal and organic matter degradation (Spearman's correlation coefficient $\rho = 0.6668$, $p\text{-value} = 0.2189$) reinforces the role of heterotrophic bacteria in the simultaneous removal of both pollutants. This correlation was identified by Haggensen, Mogensen et al. (2002), during their study on the anaerobic degradation of LAS in CSTR reactors, where they observed an apparent relationship between the extent of organic matter degradation and the anaerobic transformation of surfactants. Additionally, statistically similar removal efficiencies were achieved in the MD condition, suggesting that the heterotrophic activity of microalgae also contributed to the degradation process (Mohsenpour, Hennige, et al., 2021).

Regarding organic matter removal under the tested operational conditions, the highest efficiency was observed in systems with CO₂/O₂ supplementation and MBpH conditions, achieving COD removal efficiencies (COD-RE) of $78 \pm 1\%$, $83 \pm 1\%$, and $87 \pm 1\%$, respectively. These results suggest that pH played a critical role in influencing the outcomes, likely by enhancing bacterial activity. In contrast, the lowest performance was recorded under the organic carbon supplementation condition, which achieved only $58 \pm 1\%$ COD-RE. Ruas, Serejo, et al. (2021), also reported enhanced COD removal of approximately 95% in microalgae-bacterial photobioreactors (PBRs) with aeration and CO₂ supplementation, as the addition of CO₂ lowered the pH, creating a more favorable environment for both microalgae and bacteria.

Table 2-2 - pH value, dissolved oxygen concentration, temperature, productivity, and chemical oxygen demand (COD) removal achieved under the tested cultivation and operational conditions

Condition	pH	DO	Temperature	Productivity	COD - RE
		mg L ⁻¹	°C	mg L ⁻¹ d ⁻¹	%
Cultivation conditions					
ML	10.8 ± 1.2	9.5 ± 2.6	33 ± 3	95 ± 0.1	50 ± 6
MB	10.7 ± 1.3	11.3 ± 3.9	34 ± 3	101 ± 0.5	61 ± 4
MD	8.3 ± 0.3	1.0 ± 1.5	29 ± 2	5 ± 3	98 ± 1
BD	8.3 ± 0.3	2.7 ± 1.8	29 ± 2	29 ± 0.2	88 ± 2
WWD	7.5 ± 0.8	1.3 ± 2.0	29 ± 2	20 ± 0.6	17 ± 1
Operational Conditions					
MB-anx	10.6 ± 1.4	13.7 ± 6.5	33 ± 2.6	65 ± 2	68 ± 1
MBcarbon	10.5 ± 1.4	11.3 ± 3.5	33 ± 2.5	81 ± 2	58 ± 1
MBO ₂	9.8 ± 1.2	7.0 ± 1.0	33 ± 3.1	125 ± 1	83 ± 1
MBCO ₂	9.7 ± 1	14.9 ± 5.2	33 ± 3.1	126 ± 1	78 ± 1
MBpH	7.4 ± 0.4	10.8 ± 4.8	33 ± 3.1	77 ± 2	87 ± 1

Among the evaluated cultivation conditions, the highest biomass productivity ($101 \pm 2 \text{ mg L}^{-1} \text{ d}^{-1}$) was observed in the microalgae-bacteria system, highlighting the potential of synergistic interactions in optimizing biomass production. Ji, Jiang, et al. (2018), reported a similar trend, observing that the peak chlorophyll-a concentration in a microalgae-bacteria inoculum (1:3) was twice as high as in isolated microalgae cultures, possibly due to bacterial induction of substances that stimulate the expression of genes involved in chlorophyll metabolism.

Although not optimal, the MBCarbon condition exhibited an increase in biomass growth with the addition of an external organic carbon source, reaching $81 \pm 2 \text{ mg L}^{-1} \text{ d}^{-1}$. The highest productivity results, however, were achieved under conditions with CO₂/O₂ supplementation, yielding values of $125 \pm 1 \text{ mg L}^{-1} \text{ d}^{-1}$ and $126 \pm 1 \text{ mg L}^{-1} \text{ d}^{-1}$, respectively. This increase was also observed by Ruas, Farias, et al. (2023), in an experiment investigating the influence of microalgal strains (*Chlorella vulgaris* and *Scenedesmus acutus* Meyen) and the addition of CO₂ and O₂ on domestic wastewater treatment. The study reported significantly higher productivity under conditions with such supplementation, attributed to the enhanced photosynthetic capacity and carbon fixation rates of these microalgal species.

CONCLUSIONS

This study demonstrated the effectiveness of different inocula and operational conditions in enhancing surfactant and organic matter removal in domestic wastewater treatment. The highest surfactant removal efficiencies were achieved under the MB (microalgae-bacteria) and BD (bacteria-only) conditions, reaching $76 \pm 7\%$ and $76 \pm 13\%$, respectively, highlighting the critical role of bacterial activity in surfactant degradation. The synergistic interaction between microalgae and bacteria in the MB condition further amplified removal efficiency, as microalgae provided oxygen and organic substrates that enhanced bacterial metabolic activity. Conversely, the lowest performance was observed under conditions with organic carbon supplementation, achieving only $55 \pm 7\%$ removal, indicating that carbon addition was not the most effective strategy in this context.

The operational conditions, particularly those involving CO_2/O_2 supplementation and pH control (MBpH), also played a pivotal role in optimizing pollutant removal. The MBO₂ condition, combining microalgae and bacteria with atmospheric air supply, achieved the highest surfactant removal efficiency of $96 \pm 1\%$, with rapid degradation observed by the fourth day. The strong negative correlation between pH and surfactant removal further underscored the importance of pH control in enhancing microbial activity and degradation pathways. Additionally, the combined anoxic-aerobic algal-bacterial system demonstrated significant potential for simultaneous nutrient and surfactant removal, achieving an overall efficiency of $66 \pm 9\%$. Conversely, the lowest performance was observed under conditions with organic carbon supplementation, achieving only $55 \pm 7\%$ removal, indicating that carbon addition was not the most effective strategy in this context.

Organic matter removal was most efficient under CO_2/O_2 supplementation and MBpH conditions, with COD removal efficiencies of $78 \pm 1\%$, $83 \pm 1\%$, and $87 \pm 1\%$, respectively. These results reinforce the importance of pH regulation and bacterial activity in optimizing wastewater treatment processes. Biomass productivity was also highest under CO_2/O_2 supplementation, yielding values of $125 \pm 1 \text{ mg L}^{-1} \text{ d}^{-1}$ and $126 \pm 1 \text{ mg L}^{-1} \text{ d}^{-1}$, attributed to enhanced photosynthetic capacity and carbon fixation rates of microalgae.

REFERENCES

- Aloui, F., Kchaou, S., and Sayadi, S. (2009) Physicochemical treatments of anionic surfactants wastewater: Effect on aerobic biodegradability. *Journal of Hazardous Materials*, **164**(1), 353–359.

- Andrade, M. V. F., Sakamoto, I. K., de Oliveira Paranhos, A. G., Silva, E. L., and Varesche, M. B. A. (2017) Bioremoval of Surfactant from Laundry Wastewater in Optimized Condition by Anoxic Reactors. *Water, Air, and Soil Pollution*, **228**(4), 1–13.
- APHA (2017) *Standard Methods for the Examination of Water and Wastewater*, Washington, American Public Health Association.
- Askari, A., Vahabzadeh, F., and Mardanpour, M. M. (2021) The identification and performance assessment of dominant bacterial species during linear alkylbenzene sulfonate (LAS)-biodegradation in a bioelectrochemical system. *Bioprocess and Biosystems Engineering*, **44**(12), 2579–2590. [online] <https://doi.org/10.1007/s00449-021-02629-0>.
- Camacho-Muñoz, D., Martín, J., Santos, J. L., Aparicio, I., and Alonso, E. (2014) Occurrence of surfactants in wastewater: Hourly and seasonal variations in urban and industrial wastewaters from Seville (Southern Spain). *Science of the Total Environment*, **468–469**, 977–984. [online] <http://dx.doi.org/10.1016/j.scitotenv.2013.09.020>.
- Dammak, I., Fersi, M., Hachicha, R., and Abdelkafi, S. (2023) Current Insights into Growing Microalgae for Municipal Wastewater Treatment and Biomass Generation. *Resources*, **12**(10).
- Fernández, F. G. A., Reis, A., Wijffels, R. H., Barbosa, M., Verdelho, V., and Llamas, B. (2021) The role of microalgae in the bioeconomy. *New Biotechnology*, **61**, 99–107.
- Frutos, O. D., Quijano, G., Pérez, R., and Muñoz, R. (2016) Simultaneous biological nitrous oxide abatement and wastewater treatment in a denitrifying off-gas bioscrubber. *Chemical Engineering Journal*, **288**, 28–37. [online] <http://dx.doi.org/10.1016/j.cej.2015.11.088>.
- García, D., Alcántara, C., Blanco, S., Pérez, R., Bolado, S., and Muñoz, R. (2017) Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading. *Chemical Engineering Journal*, **313**, 424–434.
- Haggensen, F., Mogensen, A. S., Angelidaki, I., and Ahring, B. K. (2002) Anaerobic treatment of sludge: Focusing on reduction of LAS concentration in sludge. *Water Science and Technology*, **46**(10), 159–165.
- Hena, S., Abida, N., and Tabassum, S. (2015) Screening of facultative strains of high lipid producing microalgae for treating surfactant mediated municipal wastewater. *RSC Advances*, **5**, 98805–98813.
- Hua, X., Li, M., Su, Y., Dong, D., Guo, Z., and Liang, D. (2012) The degradation of linear alkylbenzene sulfonate (LAS) in the presence of light and natural biofilms: The important role of photosynthesis. *Journal of Hazardous Materials*, **229–230**, 450–454. [online] <http://dx.doi.org/10.1016/j.jhazmat.2012.06.005>.
- Ji, X., Jiang, M., Zhang, J., Jiang, X., and Zheng, Z. (2018) The interactions of algae-bacteria symbiotic system and its effects on nutrients removal from synthetic wastewater. *Bioresource Technology*, **247**(July 2017), 44–50. [online] <http://dx.doi.org/10.1016/j.biortech.2017.09.074>.
- Jurado, E., Fernández-Serrano, M., Núñez-Olea, J., Luzón, G., and Lechuga, M. (2006) Simplified spectrophotometric method using methylene blue for determining anionic surfactants: Applications to the study of primary biodegradation in aerobic screening tests. *Chemosphere*, **65**(2), 278–285.

- Katam, K., Shimizu, T., Soda, S., and Bhattacharyya, D. (2020) Performance evaluation of two trickling filters removing LAS and caffeine from wastewater: Light reactor (algal-bacterial consortium) vs dark reactor (bacterial consortium). *Science of the Total Environment*, **707**, 135987. [online] <https://doi.org/10.1016/j.scitotenv.2019.135987>.
- López-Serna, R., Posadas, E., García-Encina, P. A., and Muñoz, R. (2019) Removal of contaminants of emerging concern from urban wastewater in novel algal-bacterial photobioreactors. *Science of the Total Environment*, **662**, 32–40. [online] <https://doi.org/10.1016/j.scitotenv.2019.01.206>.
- Macedo, T. Z., Okada, D. Y., Delforno, T. P., Braga, J. K., Silva, E. L., and Varesche, M. B. A. (2015) The comparative advantages of ethanol and sucrose as co-substrates in the degradation of an anionic surfactant: microbial community selection. *Bioprocess and Biosystems Engineering*, **38**(10).
- Macedo, T. Z., Silva, E. L., Sakamoto, I. K., Zaiat, M., and Varesche, M. B. A. (2019) Influence of linear alkylbenzene sulfonate and ethanol on the degradation kinetics of domestic sewage in co-digestion with commercial laundry wastewater. *Bioprocess and Biosystems Engineering*, **42**(9), 1547–1558. [online] <https://doi.org/10.1007/s00449-019-02152-3>.
- Matamoros, V., Uggetti, E., García, J., and Bayona, J. M. (2016) Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study. *Journal of Hazardous Materials*, **301**, 197–205. [online] <http://dx.doi.org/10.1016/j.jhazmat.2015.08.050>.
- Mohsenpour, S. F., Hennige, S., Willoughby, N., Adeloye, A., and Gutierrez, T. (2021) Integrating microalgae into wastewater treatment: A review. *Science of the Total Environment*, **752**(September 2020), 142168. [online] <https://doi.org/10.1016/j.scitotenv.2020.142168>.
- Motteran, F., Nadai, B. M., Braga, J. K., Silva, E. L., and Varesche, M. B. A. (2018) Metabolic routes involved in the removal of linear alkylbenzene sulfonate (LAS) employing linear alcohol ethoxylated and ethanol as co-substrates in enlarged scale fluidized bed reactor. *Science of the Total Environment*, **640–641**, 1411–1423. [online] <https://doi.org/10.1016/j.scitotenv.2018.05.375>.
- Mungray, A. K. and Kumar, P. (2008) Anionic surfactants in treated sewage and sludges: Risk assessment to aquatic and terrestrial environments. *Bioresource Technology*, **99**(8), 2919–2929.
- Nirmalakhandan, N., Selvaratnam, T., Henkanatte-Gedera, S. M., Tchinda, D., Abeywardana-Arachchige, I. S. A., Delanka-Pedige, H. M. K., Munasinghe-Arachchige, S. P., Zhang, Y., Holguin, F. O., and Lammers, P. J. (2019) Algal wastewater treatment: Photoautotrophic vs. mixotrophic processes. *Algal Research*, **41**(May), 101569. [online] <https://doi.org/10.1016/j.algal.2019.101569>.
- Palmer, M. and Hatley, H. (2018) The role of surfactants in wastewater treatment : Impact , removal and future techniques : A critical review. *Water Research*, **147**, 60–72. [online] <https://doi.org/10.1016/j.watres.2018.09.039>.
- Pandey, A., Katam, K., Joseph, P., Soda, S., Shimizu, T., and Bhattacharyya, D. (2020) Micropollutant Removal from Laundry Wastewater in Algal-Activated Sludge Systems: Microbial Studies. *Water, Air, and Soil Pollution*, **231**(7).

- Park, J. B. K. and Craggs, R. J. (2010) Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Science & Technology*, **5**, 633–640.
- Phong Vo, H. N., Ngo, H. H., Guo, W., Nguyen, K. H., Chang, S. W., Nguyen, D. D., Liu, Yiwen, Liu, Yi, Ding, A., and Bui, X. T. (2020) Micropollutants cometabolism of microalgae for wastewater remediation: Effect of carbon sources to cometabolism and degradation products. *Water Research*, **183**, 115974. [online] <https://doi.org/10.1016/j.watres.2020.115974>.
- Pirsaheb, M., Khamutian, R., and Khodadadian, M. (2014) A comparison between extended aeration sludge and conventional activated sludge treatment for removal of linear alkylbenzene sulfonates (Case study: Kermanshah and Paveh WWTP). *Desalination and Water Treatment*, **52**(25–27), 4673–4680.
- Posadas, E., Morales, M. M., Gomez, C., Acien, F. G., and 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. [online] <http://dx.doi.org/10.1016/j.cej.2014.12.059>.
- Ruas, G., Farias, S. L., Nantes, M. A., Serejo, M. L., Henrique, G., and Boncz, M. Á. (2022) CO₂ Addition and Semicontinuous Feed Regime in Shaded HRAP — Pathogen Removal Performance. *Water*, **14**(24), 4047.
- Ruas, G., Farias, S. L., Reis, B. A. B., Serejo, M. L., Henrique, G., and Boncz, M. Á. (2023) 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., López-Serna, R., Scarcelli, P. G., Serejo, M. L., Boncz, M. Á., and Muñoz, R. (2022) Influence of the hydraulic retention time on the removal of emerging contaminants in an anoxic-aerobic algal-bacterial photobioreactor coupled with anaerobic digestion. *Science of the Total Environment*, **827**, 154262.
- Ruas, G., Serejo, M. L., Farias, S. L., Scarcelli, P., and Boncz, M. Á. (2021) Removal of pathogens from domestic wastewater by microalgal - bacterial systems under different cultivation conditions. *International Journal of Environmental Science and Technology*, (0123456789). [online] <https://doi.org/10.1007/s13762-021-03820-2>.
- Serejo, M. L., Farias, S. L., Ruas, G., Paulo, P. L., and Boncz, M. A. (2020) Surfactant removal and biomass production in a microalgal-bacterial process: effect of feeding regime. *Water Science and Technology*, **82**(6), 1176–1183.
- Shahid, A., Malik, S., Zhu, H., Xu, J., Nawaz, M. Z., Nawaz, S., Asraful Alam, M., and Mehmood, M. A. (2020) Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Science of the Total Environment*, **704**, 135303. [online] <https://doi.org/10.1016/j.scitotenv.2019.135303>.
- Sournia, A. (1978) *Phytoplankton manual* (A. Sournia, ed.), Paris, United Nations Educational, Scientific and Cultural Organization (UNESCO).
- Tran, N. H., Urase, T., Ngo, H. H., Hu, J., and Ong, S. L. (2013) Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. *Bioresource Technology*, **146**, 721–731. [online] <http://dx.doi.org/10.1016/j.biortech.2013.07.083>.
- Uggetti, E., Sialve, B., Hamelin, J., Bonnafous, A., and Steyer, J. P. (2018) CO₂ addition to increase biomass production and control microalgae species in high rate algal ponds

- treating wastewater. *Journal of CO₂ Utilization*, **28**(July), 292–298. [online] <https://doi.org/10.1016/j.jcou.2018.10.009>.
- Wang, H., Liu, T., Ding, Y., Wang, Z., Zhang, Z., Lei, Z., Shimizu, K., and Yuan, T. (2023) Enhanced performance of algal-bacterial aerobic granular sludge in comparison to bacterial aerobic granular sludge for treating surfactant-containing wastewater. *Bioresource Technology Reports*, **22**(April), 101462. [online] <https://doi.org/10.1016/j.biteb.2023.101462>.
- Zhang, Z. Z., Zhang, Y., Cheng, Y. F., and Jin, R. C. (2021) Linear anionic surfactant (SDBS) destabilized anammox process through sludge disaggregation and metabolic inhibition. *Journal of Hazardous Materials*, **403**(July 2020), 123641. [online] <https://doi.org/10.1016/j.jhazmat.2020.123641>.
- Zhu, F. J., Ma, W. L., Xu, T. F., Ding, Y., Zhao, X., Li, W. L., Liu, L. Y., Song, W. W., Li, Y. F., and Zhang, Z. F. (2018) Removal characteristic of surfactants in typical industrial and domestic wastewater treatment plants in Northeast China. *Ecotoxicology and Environmental Safety*, **153**(February), 84–90. [online] <https://doi.org/10.1016/j.ecoenv.2018.02.001>.

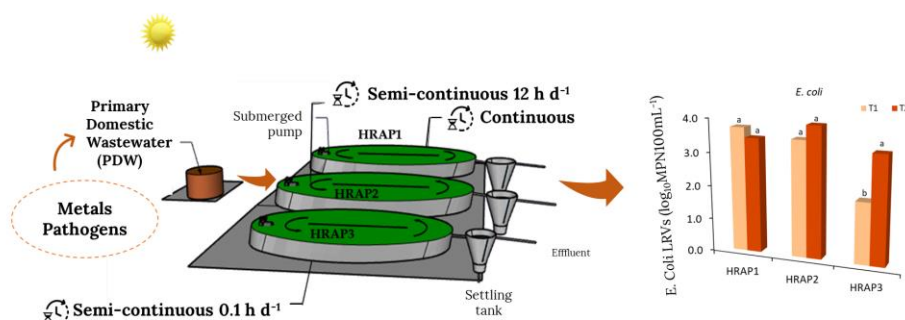
CHAPTER 3 :

3.1 EVALUATION OF THE EFFECT OF THE FEEDING REGIME ON THE REMOVAL OF PATHOGENS IN MICROALGAE-BACTERIAL SYSTEMS^{3,4,5}

ABSTRACT

Mixed microalgae-bacteria cultures in HRAPs are used for the treatment of effluents, a technology that has stood out with excellent results, as reported in the literature. However, investigating these systems in more depth can improve our understanding of the removal mechanisms for a wide range of existing and emerging pollutants, and help improve the guidelines for design and operation, in order to improve the treatment efficiency as well as biomass productivity. This work studied the impact of the feeding regime on the removal of pathogens from primary domestic wastewater in High Rate Algal Ponds (HRAPs). For this, one reactor was fed continuously (HRAP₁) while two reactors were fed in semi-continuous mode, during 12 h d⁻¹ (HRAP₂) and 0.1 h d⁻¹ (HRAP₃). For *E. coli* were reached in the semi-continuously fed reactors, there was no significant difference between the conditions studied. On the other hand, for biomass productivity, the semi-continuous feeding regime was more advantageous with a growth of $\approx 22 \text{ mg L}^{-1} \text{ d}^{-1}$.

GRAPHICAL ABSTRACT



³ This chapter is based on a previously published article. For the purposes of this thesis, only the results concerning pathogen removal, one of the main target pollutants of this research, were retained. The analysis of metals, which was beyond the scope of this thesis, was excluded.

⁴ Paper presented at the IWAlgae 2022 Conference (13th IWA Specialist conference on Wastewater Ponds and Algal Technologies), July 2022, Melbourne, Australia.

⁵ Article published as: Sarah Lacerda Farias, Grazielle Ruas, Mayara L. Serejo, Marc A. Boncz. (2023) Evaluation of the effect of the feeding regime on the removal of metals and pathogens in microalgae – bacterial systems. Water Sci Technol 88:11–22. <https://doi.org/10.2166/wst.2023.194>

INTRODUCTION

Microalgae bacterial processes, such as those commonly used in High Rate Algal Ponds (HRAPs), have shown to be, when compared to conventional treatments, a promising technology. This system has stood out because it is able to effectively and economically remove many types of pollutants, including metals and pathogens, the focus of this study. One of the main advantages of these systems is the potential to recover resources - water, energy, and nutrients - as they generate biomass as a product that can be used in the production of fuels, fertilizers, and biogas, promoting sustainability and enhancing the application of the circular economy (Muñoz and Guieysse, 2006). Life-cycle assessment (LCA) studies show that HRAPs coupled with the production of biofertilizer or biogas, when compared to typical small-sized activated sludge system, is potentially of lower environmental impact for categories such as climate change and fossil fuel consumption (Arashiro et al., 2018).

In HRAP reactors, in addition to other types of interactions, there is a symbiosis between microalgae and bacteria, which is a cooperative type of interaction among many other complex interactions, established between these types of microorganisms. The bacteria use O₂ to convert organic matter into CO₂, among others, while the microalgae consume this CO₂ and the nutrients present for photosynthesis, producing O₂ and biomass (Muñoz and Guieysse, 2006). Thus, the process decreases energy costs and carbon footprint of wastewater treatment, while fixing nutrients in biomass (García et al., 2018).

Among the pollutants present in sewage, pathogens, including a wide variety of bacteria, viruses, helminth eggs, and protozoa, are responsible for numerous diseases of oral-fecal transmission and represent a significant hazard associated with sewage, and one of the main functions of wastewater treatment is reducing this hazard (Curtis, 2003). The ease and low cost of quantification of the *Escherichia coli* bacteria, as well as the reliability of this method, make this one of the main indicator organisms for the presence of fecal material from warm-blooded animals and pollution by wastewater, considered more specific than total and fecal coliforms (Liu et al., 2020).

In addition to the symbiotic biodegradation mentioned before, the removal of pollutants in microalgae-bacteria systems can occur through other mechanisms as well. In the case of pathogens, their deactivation and consequent disinfection can happen by photo-oxidation, the effect of constantly fluctuating environmental conditions such as pH and temperature, and as a result of the presence of bactericidal substances produced by microalgae (Chambonniere et al., 2021).

Several researchers already obtained good results considering pathogen in HRAPs. Among the operational conditions, it is already known that factors such as pH, temperature, and dissolved oxygen are crucial for the removal of these pollutants in these systems (Couto et al., 2015; Muñoz and Guieysse, 2006). As the removal mechanisms are complex, involving many factors, conducting studies under real-life conditions is fundamental to optimize the treatment through these types of processes.

An important parameter that can influence the cost and quality of treatment in HRAPs is the feeding regime, with both continuous and semi-continuous cultivation being used in larger-scale applications (Lu et al., 2021). Although in batch systems sometimes more concentrated biomass may be obtained, they are limited to bench-scale applications due to the periodic need to prepare new cultures at low volumes. (Do et al., 2020). A semi-continuous feeding regime has the potential to increase productivity while reducing costs (Pereira et al., 2020), therefore potentially increasing the efficiency of pollutant removal. However, for both continuous and semi-continuous systems, results may depend on the feeding rate applied, as stability in the growth of algae cultures needs to be ensured to maintain the treatment capacity (Do et al., 2020). In this context, this work aimed to study the influence of the feeding regime - continuous or semi-continuous - on the removal of metals and pathogens (*Escherichia coli*) from primary domestic wastewater, using three HRAP-type reactors in parallel, of which one fed continuously, and the other two semi-continuously.

MATERIALS AND METHODS

Microorganisms and domestic wastewater

A microalgae culture ($\approx 98\%$ predominance of individuals of the gender *Scenedesmus* sp.) with 1.4 g L^{-1} of total suspended solids (TSS), originating from an outdoors HRAP used for the treatment of domestic wastewater was used as inoculum. *Scenedesmus* sp. stands out as one of the most important microalgae genera that naturally predominate in HRAP treatment systems due to its fast growth and high resistance characteristics (Muñoz and Guieysse, 2006). The reactors were also inoculated with a nitrifying-denitrifying activated sludge from a wastewater treatment plant (WWTP) treating domestic wastewater, with 4.2 gTSS L^{-1} . The system was fed with primary domestic wastewater (PDW) collected from a municipal WWTP and stored at 4°C in a cooled agitated storage tank (Implemis, Brazil). The average concentrations of the microbiological and physico-chemical properties of the influent are summarized in Table 3-1.

Table 3-1 - Physical-chemical and microbiological characterization of the primary domestic wastewater.

Parameter	Unit	Value
pH	-	8.0 ± 0.1
Chemical oxygen demand (COD)	mg O ₂ L ⁻¹	127 ± 11
Total organic carbon (TOC)	mg C L ⁻¹	119 ± 9
Inorganic carbon (IC)	mg C L ⁻¹	53±11
Total organic nitrogen (TN)	mg N L ⁻¹	66±15
Ammonium nitrogen (N-NH ₄ ⁺)	mg N-NH ₄ ⁺ L ⁻¹	20±5
Total phosphorus as P-PO ₄ ³⁻ (TP)	mg P-PO ₄ ³⁻ L ⁻¹	6.1 ± 0.4
C:N:P	-	28/14/1
C/N		2
<i>Escherichia coli</i>	MPN 100 mL ⁻¹	(2.5 ± 1.3) × 10 ⁶

MPN: most probable number.

Experimental setup

The experimental setup, a small pilot scale system, consisted of three outdoors reactors (HRAP₁, HRAP₂, and HRAP₃), of 20 L each, with 0.32 m² of illuminated area and 16 cm culture depth. Continuous agitation of the HRAPs was maintained by a submersible pump with a nominal flow rate of 650 L.h⁻¹ (Sarlo Better B650, Brazil), resulting in a recirculation velocity of 20 ± 2 cm s⁻¹ (Ruas *et al.*, 2020). Hydraulic tests prior to the operation of the systems ensured that there were no dead zones or hydraulic short circuits throughout the entire extension of the reactors, including the inlet and outlet devices. The study was conducted at the Federal University of Mato Grosso do Sul (Campo Grande-MS, Brazil) for 36 days, at ≈ 29°C.

Operational conditions and sampling

To assess the influence of the feeding regime, reactor HRAP₁ was fed continuously, while HRAP₂ and HRAP₃ were fed in a semi-continuous regime. HRAP₂ was fed 12 h per day (between 09:00 and 21:00) while HRAP₃ was fed 0.1 h per day (between 09:00 and 09:06). Only for reactor HRAP₃, in order to avoid short-circuiting, when applying the daily feed of influent, a waiting time of 5 minutes was applied, before the excess liquid was drained from the reactor. The same HRT of 7 days was applied to all three reactors.

Samples of feed, cultivation broth, and effluent were collected three days per week, at 09:10 (T1) and 16:00 (T2). Turbidity, pH, dissolved oxygen (DO), temperature, and light intensity

were determined in samples T1 and T2 of the culture broth. *E. coli* was also determined at both collection times from the feed and the effluent. T1 samples (feed and effluent) were also used to determine soluble concentrations of chemical oxygen demand (COD), total organic carbon (TOC), inorganic carbon (IC), total organic nitrogen (TN), ammonium nitrogen (N-NH_4^+), nitrite nitrogen (N-NO_2^-), nitrate nitrogen (N-NO_3^-), total phosphorus as P-PO_4^{3-} (TP), and metals. For these analysis of dissolved compounds, the samples were previously filtered with 0.45 μm glass fiber filters.

The applied semi-continuous feeding regime approximates the chemostat culture method (keeping the culture volume constant, adding culture medium at a constant rate), and therefore the systems reach a quasi-steady state (Boraas 1993). The steady state was determined based on graphical and numerical analysis of the physico-chemical parameters (pH, DO, TSS, etc.) and nutrient removals (P and N), being reached when the variation ($dX=dt$) of most monitored concentrations tended to zero (Ruas et al. 2022).

Analytical procedures

Analyses were performed according to *Standard Methods* (APHA, 2017). Light intensity (Photosynthetically Active Radiation - PAR), was determined using a PAR MQ-200 radiation meter (Apogee instruments, USA). Concentrations of TOC, TC, IC, and TN were determined using a Vario TOC cube (Elementar, Germany) organic carbon analyzer. Turbidity, DO and pH were measured using Hanna HI98703-01, Hanna HI2004-02 and Hanna HI2002-02 bench meters, respectively (Hanna instruments, USA). N-NH_4^+ was measured using an Orion Five Star (Thermo Scientific, USA) multiparameter analyzer with ammonia electrode. Anions (N-NO_3^- , N-NO_2^- and P-PO_4^{3-}) were analyzed using a Dionex UltiMate ICS 1100 ion chromatograph with an IonPac AG19/AS19 column (Thermo Scientific, USA). Microalgae were identified through microscopic examination (Leica DM5500B, Germany).

Escherichia coli was determined using Colilert® quantification kits (IDEXX Laboratories, USA), and the results were reported as *E. coli* Most Probable Number (MPN) per 100 mL.

Data analysis and statistical treatment

The Removal Efficiency in % (%RE) was calculated from concentrations in feed and effluent according to equation 1, while the uptake capacity (q , in mg g^{-1}) of each metal by microalgae was determined using equation 2:

$$\%RE = \left(\frac{C_{inf} - C_{eff}}{C_{inf}} \right) \cdot 100 \quad (1)$$

$$q = \frac{V(C_{inf} - C_{eff})}{M} \quad (2)$$

In both equations, C_{inf} and C_{eff} are influent and effluent concentrations (mg L^{-1}) respectively, V is the working volume of the reactor (L), and M is the dry weight of the culture biomass (g).

E. coli removal was quantified as $\log_{10}$ reduction values (LRV), calculated from the difference between the $\log_{10}$ concentrations (*E. coli* $\log_{10}$ MPN 100 mL^{-1}) in influent and effluent. The *E. coli* decay rates K (d^{-1}), were calculated using the regression line of the first order decay equation (Pereira, Dassoler, et al., 2020):

$$K = \frac{\log(N_0) - \log(N_t)}{\text{HRT}} \quad (3)$$

Where N_0 and N_t are the *E. coli* MPNs for influent and effluent, respectively, and HRT is the hydraulic retention time (d).

Statistical analyses were performed using version 3.2.2 of the R statistical software. The normality of all the data sets was checked using the Shapiro-Wilk test, and the comparison of means was conducted by analysis of variance (ANOVA) followed by the Tuckey hypothesis test, at a 95% confidence level. In the case of normal distribution, Pearson's Correlation was used to verify the influence of environmental parameters on the removals obtained. Otherwise, Spearman's rank correlation was used.

RESULTS AND DISCUSSION

Environmental conditions and biomass growth

During the experiment, temperatures and evaporation losses for all three reactors were similar at $\approx 27^\circ\text{C}$ and $\approx 0.9 \text{ L m}^{-2} \text{ d}^{-1}$, respectively (Table 3-2). The average PAR ($1083 \pm 490 \mu\text{E. m}^{-2} \text{ s}^{-1}$) was similar to the $1087.3 \pm 697.6 \mu\text{E. m}^{-2} \text{ s}^{-1}$ reported by Couto et al. (2015) for an experiment under outdoor conditions with a local climate characterized as tropical altitude.

With continuous feeding, the pH at T1 (morning) was 8.6 ± 0.5 , while in the reactors with semi-continuous feeding, HRAP₂, and HRAP₃, had similar values of 10.4 ± 0.1 and 10.1 ± 0.3 , respectively, as shown in Table 3-2. In the afternoon (T2), the pH values followed the same trend, with even higher averages in the reactors operated in a semi-continuous feeding regime. Likewise, DO concentrations ($7.6 \pm 1.3 \text{ mgO}_2 \text{ L}^{-1}$ s in HRAP₁ at T1) were also

comparable in HRAP₂ and HRAP₃, and higher than in in HRAP₁ at $13.4 \pm 1.3 \text{ mgO}_2 \text{ L}^{-1}$ and $11.8 \pm 1.6 \text{ mgO}_2 \text{ L}^{-1}$, respectively, indicating a higher bacterial oxidation activity in this reactor (HRAP₁) overnight, when HRAP₁ was receiving influent, but HRAP₂ and HRAP₃ not (Table 3-2).

DO concentrations at T2 were lower for all three reactors. The results in HRAP₂ and HRAP₃ show a positive correlation between the DO and pH in these HRAPs, in line with the consideration that the increase in DO is related to the photosynthetic activity of the algae, which removes CO₂ from the culture medium, and consequently raising the pH (Dias et al. 2017). The observed variations in turbidity at times T1 and T2 are related to natural fluctuations in light intensity, where the photosynthetic apparatus tends to decrease after higher light intensities (T2) and also by the cessation of photosynthesis in periods of the low incidence of light (hours before T1) (Muñoz & Guieysse 2006).

Higher biomass productivities, of 21.9 ± 0.3 and $21.2 \pm 0.1 \text{ mg L}^{-1} \text{ day}^{-1}$, respectively, were achieved in HRAP₂ and HRAP₃, as shown in Table 3-2. The areal productivity was 1.5, 3.6, and 3.3 g m² day for HRAP₁, HRAP₂, and HRAP₃, in that order. The C:N ratio of the primary sewage used (Table 3-1), although being close to that expected for this matrix (C:N $\approx$ 3:0), indicates carbon limitation, and this may have reduced the growth of biomass in the three conditions studied and consequently the treatment efficiencies (Couto et al. 2015; Ruas et al. 2020). Pereira et al. (2020) obtained higher biomass productivities in experiments in continuous mode using anaerobic secondary effluent and growth of five microalgae strains, including *Chlorella* sp. and *Scenedesmus* sp., with a peak volumetric productivity of $283 \text{ mg L}^{-1} \text{ day}^{-1}$ at a dilution rate of 0.3 day^{-1} for *Chlorella* sp. L06, which compared to other higher dilution rates, was more favorable because the biomass is in a constant state of exponential growth (steady state growth). Do et al. (2020) observed in an experiment in raceway ponds with a *C. variabilis* TH03-bacteria consortium in the semi-continuous mode that optimal replacement was achieved when replacing 80% of the culture volume by new sewage, reaching a stable biomass productivity of $66.2\text{--}1,189 \text{ mg L}^{-1} \text{ day}^{-1}$ where higher substitution rates impaired stable growth.

Table 3-2 - Concentration of DO, pH and turbidity at T1 (morning) and T2 (afternoon), and volumetric productivity (n=5), temperature, and evaporation rate at T1 during the operation of the three HRAPs (average±standard deviation, n=10)

Parameters	HRAP ₁		HRAP ₂		HRAP ₃	
	T1	T2	T1	T2	T1	T2
pH	8.6 ± 0.5	8.73 ± 0.8	10.4 ± 0.1	10.50 ± 0.3	10.1 ± 0.3	10.55 ± 0.3
DO (mg L⁻¹)	7.6 ± 1.3	6.08 ± 2.4	13.4 ± 1.3	7.90 ± 3.9	11.8 ± 1.6	6.69 ± 1.8
Turbidity (NTU)	53 ± 12	195.9 ± 6	100 ± 22	262.3 ± 9	88 ± 10	228.4 ± 31
Temperature (°C)	27.4 ± 1.9		27.3 ± 1.8		27.3 ± 1.8	
Productivity (mg L⁻¹ d⁻¹)	13.9 ± 0.4		21.9 ± 0.3		21.2 ± 0.1	
Evaporation rate (L m⁻² d⁻¹)	0.9 ± 0.7		0.9 ± 0.7		1.0 ± 0.7	

Removal efficiency of pathogens (*Escherichia coli*)

The removal of *E. coli* at time T1 was statically similar for the HRAP₁ and HRAP₂ (no significant difference, $p > 0.05$), with mean LRV of 3.7 ± 0.2 and 3.5 ± 0.8 , corresponding to $\approx 99\%$, while HRAP₃ underperformed with $\approx 94\%$; LRV of $1.8 \pm 0.4 \log_{10}$ MPN 100 ml⁻¹ (Figure 3-1). Minor removal was reported by Young *et al.* (2016), with a mean $\log_{10}$ reduction value of $2.13 \pm 0.55 \log_{10}$ MPN 100 ml⁻¹, in HRAP using wastewater pretreated in on-site septic tanks as influent. A similar result was obtained by Ruas *et al.* (2020), in an experiment with HRAPs reactors operating with configurations similar to this study and treating primary domestic effluent, but varying supplementation with CO₂ and TDH, reaching LRVs of 2.5–3.7 $\log_{10}$ MPN 100 ml⁻¹. At time T2 the results followed the same trend as T1, with LRV of 3.9 ± 1 and 3.2 ± 1 for HRAPs with semi-continuous feeding and 3.4 ± 1 for HRAP₁, with no significant difference between them ($p > 0.05$).

Liu *et al.* (2020), observed in a study of pathogen removal in wastewater stabilization pond systems (WSPs), that the increase in pH favored the inactivation of *E.coli* with good results with pH from 8, similar to that obtained in the three conditions studied. However, Spearman's rank correlation coefficient for the results achieved at times T1 and T2 showed that there was a weak negative linear correlation between pH and removal efficiencies in the three reactors (-0.4241 , P-value= 0.1016) and negligible correlation (-0.1436 , P-value= 0.6096), respectively, indicating that the greatest removals occurred at lower pHs, but still in the alkaline range. The reduction related to the pH parameter is associated with the death of the bacterium when it is no longer able to acidify its own cytoplasm, being for this aspect a non-linear relationship because the limit will be when the homeostatic mechanism of this microorganism is overloaded and can occur at different levels of pH (Curtis, 2003).

The increase in DO concentrations is related to photosynthesis performed by algae and the combination with sunlight conditions causes the decrease of pathogenic organisms in ponds (Dias, Passos, *et al.*, 2017), however, this parameter at time T1 showed a weak negative linear

correlation (Pearson's linear correlation test, $p = 0.0952$, $r = -0.4314$, $r^2 = 0.1861$) and at time T2 a negligible negative correlation ($p = 0.5844$, $r = -0.1537$, $r^2 = 0.0236$). The high concentration of DO can be related to the increase in photo-oxidation, a disinfection mechanism in which the bacterial cell damage is caused by reactive oxygen species (ROS) that can be formed by all sensitizers in the presence of oxygen (Chambonniere, Bronlund, et al., 2021). However, like in this study, Liu et al. (2016), also did not find a good correlation of this parameter with *E. coli* removal, indicating that disinfection induced by photo-oxidation is not only dependent on DO concentrations but is also influenced by other factors such as solar light intensity and pH.

The feeding regime can impact the pH and DO levels in an HRAP system, as a result of the volume of fresh effluent added, which may cause a marked decrease in pH due to nitrification and of the DO level due to an acceleration in bacterial oxidation processes (Pham et al. 2021). However, the pH and DO concentrations obtained in the HRAP₂ and HRAP₃ reactors rule out this possibility of nitrification with the decrease in the values of these two parameters. Do et al. (2020) observed in an experiment performed in semi-continuous mode with different percentages of replacement of the culture volume by new wastewater, that in substitutions of more than 90%, despite showing good biomass growth, the algal–bacteria culture experienced a long lag phase, weakening the health of this consortium and affecting the treatment capacity, this being a possibility for the results obtained in HRAP₃, in which feeding at 0.1 h day^{-1} resulted in a greater dilution of the cultivation broth (around 14%) in a short time.

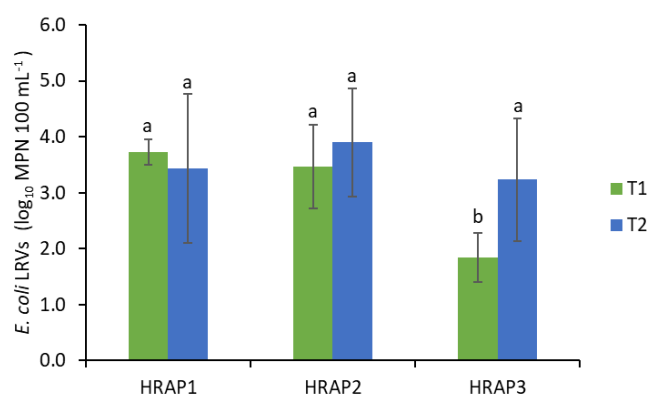


Figure 3-1 - Removal efficiencies represented by their average and standard deviations in log₁₀ reduction values (LRV) of *Escherichia coli* at times T1 (morning) and T2 (afternoon) (n=5). Means followed by the same letter do not statistically differ

For the Photosynthetically Active Radiation (PAR) parameter, Spearman's rank correlation coefficient was negligible at T1 but moderately positive at time T2 (Spearman's correlation coefficient $\rho = 0.4493$, $p\text{-value} = 0.0928$), indicating that sunlight-mediated inactivation was

possibly an active mechanism in removing the pathogens. This mechanism may have occurred by direct absorption of solar UV-B or photo-oxidation (Dias et al., 2017). The action of pathogen removal mechanisms mediated by sunlight is affected by the attenuation of light in HRAPs due to algae growth, dependent therefore on the operational and environmental parameters and the composition of the influent. However, this negative impact is neutralized due to the constant recirculation and mixing of the culture broth in the reactor, allowing greater exposure of pathogens near the surface of the HRAP (Chambonniere et al., 2021).

Figure 3-2 shows the variations in *E. coli* decay rates at times T1 and T2, where a subtle increase is observed at time T2, especially for HRAP3 (means of 0.54 ± 0.7 and $1.06 \pm 0.4 \text{ d}^{-1}$, respectively), but with no significant difference in the disinfection coefficients in the two periods analyzed. Craggs et al. (2004), observed in a pilot scale HRP treating wastewater from a dairy farm that despite the short wavelength components of sunlight in solar UV, especially those in the UVB range, are a major cause of reduced *E. coli*, a situation that is favorable when the sun is at its maximum altitude, no increase in the rate of inactivation of *E. coli* near solar noon has been identified, which can be justified by the fact that the substance more intrinsically harmful UVB was attenuated so quickly in highly pigmented dairy. Contrary to what was obtained in this study, Pereira et al. (2020), in an experiment in continuous reactors using unsterilized wastewater effluent at different dilutions, observed that higher *E. coli* decay rates were obtained at higher dilution rates.

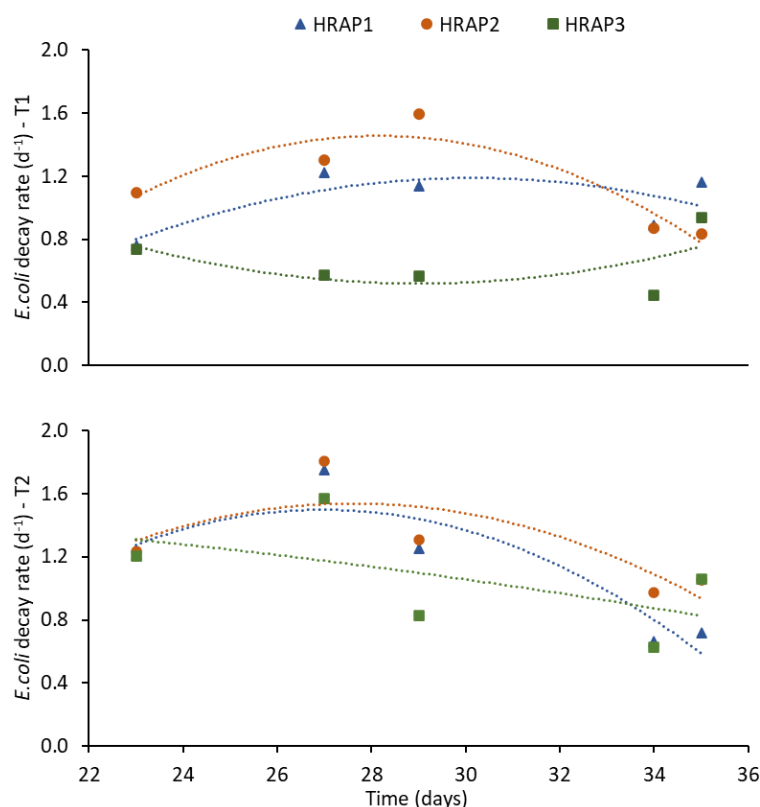


Figure 3-2 - Variations of the *Escherichia coli* decay rates (d^{-1}) represented by a second-order polynomial fit at times T1 and T2 for the HRAP₁, HRAP₂, and HRAP₃ reactors during the stationary growth phase of microalgae.

CONCLUSIONS

The removal efficiencies of *E. coli* were similar for both continuous and semi-continuous feeding regimens. The lowest removal was obtained in the reactor with a semi-continuous feed of $0.1 \text{ h}^{-1} \text{ d}^{-1}$, with an LRV of $1.8 \pm 0.4 \log_{10} \text{MPN } 100 \text{ ml}^{-1}$, indicating that the highest dilution in a short space of time may have affected the treatment capacity. Sunlight-mediated inactivation was possibly a relevant mechanism in removing the pathogens in this experiment. The semi-continuous feeding regime was beneficial for increasing the biomass productivity, with growth rates of 21.9 ± 0.3 and $21.2 \pm 0.1 \text{ mg L}^{-1} \text{ d}^{-1}$. In order to further quantify the advantages of the semi-continuous feeding regime in these systems, future studies with other pollutants will be carried out, combining the presented research with a study of other parameters, as well as an economic feasibility analysis.

REFERENCES

- APHA. (2017), *Standard Methods for the Examination of Water and Wastewater*, 23rd ed., American Public Health Association, Washington-DC, USA. ISBN:
- Arashiro, L.T., Montero, N., Ferrer, I., Acien, F.G., Gómez, C. and Garfí, M. (2018), "Life cycle assessment of high rate algal ponds for wastewater treatment and resource

- recovery”, *Science of the Total Environment*, 622–623, 1118–1130, DOI 10.1016/j.scitotenv.2017.12.051.
- Boraas, M.E. (1993), “Semicontinuous Culture Methods”, in Walz, N. (Ed.), *Plankton Regulation Dynamics. Ecological Studies*, Springer Berlin Heidelberg, 13–20.
- Chambonniere, P., Bronlund, J. and Guieysse, B. (2021), “Pathogen removal in high-rate algae pond: state of the art and opportunities”, *Journal of Applied Phycology*, 33, 1501–1511. DOI 10.1007/s10811-020-02354-3.
- Couto, E. de A., Calijuri, M.L., Assemany, P.P., Tango, M.D. and Santiago, F. (2015), “Influence of solar radiation on nitrogen recovery by the biomass grown in high rate ponds”, *Ecological Engineering*, 81, 140–145, DOI 10.1016/j.ecoleng.2015.04.040.
- Craggs, R.J., Zwart, A., Nagels, J.W. and Davies-Colley, R.J. (2004), “Modelling sunlight disinfection in a high rate pond”, *Ecological Engineering*, 22(2), 113–122, DOI 10.1016/j.ecoleng.2004.03.001.
- Curtis, T. (2003), “Bacterial pathogen removal in wastewater treatment plants”, in Mara, D. and Honran, N. (Eds.), *Handbook of Water and Wastewater Microbiology*, Academic Press, London, UK, ISBN: . pp 477–490.
- Dias, D.F.C., Passos, R.G. and von Sperling, M. (2017), “A review of bacterial indicator disinfection mechanisms in waste stabilisation ponds”, *Reviews in Environmental Science and Biotechnology*, 16(3), 517–539, DOI 10.1007/s11157-017-9433-2.
- Do, T.C. Van, Nguyen, T.N.T., Tran, D.T., Le, T.G. and Nguyen, V.T. (2020), “Semi-continuous removal of nutrients and biomass production from domestic wastewater in raceway reactors using *Chlorella variabilis* TH03-bacteria consortia”, *Environmental Technology and Innovation*, 20, 101172, DOI 10.1016/j.eti.2020.101172.
- García, D., Posadas, E., Blanco, S., Acien, G., García-Encina, P., Bolado, S. and Muñoz, R. (2018), “Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors”, *Bioresource Technology*, 248, 120–126, DOI 10.1016/j.biortech.2017.06.079.
- Liu, L., Hall, G. and Champagne, P. (2016), “Effects of environmental factors on the disinfection performance of a wastewater stabilization pond operated in a temperate climate”, *Water (Switzerland)*, 8(1), 5, DOI 10.3390/w8010005.
- Liu, L., Hall, G. and Champagne, P. (2020), “The role of algae in the removal and inactivation of pathogenic indicator organisms in wastewater stabilization pond systems”, *Algal Research*, 46, 101777, DOI 10.1016/j.algal.2019.101777.
- Lu, M.M., Gao, F., Li, C. and Yang, H.L. (2021), “Response of microalgae *Chlorella vulgaris* to Cr stress and continuous Cr removal in a membrane photobioreactor”, *Chemosphere*, 262, 128422, DOI 10.1016/j.chemosphere.2020.128422.
- Muñoz, R. and Guieysse, B. (2006), “Algal-bacterial processes for the treatment of hazardous contaminants: A review”, *Water Research*, 40(15), 2799–2815, DOI 10.1016/j.watres.2006.06.011.
- Pereira, M.V., Dassoler, A.F., Antunes, P.W., Gonçalves, R.F. and Cassini, S.T. (2020), “Indigenous microalgae biomass cultivation in continuous reactor with anaerobic effluent: effect of dilution rate on productivity, nutrient removal and bioindicators”, *Environmental Technology (United Kingdom)*, 41(14), 1780–1792, DOI 10.1080/09593330.2018.1549105.

- Pham, L.A., Laurent, J., Bois, P., Teshome, T.M. and Wanko, A. (2021), “Operating a semi-continuous raceway pond allows to link pH and oxygen dynamics to the interaction between microalgae and bacteria”, *Desalination and Water Treatment*, 211, 105–116, DOI 10.5004/dwt.2021.26506.
- Ruas, G., Farias, S.L., Scarcelli, P.G., Serejo, M.L. and Boncz, M.A. (2020), “The effect of CO₂ addition and hydraulic retention time on pathogens removal in HRAPs”, *Water Science & Technology*, 82(6), 1184–1192, DOI 10.2166/wst.2020.255.
- Ruas, G., López-Serna, R., Scarcelli, P. G., Serejo, M. L., Boncz, M. Á., & Muñoz, R. (2022). Influence of the hydraulic retention time on the removal of emerging contaminants in an anoxic-aerobic algal-bacterial photobioreactor coupled with anaerobic digestion, *Science of the Total Environment*, 827, 154262. <https://doi.org/10.1016/j.scitotenv.2022.154262>
- Young, P., Buchanan, N. and Fallowfield, H.J. (2016), “Inactivation of indicator organisms in wastewater treated by a high rate algal pond system”, *Journal of Applied Microbiology*, 121(2), 577–586, DOI 10.1111/jam.13180.

CHAPTER 3

3.2 SURFACTANT REMOVAL AND BIOMASS PRODUCTION IN A MICROALGAL-BACTERIAL PROCESS: EFFECT OF FEEDING REGIME⁶⁷

ABSTRACT

The influence of the feeding regime on surfactant and nutrient removal and biomass production was evaluated in three high rate algal ponds for treatment of primary domestic wastewater. Feeding times of 24, 12 and 0.1 hours per day were studied in each reactor at a similar hydraulic retention time of 7.0 days and organic load of $2.3 \text{ mg m}^{-2} \text{ d}^{-1}$. Semi-continuous feeding at 12 and 0.1 h d^{-1} showed better microalgal biomass production ($0.21\text{--}0.23 \text{ g L}^{-1}$) and nutrient removal, including nitrogen (74–76%) and phosphorus (80–86%), when compared to biomass production (0.13 g L^{-1}) and nitrogen (69%) and phosphorus (46%) removals obtained at continuous feeding (24 h d^{-1}). Additionally, the removal efficiency of surfactant in the three reactors ranged between 90 and 97%, where the best result was obtained at 0.1 h d^{-1} , resulting in surfactant concentrations in the treated effluent (0.3 mg L^{-1}) below the maximum freshwater discharge limits.

GRAPHICAL ABSTRACT



⁶ Paper presented at the IWAAlgae 2019 Conference (*IWA Conference on Algal Technologies and Stabilization Ponds for Wastewater Treatment and Resource Recovery*), July 2022, Melbourne, Australia.

⁷ Article published as: Serejo, M.L., Farias, S.L., Ruas, G., *et al.* (2020) Surfactant removal and biomass production in a microalgal-bacterial process: effect of feeding regime. *Water Sci Technol* 82:1176–1183. <https://doi.org/10.2166/wst.2020.276>.

Introduction

According to the World Health Organization (WHO), there are still many countries that treat less than half of the wastewater generated. In Brazil, recent data indicate that 62.5% of the sewage generated is collected, while only 49% of the total sewage generated is effectively treated (UNICEF and WHO, 2019; Brasil, 2024). Furthermore, conventional treatment processes do not completely remove nutrients and emerging contaminants, including surfactants, which as a result are discharged in water bodies continuously, increasing the damage done to aquatic ecosystems. Surfactants comprise a vast number of chemical compounds and are divided into the classes of anionic, cationic and non-ionic surfactants. Anionic surfactants are traditionally the most used surfactants (around 60%) due to their deterative properties and lower costs (Pirsaheb et al., 2014; Palmer & Hatley, 2018). According to Market Wired (2017), about 24.2 million tons of surfactant are expected to be produced in 2022, and the largest volumes of surfactant-containing products come from the cleaning products (detergents and soaps), petroleum and personal care products industries (Nitschke & Pastore, 2002). As a result of their household use, these compounds are found in domestic sewage, and as a consequence of incomplete removal in WWTPs, they are continuously discharged in water bodies, increasing the damage done to aquatic ecosystems (Scott & Jones, 2000). Their deposition agricultural lands from waste sludge (Scott & Jones, 2000), also represents a potential environmental risk, depending on the concentration and type of surfactant (Lechuga et al., 2016). The main environmental damage resulting from the discharge of these surfactants in water bodies includes: reduced surface tension of the water, reduced breeding ability of aquatic organisms, and reduced oxygen levels in water bodies as a result of their degradation, among others (Palmer & Hatley, 2018).

Traditionally, technologies based on physical-chemical methods, such as chemical coagulation, electrochemical oxidation, photocatalytic degradation, etc., have been used to remove the surfactants from water (Aboulhassan et al. 2006; Palmer and Hatley 2018). However, these technologies have drawbacks, like high operational costs and in some cases the production of hazardous by-products (Palmer and Hatley, 2018). On the other hand, biological treatment of surfactants by aerobic microorganisms requires a lot of energy for aeration, while degradation by anaerobic microorganisms has only a limited removal efficiency, of around 40-85%, depending on the type of surfactant (Palmer and Hatley, 2018). In contrast, microalgal-bacterial processes in high rate algal pond (HRAP) systems, may represent a less energy intensive and more environmentally friendly alternative for an efficient

removal of these contaminants. Contextually, this process is based on the cooperative interactions between microalgae and bacteria, with CO₂ and O₂ exchanges resulting from oxidation of organic matter by bacteria and concomitant assimilation of CO₂ and nutrients by microalgae' photosynthesis (Muñoz and Guieysse, 2006). Thus, in HRAP systems, mechanical aeration may not be required, as microalgal photosynthesis provides oxygen for the aerobic bacteria, while at the same time nutrients such as nitrogen and phosphate, responsible for eutrophication, are taken up by the microalgae and removed. Other pollutants can be removed in this process as well, including surfactants. The microorganisms may use the surfactants as an energy or as a nutrient source, by (co-) metabolization (Palmer and Hatley, 2018). In this context, surfactant removal by microalgae-based technologies can occur via abiotic (sorption, volatilization or photodegradation) as well as biotic (biodegradation, microalgae uptake or metabolization) mechanisms (Matamoros et al., 2015).

Hena et al. (2015), show high growth rates of *Scenedesmus* sp., *Chlamydomonas* sp., *Chlorococcum humicola*, *Botryococcus braunii* and *Chlorella* sp. in batch experiments using municipal wastewater with a high anionic surfactant content ($\approx 51 \text{ mg L}^{-1}$), reaching removal efficiencies of above 97.9% in 10 days. Katam et al. (2018), compared the anionic surfactant removal in a microalgal reactor with that in an activated sludge process: removal efficiencies reached up to 80 and 95%, respectively. In spite of these promising results, the effectiveness of HRAPs for anionic surfactant removal is not yet considered proven in the literature. Furthermore, despite the promising results with wastewater treatment obtained in HRAPs with continuous and semi-continuous feeding (de-Bashan et al., 2002; Kim et al., 2014; Posadas et al., 2014 and 2015; Beydes and Kapdan 2018; Ruas et al., 2018; Salgueiro et al., 2018), variations of the feeding regime in HRAPs affect pollutant removal and biomass productivity, but the exact effects of these variations are still barely known, while this knowledge is crucial to understand and optimize the performance of microalgal-bacterial systems.

The objective of this work was thus to evaluate the influence of different feeding regimes (continuous versus semi-continuous) on the removal of surfactants and nutrients, as well as on the biomass production in three identical HRAPs treating primary domestic wastewater (PDW), at identical organic loading rates.

MATERIAL AND METHODS

Inoculum

The HRAPs were inoculated with a consortium formed mainly by *Scenedesmus* sp. ($\approx 98\%$) previously cultivated in outdoor reactors treating domestic wastewater, with a total suspended solids (TSS) concentration of 1.4 g L^{-1} . Activated sludge, collected from the Imbirussu WWTP in Campo Grande-MS (Brazil), with 4.2 gTSS L^{-1} was also inoculated in the HRAPs.

Primary domestic wastewater

Primary domestic wastewater (PDW) was collected from a primary treatment tank of a WWTP located in Campo Grande-MS, Brazil, and stored in a 300 L agitated cooling tank (Implemis, Brazil) at 4°C prior to feeding into the HRAPs. Influent soluble concentrations of surfactant, chemical oxygen demand (COD), total organic carbon (TOC), inorganic carbon (IC), total organic nitrogen (TN), ammonium ion (N-NH_4^+) and total phosphorus as P-PO_4^{3-} (TP) in the PDW are summarized in the Table 3-1. All parameters were analysed according to *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.* 2012). Nitrite (N-NO_2^-) and nitrate (N-NO_3^-) concentrations were below detection limits using ion chromatography (see section ‘Analytical procedures’).

Table 3-1 - Physical-chemical characteristics of the primary domestic wastewater during the experiment

Parameter	Unit	Value
Surfactant	mg L^{-1}	9.9 ± 0.7
COD	mg L^{-1}	127 ± 11
TOC	mg L^{-1}	119 ± 9
IC	mg L^{-1}	53 ± 11
TN	mg L^{-1}	66 ± 15
N-NH_4^+	mg L^{-1}	20 ± 5
TP	mg L^{-1}	6.1 ± 0.4
pH	-	8.0 ± 0.1

Experimental setup

The experimental setup consisted of three polypropylene 21 L HRAPs (R1, R2 and R3), with an illuminated surface of $\approx 0.13 \text{ m}^2$ and 16 cm cultivation broth depths (Figure 3-1), installed outdoors. A submerged pump with a nominal flow rate of 540 L h^{-1} (Sarlo Better B500, Brazil) was located at the bottom of each reactor, maintaining a liquid recirculation velocity

of $20 \pm 2 \text{ cm s}^{-1}$ in order to promote complete agitation (Ruas et al., 2018) Each HRAP was followed by a 1 L sedimentation tank (S1, S2 and S3), with a hydraulic retention time (HRT) of $\approx 8 \pm 0 \text{ h}$.

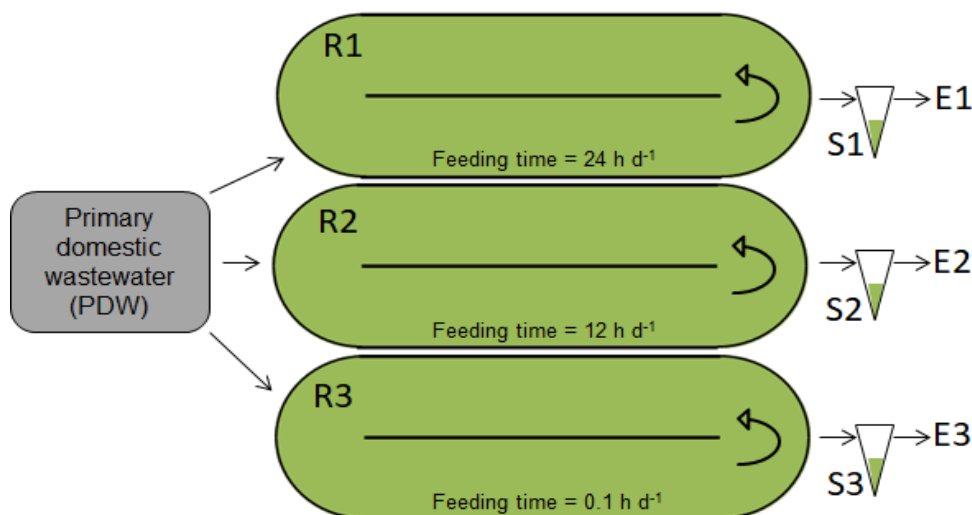


Figure 3-1- Experimental setup of the three 21 L HRAPs for primary domestic wastewater treatment

Operational conditions

The HRAPs were operated at similar organic loading rate and HRT, of $2.3 \pm 0.4 \text{ mgCOD m}^{-2} \text{ d}^{-1}$ and 7.0 ± 0.2 days, respectively, but with different feeding regimes, in order to evaluate the effect of feeding regime on PDW treatment. R1 was fed continuously (24 hours per day), whilst R2 and R3 were fed semi-continuously, during 12 and 0.1 hours per day, respectively. The feeding of R2 and R3 both started at 9:00 a.m but finished at 9:00 pm and 9:06 am, respectively. The experiment was conducted at the Effluents Laboratory of the Federal University of Mato Grosso do Sul (Campo Grande-MS, Brazil) for 36 days at a temperature of $\approx 29 \text{ }^{\circ}\text{C}$.

Sampling

Two samplings were performed three times a week to elucidate the performance of the HRAPs: in the morning (T1) and afternoon (T2). The first sampling, T1, occurred at 9:00 a.m. in the cultivation broth of R1, R2 and R3, before starting feeding of R2 and R3; and also at 9:06 a.m. in the effluent (E1, E2 and E3) of the settlers, after stopping feeding of R3. The second sampling, T2, occurred at 4:00 p.m., only in the cultivation broths of R1, R2 and R3, after several hours of exposure to sunlight. At T1, samples of 200 mL were taken to determine TSS and soluble concentrations of COD, TOC, IC, TN, N-NH_4^+ , N-NO_2^- , N-NO_3^- , TP and anionic surfactant. At T2, samples of 20 mL were drawn to determine the soluble anionic

surfactant. Samples of dissolved compounds were obtained from the samples by filtering through 0.45 μm glass fibre filters prior to analysis. The temperature, pH and dissolved oxygen (DO) concentrations were monitored daily at T1 and T2. All parameters were analysed according to *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.* 2012). Furthermore, the daily evaporation rate was determined from the difference between the influent and effluent flow rates.

Analytical procedures

The TOC, IC and TN were determined using a total organic carbon analyzer (Vario TOC Cube, Elementar, Germany). COD was analyzed by means of the closed reflux acid digestion method using dichromate reagent (APHA *et al.*, 2012). Both N-NH_4^+ and pH were measured using Orion Dual Star (Thermo Scientific, The Netherlands) ammonia and pH electrodes, respectively, while N-NO_2^- , N-NO_3^- and P-PO_4^{3-} were analyzed using a Dionex UltiMate ICS 1100 ion chromatography system with an IonPac AG19/AS19 column (Thermo Scientific, USA). The anionic surfactant concentration was determined using methyl dodecylbenzene sulphonate (MBAS) reagent in MN Nanocolor® Tube Tests. Temperature and DO were measured using a Jenway 9500 DO2 oximeter (Jenway, UK). The light intensity (PAR: photosynthetically active radiation) was recorded with a Quantum meter MQ-200 (Apogee Instruments, USA). The microalgae identification was carried out by microscopic examination (Olympus BX41, USA) of samples fixed with 5% lugol acid and stored at 4 °C prior to analysis.

RESULTS AND DISCUSSION

Surfactant removal efficiency

The influent surfactant concentration in this study, of $9.9 \pm 0.7 \text{ mg L}^{-1}$, was in the same range as in previous studies indicating 2–21 mg L^{-1} concentrations in domestic wastewater (Pirsaheb *et al.*, 2014). Surfactant concentrations comparable to the discharge limit in fresh water in Brazil of 0.5 mg L^{-1} (Brazil 2005) were found in all samples from the cultivation broth of R1 and R2, at T1 and T2 (Figure 3-2, left), and from the effluent of the settler E1 and E2 (Figure 3-2, right). This corresponds to an approximately 95% removal efficiency, suggesting continuous removal of surfactants from both reactors. Concentrations below the discharge limit were only recorded in R3 ($0.3 \pm 0.1 \text{ mg L}^{-1}$) just before feeding at T1, corresponding to a $97 \pm 1\%$ removal. On the other hand, surfactant concentrations in R3 effluent reached $1.5 \pm 0.3 \text{ mg L}^{-1}$ just after feeding (E3) (Figure 3-3), and reduced to $1.0 \pm 0.4 \text{ mg L}^{-1}$ after 7 hours of daylight at T2. This result shows that bacteria are responsible for the removal

(biodegradation) as even in R1 (continuous feeding) the concentrations at the end of the day are slightly higher than results obtained in the morning.

High removal efficiencies of anionic surfactants from municipal wastewater by *Scenedesmus* sp. (97.5%), *Chlamydomonas* sp. (98.0%), *Chlorella* sp. (99.4%), *Chlorococcum humicola* (97.9%) and *Botryococcus braunii* (99.1%) were recorded by Hena *et al.* (2015) in 10 days batch experiments, using municipal wastewater with an initial concentration of 50 mg L⁻¹. Katam *et al.* (2018), studied the effect of solid retention times (STR) (2–12 days) on anionic surfactant removal in a microalgal reactor and an aerobic bacterial reactor. Removal efficiencies reached up to 80% and 95%, respectively, at 10 days SRT. A wastewater treatment plant using wetlands also removed anionic surfactants from domestic wastewater, with 98.3% efficiency (Kruszelnicka *et al.*, 2019), while removal in continuous activated sludge systems ranged from 93.7 to 96.7% (initial concentration of ≈ 16 mg L⁻¹) (Pirsaheb *et al.*, 2014). On the other hand, Matamoros *et al.* (2015) obtained removal efficiencies of a non-ionic surfactant in HRAPs varying between 59% (cold season, 4 days HRT) and 93% (warm season, 8 days HRT).

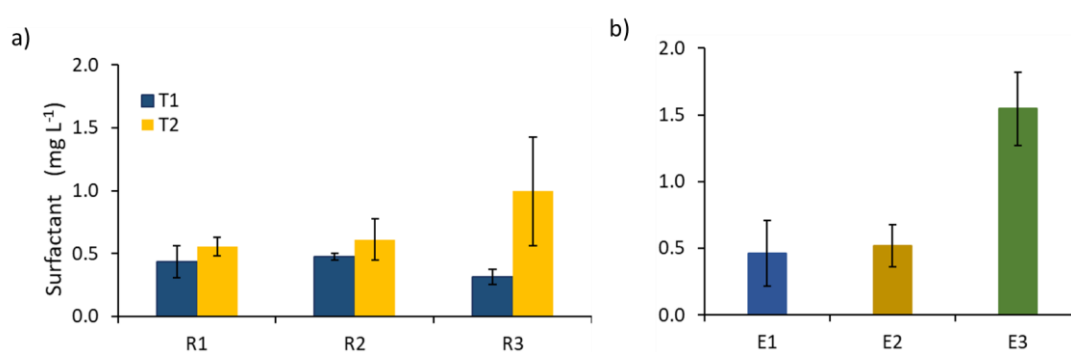


Figure 3-2 - Surfactant concentration of (left) the cultivation broth of R1, R2 and R3 at T1 and T2, and (right) the effluent E1, E2 and E3 from the settler.

Finally, the surfactant concentration in R3 clearly shows first-order degradation kinetics (Figure 3-3), with a rate constant of 1.18 d⁻¹. This biodegradation rate constant is similar to that obtained by Andrade *et al.* (2017), of 0.91–1.30 d⁻¹, using activated sludge for linear alkyl benzene sulfonate (LAS) removal; however lower than the rate constant calculated from results of Hena *et al.* (2015), of 2.72–4.98 d⁻¹, using different microalgae for municipal wastewater treatment.

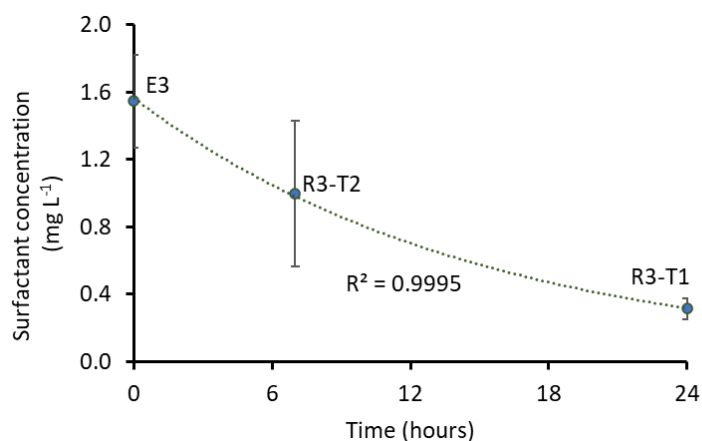


Figure 3-3 - Time course of surfactant degradation of R3 in the effluent E3 (after feeding) and cultivation broth at T1 and T2.

Biomass productivity and settleability

The different feeding regimes in R1, R2 and R3 promoted mixed culture biomass concentrations of 0.13 ± 0.02 , 0.23 ± 0.03 and 0.21 ± 0.01 g L⁻¹, corresponding to biomass productivity rates of 1.5, 3.6 and 3.3 g m⁻² d⁻¹, respectively. These rates were lower than those found by Ruas et al. (2018) and Posadas et al. (2015) of ≈ 4 and 5 g m⁻² d⁻¹ in continuous HRAPs treating domestic wastewater at 5 and 6 days HRT, respectively. The low carbon and nutrient loading rates applied to the HRAPs probably explain these lower recorded biomass productivities, as already reported by Posadas et al. (2014). On the other hand, the higher biomass production rate obtained with the semi-continuous feeding regime, when compared to the continuous feeding regime, may be directly related to two factors: i) the operation mode, as according to Beydes and Kapdan (2018), an intermittent feeding mode provides higher biomass concentrations and easier control of environmental conditions, as well as a better resistance to toxic or inhibitory compound loadings; and ii) the higher nutrient concentration available for microalgal growth during the period of exposure to sunlight. Kim et al. (2014) studied biomass growth in, and nutrient removal from raw municipal wastewater in a 60 L HRAP operated semi-continuously at HRTs of 2, 4, 6, and 8 days, and obtained a positive correlation between these parameters and increasing HRT, producing biomass concentrations of about 1.00, 1.26, 1.45 and 1.74 g L⁻¹, respectively. On the other hand, Ruas et al. (2018), found concentrations of 0.11–0.12 g L⁻¹ in continuously operating HRAPs treating domestic wastewater at a 5 days HRT, while 0.32–0.49 g L⁻¹ were recorded by Posadas et al. (2015) at 2.7–6.7 days HRT.

A good settleability of 81% was obtained in R2, followed by R1 (35%) and R3 (18%), however settleability was not correlated with the microalgal population found in the reactors (Figure 3-4, right). In all three reactors, *Scenedesmus* sp. was the main specie (> 98%) found after 36 days of operation, in line with literature: this specie has been commonly reported in continuous photobioreactors treating domestic wastewater (Muñoz and Guieysse, 2006; Posadas et al., 2015), but also in a semi-continuous HRAP treating raw municipal wastewater, together with *Chlorella* sp. and *Stigeoclonium* sp. (Kim et al., 2014). Settleability in this last case was very high, at 99%.

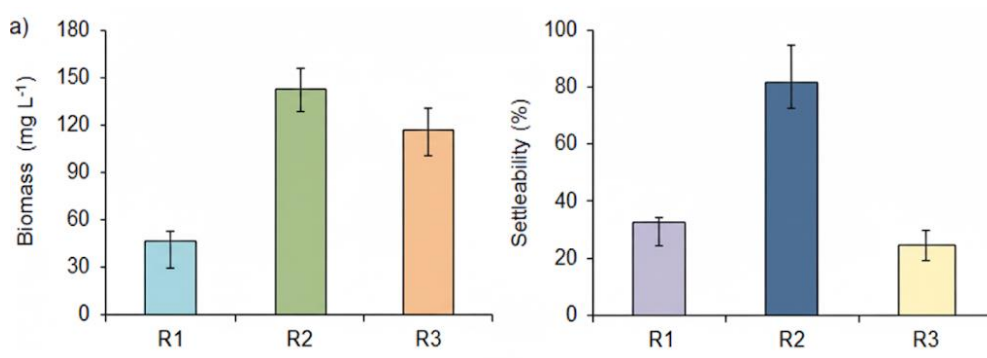


Figure 3-4 - left: Biomass concentration and right: settleability obtained in R1, R2 and R3 during secondary domestic wastewater treatment.

Removal efficiency of COD and nutrients

Despite the elevated temperatures (≈ 27.2 °C) obtained in the reactors (Table 3-2), recorded evaporation losses were relatively low (≈ 0.9 L m⁻² d⁻¹), when compared to the 1.3 L m⁻² d⁻¹ estimated by Guieysse et al. (2013) for outdoor conditions in tropical climates. On the other hand, in the cultivation broth of R1, a lower pH and DO were recorded than in R2 and R3, which was directly related to higher microalgal-bacterial growth in these reactors (Muñoz and Guieysse, 2006).

Similar COD and TOC removals of 70–74 and 37–42% respectively were found in all reactors, due to similar bacterial activity. Kim et al. (2014), found slightly lower COD and TOC removal efficiencies, of 63 and 34%, respectively, in a semi-continuous HRAP treating raw municipal wastewater at 8 days HRT (initial COD and TOC of 110 and 60 mg L⁻¹, respectively). In continuous HRAPs, COD and TOC removals ranged between 66–86 and 54–70%, respectively, at 6.0–6.7 days HRT (Posadas *et al.* 2015). Apart from organic carbon, microalgal processes may reduce inorganic carbon as well, as a result of photosynthesis. In R1, the removal of IC was higher ($67 \pm 4\%$) than observed in R2 ($59 \pm 5\%$) and in R3 ($58 \pm 8\%$). Based on the C-content of *Scenedesmus* sp. biomass cultivated in domestic

wastewater, around 50–53% was found by Posadas et al. (2015); the data suggest that in all reactors the main mechanism of C removal was assimilation into biomass. Stripping of carbon was also recorded, but, in line with expectations, this happens only in R1 (11–16%), and not in reactors R2 and R3, where the higher pH results in CO₂ retention. The pH can thus explain the higher removal of IC in R1.

Table 3-2 - Environmental conditions and COD and nutrient removal efficiencies found in the three HRAPs at T1.

	Unit	R1	R2	R3
Environmental conditions				
pH	-	8.6 ± 0.5	10.4 ± 0.1	10.1 ± 0.3
DO	mg L ⁻¹	7.6 ± 1.3	13.4 ± 1.3	11.8 ± 1.6
Temperature	°C	27.4 ± 1.9	27.3 ± 1.8	27.3 ± 1.8
Evaporation losses	L m ⁻² d ⁻¹	0.9 ± 0.7	0.9 ± 0.7	1.0 ± 0.7
Removal efficiencies				
COD	%	74 ± 8	73 ± 8	70 ± 13
TOC	%	41 ± 8	37 ± 10	42 ± 14
IC	%	67 ± 4	59 ± 5	58 ± 8
TN	%	65 ± 8	74 ± 6	76 ± 8
N-NH ₄ ⁺	%	100 ± 0	100 ± 0	100 ± 0
TP	%	46 ± 5	86 ± 1	80 ± 7

The TN removal efficiency as obtained in R1 (69 ± 8%) was slightly lower than in R2 (74 ± 6%) and in R3 (76 ± 8%), which can be related to the higher biomass productivity recorded in R2 and R3. Ammonia (N-NH₄⁺) was completely removed in all reactors, and nitrite (N-NO₂) and nitrate (N-NO₃) were not detected in the effluent. Phosphate removal in R1 (46 ± 5%) was also lower than in R2 and R3, with the removal from R2 (86 ± 1%), slightly higher than from R3 (80 ± 7%). Kim et al. (2014), found TN and TP removal efficiencies of 92–95 and 81–95%, respectively, in a semi-continuous HRAP treating raw municipal wastewater at 2–8 days HRT, with an initial TN of 44.8 mg L⁻¹ and an initial TP of 4.7 mg L⁻¹. Alternatively, Posadas et al., (2015) recorded TN and TP removals of 60–97% and 33–70%, respectively, in continuous HRAPs treating primary domestic wastewater during different seasons, at 2.8–6.7 days HRT and using a controlled pH (initial TN of 52–70 mg L⁻¹ and TP of 9–11 mg L⁻¹). Higher ammonium and phosphorus removal efficiencies from synthetic wastewater, using *Chlorella vulgaris* (UTEX 2714), were also found by de-Bashan

et al. (2002) in semi-continuous cultures, when compared to the removal in continuous and batch cultures.

Based on the biomass N (8.4–9.0%) and P (0.8–1.3%) content of *Scenedesmus* sp. cultivated in domestic waste- water as obtained by Posadas *et al.* (2015), we can infer that the main mechanism of N removal from R1 was ammonia stripping (>73%), while in R2 and R3 it was ammonia stripping (54–57%) and assimilation into biomass (43– 46%). Considering the pH of the reactors, a higher share of ammonium ion in the cultivation broth was evidenced in R1, while ammonia was recorded in R2 and R3. In contrast, assimilation into biomass accounts for the same range in the three reactors (34–58%); however, the high pH recorded may have promoted significant precipitation of P (Muñoz & Guieysse, 2006).

CONCLUSIONS

Semi-continuous feeding in HRAPs operated during the day- light period showed to be more advantageous than the usual continuous operation, with respect to both the microalgal biomass productivity and the nutrient removal efficiencies. Especially considering surfactant removal, a feeding for only 0.1 h d⁻¹ may give the best results when the treated effluent is withdrawn from the system before admitting new influent (batch operation). However, further research is still needed to increase the performance of microalgal-bacterial systems operated in batch or semi-continuously for domestic wastewater treatment, as well as an investigation into the removal of other groups of surfactants and an improvement of nutrient recovery into biomass, and also an analysis of economic viability.

REFERENCES

- Aboulhassan, M.A., Souabi, S., Yaacoubi, A. and Baudu, M. (2006) Removal of surfactant from industrial wastewaters by coagulation flocculation process. *International Journal of Environmental Science and Technology*, **3**(4), 327-332.
- Andrade, M.V.F., Sakamoto, I.K., Corbi, J.J., Silva, E.L. and Varesche, M.B.A. (2017) Effects of hydraulic retention time, co-substrate and nitrogen source on laundry wastewater anionic surfactant degradation in fluidized bed reactors. *Bioresource Technology*, **224**, 246-254.
- APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environment Federation), *Standard Methods for the Examination of Water and Wastewater* (2012), 22th edn, Washington DC, USA, ISBN 08-7553-047-8
- Beydes, H. and Kapdan, I.K. (2018) Algal Nutrient Removal from Wastewater in Fed-Batch Operated Photobioreactor. *International Journal of Environmental Research*, **12**(3), 303–311.
- Brasil (2005) National Environment Council (Conselho Nacional do Meio Ambiente - CONAMA), Resolution No. 357.

- Brasil (2024) Relatório dos Serviços de Esgotamento Sanitário - Ano de Referência 2023, Brasília. [online]
- de-Bashan, M.M., Hernandez, J.-P. and Bashan, Y. (2002) Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. *Water Research*, **36**, 2941–2948.
- Guieysse, B., Béchet, Q. and Shilton, A. (2013) Variability and uncertainty in water demand and water footprint assessments of fresh algae cultivation based on case studies from five climatic regions. *Bioresource Technology*, **128**, 317–323.
- Hena, S., Abida, N. and Tabassum, S. (2015) Screening of facultative strains of high lipid producing microalgae for treating surfactant mediated municipal wastewater. *RSC Adv.*, **5**, 98805–813.
- Katam, K. and Bhattacharyya, D. (2018) Comparative study on treatment of kitchen wastewater using a mixed microalgal culture and an aerobic bacterial culture: kinetic evaluation and FAME analysis. *Environmental Science and Pollution Research*, **25**, 20732–742.
- Kim, B.-H., Kang, Z., Ramanan, R., Choi, J.-E., Cho, D.-H., Oh, H.-M. and Kim, H.-S. (2014) Nutrient Removal and Biofuel Production in High Rate Algal Pond using real municipal wastewater. *Journal of Microbiology and Biotechnology*, **24**(8), 1123–1132.
- Kruszelnicka, I., Ginter-Kramarczyk, D., Wyrwas, B. and Idkowiak, J. (2019) Evaluation of surfactant removal efficiency in selected domestic wastewater treatment plants in Poland. *Journal of Environmental Health Science and Engineering*, 1–8.
- Lechuga, M., Fernández-Serrano, M., Jurado, E., Núñez-Olea, J., and Ríos, F. (2016) Acute toxicity of anionic and non-ionic surfactants to aquatic organisms. *Ecotoxicology and Environmental Safety*, **125**, 1–8.
- Market Wired (2017) Surfactants market to be worth \$ 46.20 billion by 2022: Grand View Research, Inc., Available at: <https://www.marketwatch.com/press-release/surfactants-market-to-reach-4620-billion-by-2022-grand-view-research-inc-2015-10-28>, Accessed 25th Nov 2019.
- Matamoras, V., Gutiérrez, R., Ferrer, I., García, J., and Bayona, J.M. (2015) Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. *Journal of Hazardous Materials*, **288**, 34–42.
- Muñoz, R. and Guieysse, B. (2006), Algal-bacterial processes for the treatment of hazardous contaminants: a review. *Water Research*, **40**, 2799–815.
- Nitschke, M. and Pastore, G.M. (2006), Production and properties of a surfactant obtained from *Bacillus subtilis* grown on cassava wastewater. *Bioresource Technology*, **97**, 336–341.
- Palmer, M. and Hatley, H. (2018) The role of surfactants in wastewater treatment: Impact, removal and future techniques: A critical review. *Water Research.*, **147**, 60–72.
- Pirsaheb, M., Dargahi, A. and Zinatizadeh, A. (2014) A comparison between extended aeration sludge and conventional activated sludge treatment for removal of linear alkylbenzene sulfonates (Case study: Kermanshah and Paveh WWTP). *Desalination and Water Treatment*, **52**, 25–27.

- Posadas, E., Morales, M. del M., Gomez, C., Acién, G. and 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.
- Posadas, E., Muñoz, A., García-González, M.C., Muñoz, R. and García-Encina, P.A. (2014) A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters. *Journal of Chemical Technology and Biotechnology*, **90**, 1094–1101.
- Ruas, G., Serejo, M.L., Paulo, P.L. and Boncz, M.Á. (2018) Evaluation of domestic wastewater treatment using microalgal-bacterial processes: effect of CO₂ addition on pathogen removal. *Journal of Applied Phycology*, **30**, 921–929.
- Salgueiro, J.L., Pérez, L., Maceiras, R., Sánchez, Á. and Cancela, Á. (2018) Semicontinuous culture of *Chlorella vulgaris* Microalgae for wastewater treatment. *International Journal of Environmental Research*. **12**, 765–772.
- Scott, M.J. and Jones, M.N. (2000) The biodegradation of surfactants in the environment. *Biochimica et Biophysica Acta*, **1508**, 235–251.
- UNICEF and WHO (2019) *Progress on household drinking water, sanitation and hygiene 2000-2017: Special focus on inequalities*, New York, United Nations Children's Fund (UNICEF) and World Health Organization (WHO).

CHAPTER 4 :

INTERMITTENT MIXING IN HIGH RATE ALGAL PONDS: IMPLICATIONS FOR SURFACTANT AND PATHOGEN REMOVAL⁸

ABSTRACT

High rate algal ponds (HRAPs) are shallow, open systems that efficiently treat wastewater using microalgae-bacteria consortia while recovering nutrients and producing biomass for biofertilizers and bioproducts. Paddlewheels, commonly used to promote continuous mixing in these systems, are essential to prevent biomass sedimentation and to enhance the distribution of light and nutrients throughout the pond. However, despite their importance, paddlewheels are among the most energy-demanding components of HRAPs. In this context, this study aimed to evaluate the impact of intermittent mixing on the removal of surfactants and pathogens from domestic sewage, as a potential strategy to reduce energy consumption without compromising treatment performance. Results showed that both continuous and intermittent agitation achieved high removal efficiencies for surfactants (> 90%) and *E. coli* (> 97%), with no significant differences between strategies. Biomass productivity reached 0.87 ± 0.5 g TSS L⁻¹, slightly higher under continuous mixing. These findings indicate that intermittent agitation is a viable strategy to reduce energy consumption in HRAPs without compromising treatment performance. These results reinforce the potential of HRAPs with intermittent mixing as a low-energy solution for decentralized wastewater treatment systems.

INTRODUCTION

High-rate algal ponds (HRAPs) have emerged as a promising and increasingly explored technology for wastewater treatment using microalgae–bacteria systems. Their shallow, open design enhances light penetration and gas exchange, optimizing microalgal photosynthesis and bacterial metabolism, contributing to more efficient removal of organic matter and nutrient uptake (Muñoz and Guieysse, 2006). These systems align with circular economy principles by integrating wastewater treatment with biomass valorization, enabling resource recovery for biofertilizers, biofuels, and bioproducts, and promoting the reuse of treated effluent in agriculture or industry (Shahid, Malik, et al., 2020).

⁸ Paper presented at the IWA 17th Small Water and Wastewater Systems and 9th Resource Oriented Sanitation Conferences, Nov. 2024, Curitiba-PR, Brazil.

Unlike conventional activated sludge processes, HRAPs operate with significantly lower energy input, as oxygen is primarily supplied via photosynthesis rather than mechanical aeration (Arashiro, Montero, et al., 2018; Delanka-Pedige, Cheng, et al., 2020). Some configurations report energy requirements below 2 W/m³, highlighting their potential for sustainable wastewater management in both decentralized and large-scale applications (Acién Fernández et al., 2018). These characteristics reinforce their potential for sustainable wastewater treatment, especially in contexts where decentralized or low-cost solutions are needed. Nevertheless, challenges such as land use, seasonal variability, and biomass harvesting efficiency still require further investigation (Sutherland and Ralph, 2020).

Despite their overall low energy requirements, HRAPs do require some mechanical energy input to maintain optimal conditions for microalgal and bacterial growth. The use of paddlewheels is essential to ensure continuous mixing of the culture, preventing the sedimentation of microalgae-bacteria aggregates and facilitating enhanced nutrient diffusion across the cellular boundary layer (Rogers, Rosenberg, et al., 2014). This mixing process represents the second highest energy demand in HRAPs, after biomass harvesting, but remains significantly lower than the aeration energy demands in activated sludge systems (Sutherland and Ralph, 2020; Kohlheb, van Afferden, et al., 2020).

Investigating operational parameters such as mixing regime, hydraulic retention time, feeding strategy, and light availability is essential to enhance the performance of HRAPs. Adjusting and optimizing these conditions can lead to improved pollutant removal, greater biomass productivity, and reduced operational costs, thereby improving the feasibility of this technology for sustainable wastewater treatment across diverse environmental and socioeconomic contexts (Ruas, Farias, et al., 2020b; Pompei, Ruas, et al., 2024; Butterworth and Fallowfield, 2024; Serejo, Farias, et al., 2020; Sivakaminathan, Wolf, et al., 2020).

Among these parameters, the mixing strategy plays a key role in maintaining biomass in suspension and ensuring uniform distribution of light and nutrients, as well as preventing thermal stratification and light limitation that can impair algal activity (Dammak, Fersi, et al., 2023). While continuous mixing is commonly used and effective, it accounts for a significant portion of the system's energy demand. Intermittent mixing has emerged as a potential alternative to reduce energy consumption while preserving treatment efficiency (Butterworth and Fallowfield, 2024).

Anionic surfactants and *Escherichia coli* were selected as target pollutants due to their widespread occurrence in domestic wastewater and their environmental and public health

relevance. Surfactants, commonly used in household and industrial products, are often present at elevated concentrations, posing challenges to conventional treatment processes (Palmer and Hatley, 2018; Li, Yuan, et al., 2025). Among them, linear alkylbenzene sulfonate (LAS)—a mixture of C₁₀ to C₁₃ isomers representing 25–30% of global surfactant production—is the main anionic surfactant in detergents, characterized by its amphiphilic structure and classified as a sulfate ester (Askari, Vahabzadeh, et al., 2021).

Escherichia coli was included as a microbiological indicator because of its established role in assessing fecal contamination and the hygienic quality of treated effluents (Abd-Elmaksoud, Abdo, et al., 2021; Farias, Ruas, et al., 2023). Compared to thermotolerant coliforms, *E. coli* provides greater public health protection, as it can be quantified more reliably and at lower cost using methods such as the Quantitray technique, and it avoids interference from non-fecal bacteria like thermotolerant *Klebsiella* (Dias, Passos, et al., 2017).

Therefore, this study evaluated the effect of intermittent mixing on the removal of surfactants and pathogens from domestic sewage in HRAPs, aiming to assess its viability as a cost-effective operational strategy.

MATERIAL AND METHODS

Microorganisms and domestic wastewater

The secondary domestic wastewater used as influent was collected from the septic tank of a local sewage treatment plant. Throughout the study, the influent was characterized for physicochemical parameters, including pH, total suspended solids (TSS), chemical oxygen demand (COD), Total organic carbon (TOC), Inorganic carbon (IC), ammonium (NH₄⁺-N), nitrate (NO₃⁻-N), nitrite (NO₂⁻-N), Total nitrogen (TN), anionic surfactants, and *Escherichia coli*, with the following average concentrations, respectively: pH 7.67; TSS 35 ± 20 mg L⁻¹, 146 ± 45 COD mg L⁻¹; TOC 81 ± 55 mg L⁻¹; IC 40 ± 12 mg L⁻¹; ammonium 23 ± 5 mg N-NH₄⁺ L⁻¹; nitrite 0.02 ± 0.01 mg N-NO₂⁻ L⁻¹; nitrate 3.8 ± 2.1 mg N-NO₃⁻ L⁻¹; NT 62 ± 7 mg L⁻¹, anionic surfactants 17 ± 5 mg L⁻¹; and *E. coli* 6.8 ± 2.0 × 10⁻¹ MPN.

The inoculum used to seed the HRAPs consisted of a microalgae culture with an initial concentration of 0.95 g TSS L⁻¹, obtained from a pre-established HRAP system. Additionally, nitrifying-denitrifying activated sludge sourced from a municipal wastewater treatment plant (WWTP) was introduced, with an initial concentration of 0.9 g TSS L⁻¹.

Experimental setup

The experimental setup comprised two High Rate Algal Pond (HRAP) type reactors, designated as R1 and R2 (Figure 4-1). Each reactor had a working volume of 40 liters, 16 cm of water depth and an illuminated surface area of 0.35 m². The hydraulic retention time (HRT) was maintained at 5 days and the experiment was conducted outdoors at a university campus located in Mato Grosso do Sul, Brazil.

To maintain appropriate mixing conditions, the HRAPs were agitated using a submersible pump (Sarlo Better B650, Brazil) with a nominal flow rate of 650 L h⁻¹. This setup ensured a consistent recirculation velocity of 20 ± 2 cm s⁻¹, which is within the optimal range for promoting biomass suspension and preventing excessive sedimentation of particulates. The agitation regime was configured differently for each reactor: R1 operated with continuous mixing throughout the experimental period, while R2 had an intermittent mixing schedule, with agitation activated only between 06:00 am and 06:00 pm. This configuration allowed for comparative analysis of the impact of mixing regimes on system performance, including the influence on microbial communities and pollutant removal efficiency.

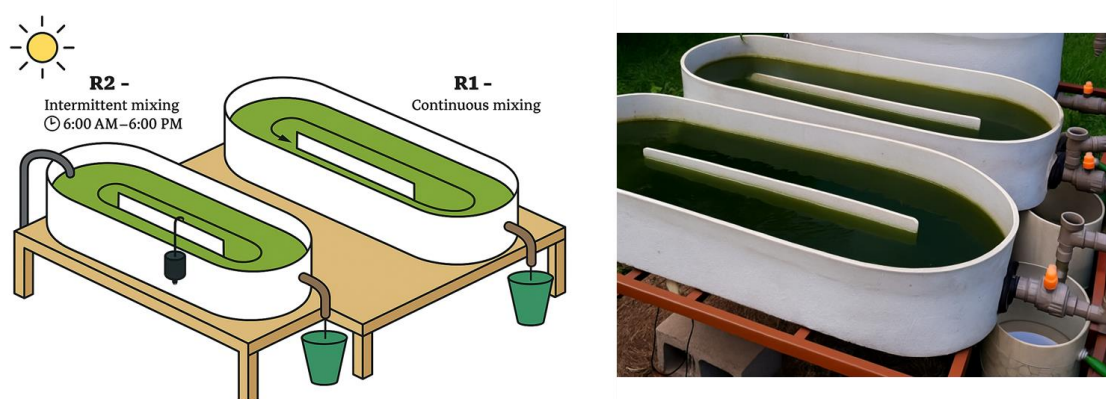


Figure 4-1 - Schematic and real design of the system of the HRAP reactors operated in parallel used in the study, with the configuration in the mixing regime: R1 with continuous mixing and R2 with intermittent mixing.

Operational conditions and sampling

Samples were collected three times a week for the determination of soluble concentrations of chemical oxygen demand (COD), total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), ammonium ion (N-NH₄⁺), nitrite (N-NO₂⁻), nitrate (N-NO₃⁻), *Escherichia coli*, and surfactants. pH, dissolved oxygen (DO), temperature, turbidity, and effluent volume were continuously monitored using sensors integrated into a system based on Arduino.

Analytical procedures

Analytical procedures followed the guidelines set by APHA, AWWA, and WEF (2012). Photosynthetically Active Radiation (PAR) was measured using a PAR MQ-200 quantum sensor (Apogee Instruments, USA). Total Organic Carbon (TOC), Inorganic Carbon (IC), Total Carbon (TC), and Total Nitrogen (TN) were quantified with a Vario TOC cube analyzer (Elementar, Germany). Parameters including pH, dissolved oxygen (DO), temperature, and turbidity were continuously monitored via sensors integrated into an online system, ensuring real-time data collection.

Ammonium nitrogen (N-NH_4^+) levels were determined using an ammonia-selective electrode connected to an Orion Five Star multiparameter meter (Thermo Scientific, USA). Nitrate (N-NO_3^-) and nitrite (N-NO_2^-) concentrations were assessed colorimetrically, measuring absorbance with a DR 3900 spectrophotometer (Hach, Germany). Turbidity was measured using Hanna HI98703-01 bench meter (Hanna Instruments, USA). Microalgal species identification followed Sournia's (1978) methodology, utilizing a Leica DM5500B microscope (Germany).

Anionic surfactant concentrations in the reactors were measured using the simplified method by Jurado, Fernández-Serrano et al. (2006), chosen for its efficiency in evaluating surfactant degradation in wastewater treatment. Chlorophyll-*a* concentrations were determined spectrophotometrically after pigment extraction with 90% acetone at 4 °C, using absorbance readings at 665 and 750 nm before and after acidification, with corrections for pheophytin interference.

Escherichia coli (*E. coli*) presence was detected using Colilert® test kits (IDEXX Laboratories, Westbrook, ME, USA), a standard approach for rapid coliform identification in water samples. The *E. coli* decay rate (k , d^{-1}) was estimated from influent and effluent concentrations and hydraulic retention time (HRT), using a first-order model assuming pseudo-steady-state conditions (Craggs, Zwart, et al., 2004; Chambonniere, Bronlund, et al., 2020).

Statistical analyses were performed using the R programming language. Analysis of variance (ANOVA) evaluated differences between reactor operating conditions. Upon identifying significant differences, Tukey's HSD post hoc test was applied for pairwise comparisons at a 95% confidence level ($p < 0.05$), providing a robust assessment of treatment performance and mixing regime impacts.

RESULTS AND DISCUSSION

Operational and environmental conditions

The experiment was conducted outdoors over a 26-day period, with an average ambient temperature of 23.9 ± 6 °C. As shown in Table 4-1, both reactors (R1 and R2) exhibited similar environmental conditions throughout the experiment, with pH values consistently above 9.0 and dissolved oxygen (DO) concentrations around 5.0 mg L^{-1} , indicative of favorable conditions for microalgae growth and aerobic microbial processes (Dammak, Fersi, et al., 2023).

Table 4-1 - Results of pH, Dissolved oxygen (DO) concentration, cultivation broth temperature, and percentage of chemical oxygen demand (COD) removal in HRAPs with continuous mixing (R1) and with intermittent mixing (R2).

Reactor	Environmental conditions		Reactor operating conditions		
	Temperature (°C)	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	pH	DO (mg L^{-1})	Temperature (°C)
R1	23.2 ± 6	3067 ± 1483	9.7 ± 1	7.4 ± 3	23.1 ± 2
R2			9.9 ± 1	5.9 ± 1	21.8 ± 1

The similar pH and DO levels observed in both reactors indicate that the two mixing strategies - continuous and intermittent - provided comparable conditions for photosynthetic activity and oxygen transfer. Alkaline pH values above 9.0 are typical in high-rate algal ponds (HRAPs) due to intense CO_2 uptake during microalgal photosynthesis (Park and Craggs, 2010), and DO concentrations above 5 mg L^{-1} suggest a well-oxygenated environment, minimizing anaerobic zones and favoring organic matter degradation.

The diurnal physicochemical profiles of the HRAPs reflected the influence of mixing regimes. R1 (continuous mixing) showed higher dissolved oxygen levels throughout the day, peaking at 14.8 mg L^{-1} , compared to 8.4 mg L^{-1} in R2 (intermittent mixing), as show in Figure 4-2. R1 also maintained slightly higher temperatures (up to 25 °C), closer to the ambient temperatures, likely due to better circulation and more intense contact with the external environment. R1 exhibited lower and more variable pH values (≈ 9.0 – 9.4), closer to the optimal range for microalgal growth, as mixing must have improved CO_2 transport from the air into the liquid phase, whereas R2 maintained a more stable but excessively alkaline profile (≈ 9.9 – 10.2). The higher pH values observed in both reactors are associated with the photosynthetic activity of microalgae, which consumes dissolved CO_2 and reduces carbonic acid formation, thereby leading to an increase in pH (Dammak, Fersi, et al., 2023),



Figure 4-2 - Daily variation of dissolved oxygen, pH, and temperature in reactors with continuous mixing (R1) and intermittent mixing (R2).

Removal efficiency of surfactants

The removal of anionic surfactants was highly efficient in both mixing regimes, with average efficiencies of $89 \pm 7\%$ in R1 and $90 \pm 6\%$ in R2, and no significant difference between them ($p > 0.05$; Figure 4-3). Final effluent concentrations were consistently below 2 mg L^{-1} , demonstrating the capacity of HRAPs to substantially reduce synthetic detergent levels in treated wastewater.

The high removal performance is mainly attributed to aerobic bacteria, which effectively metabolize surfactants as a source of carbon and energy, taking advantage of the increased oxygen concentrations resulting from microalgal photosynthesis, while the microalgae

provide a secondary contribution through biomass-driven uptake and photo-oxidative processes (Katam and Bhattacharyya, 2018). The action of the microalgae may go beyond synergistic mechanisms in surfactant removal, as demonstrated by the high efficiency of facultative heterotrophic strains (e.g., *Scenedesmus* sp., *Chlorella* sp.) that can remove surfactants from municipal wastewater, achieving removal efficiencies above 90% (Hena, Abida, et al., 2015). LAS removal by microalgae is primarily linked to the production of reactive oxygen species (ROS) during photosynthesis, which drive surfactant breakdown beyond the effects of pH and dissolved oxygen (Hua, Li, et al., 2012).

Similar efficiencies were observed in our earlier experiments (Serejo, Farias, et al., 2020; see also Chapter 3 of this thesis), when removal efficiencies of 90–97% were observed in HRAPs operated under different feeding regimes. In those experiments, emphasis was on the influence of photoperiod and microbial adaptation on the degradation of synthetic organic compounds though. The present results again indicate that the temporal mixing pattern (continuous or intermittent) does not markedly influence the microbial capacity to degrade anionic surfactants, as long as light availability and pH conditions remain conducive to metabolic activity.

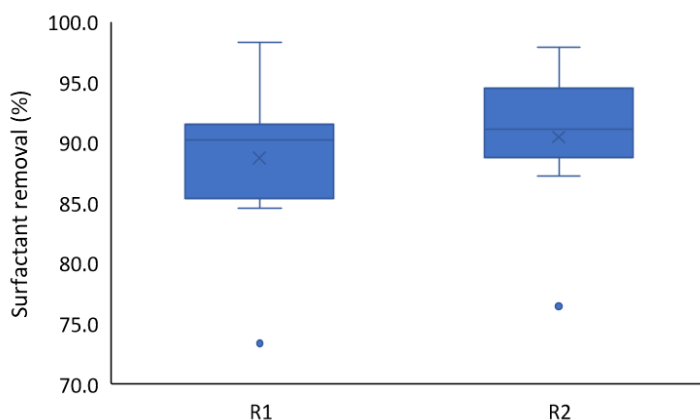


Figure 4-3- Removal efficiencies (average values and standard deviations) in percentages for surfactants (n=10) in HRAPs R1 (continuously mixed) and R2 (intermittently mixed).

Removal efficiency of pathogens (*E.coli*)

E. coli inactivation was high in both reactors, with no statistically significant difference between them ($p > 0.05$). R2 achieved a log removal value (LRV) of 1.8 ± 1.3 , corresponding to a $98 \pm 2\%$ removal efficiency (Figure 4-4, above). These results are consistent with those obtained by Butterworth and Fallowfield (2024), who observed comparable *E. coli* reductions in HRAPs operated with 10 days of mixing cessation, reporting no significant differences in

LRVs between continuously and intermittently mixed systems. Equivalent results were reported by Ruas, Farias, et al. (2022), who investigated the effects of CO₂ addition and feeding regimes (continuous versus semicontinuous) on pathogen removal in HRAPs treating raw sewage; no significant differences in *E. coli* removal efficiency were found, with values consistently above 98%.

The effectiveness of pathogen removal in both reactors can be attributed to the synergistic action of multiple factors, including high dissolved oxygen levels, alkaline pH, extended hydraulic retention time (HRT), and continuous exposure to solar radiation (Nirmalakhandan, Selvaratnam, et al., 2019). The elevated pH (> 9.0) observed in both treatments likely played a key role in microbial inactivation, since photosynthetic activity can render the water environment alkaline, beyond the tolerance range of many pathogens (Dammak, Fersi, et al., 2023). This agrees with Chambonniere, Bronlund, et al. (2022), who demonstrated that alkaline pH-induced toxicity is a major driver of *E. coli* decay, highlighting its relevance as a dominant disinfection pathway in HRAPs.

Additionally, oxygen-rich conditions in HRAPs favor the production of reactive oxygen species (ROS) via photochemical processes, which can further enhance pathogen inactivation (Chambonniere, Bronlund, et al., 2021). Previous studies have highlighted the importance of sunlight exposure and algal photosynthesis in achieving high disinfection rates (Fallowfield et al., 2022; Park et al., 2011). Light exposure is a key driver, as Pompei, Ruas, et al. (2023), demonstrated significantly reduced *E. coli* removal under shading, highlighting the importance of both direct UVB damage and ROS-mediated oxidative stress. In the present study, even though intermittent mixing may have influenced biomass shading, *E. coli* removal remained high ($\approx 98\%$), suggesting that photo-driven disinfection mechanisms were not substantially hindered.

The *E. coli* decay rates (k , d^{-1}) fluctuated throughout the monitoring period, ranging from approximately 0.57 to 1.04 d^{-1} in both reactors (Figure 4-4, below). Although R2, operated with intermittent mixing during daylight hours, exhibited slightly higher decay rates on most days, the differences between both reactors were not statistically significant ($p > 0.05$). These findings are consistent with our earlier work (Farias, Ruas, et al., 2023; see also Chapter 3 of this thesis), which reported similar temporal fluctuations in decay coefficients in HRAPs (0.54 ± 0.7 to $1.06 \pm 0.4 d^{-1}$) with no significant differences.

Intermittent mixing did not compromise the hydrodynamic conditions needed for pathogen contact with light and oxygen throughout the pond volume. This suggests that daytime mixing is sufficient to maintain a well-distributed biomass layer, promoting uniform exposure to disinfection mechanisms. These findings reinforce the potential of HRAPs as effective, low-energy systems for pathogen removal, even under simplified operational strategies.

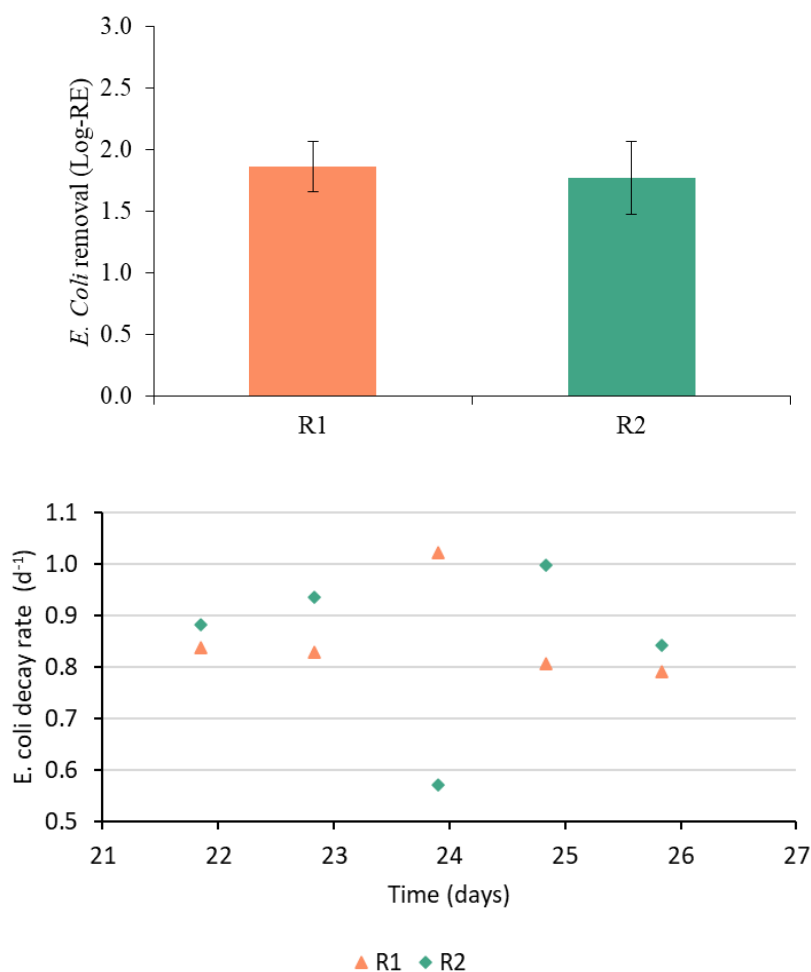


Figure 4-4 – above: Removal efficiencies (average values and standard deviations) in logarithmic units (LRV) of *Escherichia coli* (n=5) in HRAPs R1 (continuously mixed) and R2 (intermittently mixed); and below: Variations of the *Escherichia coli* decay rates (day⁻¹) during the experiment.

Removal efficiency of COD and nutrients, Productivity and Microalgae Population

Regarding chemical oxygen demand (COD), the reactor operated under continuous mixing (R1) achieved a mean removal efficiency of $59 \pm 6\%$, while the reactor with intermittent mixing (R2) reached $50 \pm 5\%$ (Table 4-2). Although R1 exhibited slightly higher average removal, statistical analysis showed no significant difference between the two operational modes ($p = 0.146$). These results indicate that intermittent mixing can deliver comparable

performance to continuous mixing in terms of organic matter removal, despite the slightly greater variability observed in R1. This outcome is consistent with previous studies demonstrating the feasibility of reducing mixing time in HRAPs without compromising treatment efficiency, especially when mixing is aligned with light availability (Young, Fallowfield, et al., 2017). Nevertheless, despite the lower performance in organic matter removal, the final COD concentrations were within the typical outflow values for HRAPs and below the limits established by discharge guidelines (Kohlheb, van Afferden, et al., 2020).

Based on the results shown in Table 4-2, the continuous mixing regime (R1) generally outperformed the intermittent regime (R2) in nitrogen removal. For TN, R1 achieved a $82 \pm 1\%$ removal efficiency, versus $77 \pm 3\%$ in R2, with Tukey's test confirming this difference as statistically significant. Ammonium nitrogen ($\text{N}-\text{NH}_4^+$) removal was also high in both regimes ($97 \pm 2\%$), consistent with the preferential assimilation of ammonia by microalgae and potential contributions from ammonia stripping under high pH and temperature (Eltanahy, Salim, et al., 2018). The superior nitrogen removal in R1 is likely linked to its higher biomass productivity, enhancing assimilation of both ammonium and other nitrogen forms. It is also possible that greater mixing promotes larger, denser colonies, which can increase nutrient removal efficiency, as reported by Sutherland, Turnbull, et al. (2014).

For carbon-related parameters, TOC and IC removals were $42 \pm 7\%$ and $39 \pm 12\%$ in R1, and $35 \pm 6\%$ and $37 \pm 5\%$ in R2, respectively, with no significant differences detected. The high daytime pH typical of HRAPs alters the carbonate equilibrium, decreasing the fraction of dissolved CO_2 available for direct uptake. During the pH rise, the observed inorganic carbon dynamics can be attributed to both photosynthetic CO_2 consumption and CO_2 stripping from the culture medium (Valdés, Hernández, et al., 2012). When compared to the 47% TOC and 45% IC removals reported in our previous study on HRAP performance (Serejo, Farias, et al., 2020; see also Chapter 3 of this thesis) under the same continuous mixing velocity of 20 cm s^{-1} , the corresponding values obtained in the present experiments were slightly lower.

The biomass productivity of the HRAPs remained relatively stable under both mixing regimes, with average values of $34.6 \pm 14 \text{ g m}^{-2} \text{ day}^{-1}$ in R1 and $27.7 \pm 22 \text{ g m}^{-2} \text{ day}^{-1}$ in R2 (Table 4-2). Although R1 exhibited a numerically higher mean productivity, the large standard deviations indicate considerable temporal variability, and no statistically significant differences were detected between the two systems. These results suggest that both continuous and intermittent mixing supported comparable biomass yields, reflecting the capacity of HRAPs to sustain robust algal growth under the tested operational conditions.

Similar productivity levels were reported by Sánchez Zurano, Garrido Cárdenas, et al. (2020), who observed photoautotrophic biomass yields ranging from 26.6 to 45.9 g m⁻² day⁻¹ in thin-layer photobioreactors treating wastewater, emphasizing the dominance of autotrophic metabolism and the feasibility of maintaining stable production under variable environmental conditions.

No statistically significant differences were observed between the continuously mixed (R1) and intermittently mixed (R2) reactors in terms of total suspended solids (TSS) or chlorophyll *a* concentration (Table 4-2). TSS values averaged 958 ± 0.4 mg L⁻¹ in R1 and 873 ± 0.6 mg L⁻¹ in R2, while chlorophyll *a* concentrations were 2.2 and 2.1 mg L⁻¹, respectively. These results are consistent with those reported by Butterworth and Fallowfield (2024), who found no significant differences in algal biomass between HRAPs under continuous and intermittent mixing. Despite favoring sedimentation, the daytime mixing in R2 was sufficient to maintain biomass suspension and light access, supporting a photosynthetic activity similar to that found in R1.

Table 4-2 - Percentages of COD, TOC, IC, TN, N-NH₄⁺, and concentrations of biomass productivity during the operation of the two HRAPs: R1 (continuously mixed) and R2 (intermittantly mixed) (avg ± sd).

	Removal efficiencies (%)					Microalgal biomass		
	COD (n=5)	TOC (n=5)	IC (n=5)	TN (n=5)	N-NH ₄ ⁺ (n=9)	TSS (mg L ⁻¹) (n=6)	Productivity (g m ⁻² d ⁻¹) (n=6)	Chlorophyll <i>a</i> (mg L ⁻¹) (n=1)
R1	59 ± 6	42 ± 7	39 ± 12	82 ± 1	97 ± 2	958 ± 0.4	34.6 ± 14	2.2
R2	50 ± 5	35 ± 6	37 ± 5	77 ± 3	97 ± 2	873 ± 0.6	27.7 ± 22	2.1

Microscopic analysis revealed that *Chlorella* sp. was the predominant microalga in both reactors throughout the experimental period, consistent with its known adaptability to high-rate algal pond (HRAP) conditions (de Cassia Soares Brandão, Oliveira, et al., 2023). Intermittent mixing did not substantially alter the species composition, indicating that both operational regimes supported a stable algal consortium capable of sustaining efficient organic matter removal and disinfection processes. This dominance aligns with global trends, as *Chlorella* sp. is the most widely employed genus for wastewater bioremediation due to its resilience and capacity to form consortia with other microalgae and bacteria, enhancing nutrient and contaminant removal (Abuhasheesh, Ghazal, et al., 2025).

CONCLUSIONS

The present study demonstrated the effectiveness of High-Rate Algal Ponds (HRAPs)

operated with intermittent agitation for domestic wastewater treatment, with a focus on surfactant and pathogen removal. The results confirm that intermittent agitation is a viable and efficient operational strategy that does not compromise treatment performance. Surfactant removal efficiencies exceeded 90% ($89 \pm 7\%$ under continuous mixing and $90 \pm 6\%$ in under intermittent mixing), with final effluent concentrations consistently below 2 mg L^{-1} , while *Escherichia coli* inactivation exceeded 97% ($97.6 \pm 1.5\%$ in R1 and $97.9 \pm 1.2\%$ in R2), confirming the effectiveness of photo-driven and alkaline-mediated disinfection mechanisms even when mixing was reduced. Biomass productivity was maintained at satisfactory levels ($0.87 \pm 0.5 \text{ g TSS L}^{-1}$), indicating that microalgal growth was not negatively affected by intermittent mixing. These findings highlight the potential of HRAPs with intermittent agitation as a low-energy, effective solution for decentralized wastewater treatment, promoting sustainable water management.

REFERENCES

- Abd-Elmaksoud, S., Abdo, S. M., Gad, M., Hu, A., El-Liethy, M. A., Rizk, N., Marouf, M. A., Hamza, I. A., and Doma, H. S. (2021) Pathogens removal in a sustainable and economic high-rate algal pond wastewater treatment system. Sustainability (Switzerland), **13**(23), 1–13.
- Abuhasheesh, Y., Ghazal, A., Tang, D. Y. Y., Banat, F., Hasan, S. W., and Show, P. L. (2025) Advances in Chlorella microalgae for sustainable wastewater treatment and bioproduction. Chemical Engineering Journal Advances, **22**(February), 100715. [online] <https://doi.org/10.1016/j.cej.2025.100715>.
- Arashiro, L. T., Montero, N., Ferrer, I., Acién, F. G., Gómez, C., and Garfi, M. (2018) Life cycle assessment of high rate algal ponds for wastewater treatment and resource recovery. Science of the Total Environment, **622–623**, 1118–1130. [online] <https://doi.org/10.1016/j.scitotenv.2017.12.051>.
- Askari, A., Vahabzadeh, F., and Mardanpour, M. M. (2021) The identification and performance assessment of dominant bacterial species during linear alkylbenzene sulfonate (LAS)-biodegradation in a bioelectrochemical system. Bioprocess and Biosystems Engineering, **44**(12), 2579–2590. [online] <https://doi.org/10.1007/s00449-021-02629-0>.
- Butterworth, S. and Fallowfield, H. (2024) Comparison of the wastewater treatment performance of continuously and discontinuously mixed high-rate algal ponds at Kingston on Murray. Water Science & Technology, **89**(3), 505–512.
- de Cassia Soares Brandão, B., Oliveira, C. Y. B., dos Santos, E. P., de Abreu, J. L., Oliveira, D. W. S., da Silva, S. M. B. C., and Gálvez, A. O. (2023) Microalgae-based domestic wastewater treatment: a review of biological aspects, bioremediation potential, and biomass production with biotechnological high-value. Environmental Monitoring and Assessment, **195**(11). [online] <https://doi.org/10.1007/s10661-023-12031-w>.
- Chambonniere, P., Bronlund, J. E., and Guieysse, B. (2022) Study from microcosms and mesocosms reveals *Escherichia coli* removal in high rate algae ponds during domestic

- wastewater treatment is primarily caused by dark decay. PLoS ONE, **17**(3 March), 1–19. [online] <http://dx.doi.org/10.1371/journal.pone.0265576>.
- Chambonniere, P., Bronlund, J., and Guieysse, B. (2020) Escherichia coli removal during domestic wastewater treatment in outdoor high rate algae ponds: Long-term performance and mechanistic implications. Water Science and Technology, **82**(6), 1166–1175.
- Chambonniere, P., Bronlund, J., and Guieysse, B. (2021) Pathogen removal in high-rate algae pond: state of the art and opportunities. Journal of Applied Phycology.
- Craggs, R. J., Zwart, A., Nagels, J. W., and Davies-Colley, R. J. (2004) Modelling sunlight disinfection in a high rate pond. Ecological Engineering, **22**(2), 113–122.
- Dammak, I., Fersi, M., Hachicha, R., and Abdelkafi, S. (2023) Current Insights into Growing Microalgae for Municipal Wastewater Treatment and Biomass Generation. Resources, **12**(10).
- Delanka-Pedige, H. M. K., Cheng, X., Munasinghe-Arachchige, S. P., Bandara, G. L. C. L., Zhang, Y., Xu, P., Schaub, T., and Nirmalakhandan, N. (2020) Conventional vs. algal wastewater technologies: Reclamation of microbially safe water for agricultural reuse. Algal Research, **51**(March).
- Dias, D. F. C., Passos, R. G., and von Sperling, M. (2017) A review of bacterial indicator disinfection mechanisms in waste stabilisation ponds. Reviews in Environmental Science and Biotechnology, **16**(3), 517–539.
- Eltanahy, E., Salim, S., Vadiveloo, A., Verduin, J. J., Pais, B., and Moheimani, N. R. (2018) Comparison between jet and paddlewheel mixing for the cultivation of microalgae in anaerobic digestate of piggy e ffl uent (ADPE). Algal Research, **35**(May), 274–282. [online] <https://doi.org/10.1016/j.algal.2018.08.025>.
- Farias, S. L., Ruas, G., Serejo, M. L., and Boncz, M. Á. (2023) Evaluation of the effect of the feeding regime on the removal of metals and pathogens in microalgae – bacterial systems. Water Science & Technology, **88**(1), 11–22.
- Hena, S., Abida, N., and Tabassum, S. (2015) Screening of facultative strains of high lipid producing microalgae for treating surfactant mediated municipal wastewater. RSC Advances, **5**, 98805–98813.
- Hua, X., Li, M., Su, Y., Dong, D., Guo, Z., and Liang, D. (2012) The degradation of linear alkylbenzene sulfonate (LAS) in the presence of light and natural biofilms: The important role of photosynthesis. Journal of Hazardous Materials, **229–230**, 450–454. [online] <http://dx.doi.org/10.1016/j.jhazmat.2012.06.005>.
- Katam, K. and Bhattacharyya, D. (2018) Comparative study on treatment of kitchen wastewater using a mixed microalgal culture and an aerobic bacterial culture: kinetic evaluation and FAME analysis. Environmental Science and Pollution Research, **25**(21), 20732–20742.
- Kohlheb, N., van Afferden, M., Lara, E., Arbib, Z., Conthe, M., Poitzsch, C., Marquardt, T., and Becker, M. Y. (2020) Assessing the life-cycle sustainability of algae and bacteria-based wastewater treatment systems: High-rate algae pond and sequencing batch reactor. Journal of Environmental Management, **264**(March 2019), 110459. [online] <https://doi.org/10.1016/j.jenvman.2020.110459>.
- Li, Z., Yuan, R., Hou, R., Zhou, B., and Chen, H. (2025) Construction of an algal-bacterial symbiosis system and its application to municipal wastewater treatment: A review.

- Process Safety and Environmental Protection, **196**(November 2024), 106846. [online] <https://doi.org/10.1016/j.psep.2025.106846>.
- Muñoz, R. and Guieysse, B. (2006) Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Research*, **40**(15), 2799–2815.
- Nirmalakhandan, N., Selvaratnam, T., Henkanatte-Gedera, S. M., Tchinda, D., Abeywardana-Arachchige, I. S. A., Delanka-Pedige, H. M. K., Munasinghe-Arachchige, S. P., Zhang, Y., Holguin, F. O., and Lammers, P. J. (2019) Algal wastewater treatment: Photoautotrophic vs. mixotrophic processes. *Algal Research*, **41**(May), 101569. [online] <https://doi.org/10.1016/j.algal.2019.101569>.
- Palmer, M. and Hatley, H. (2018) The role of surfactants in wastewater treatment : Impact , removal and future techniques : A critical review. *Water Research*, **147**, 60–72. [online] <https://doi.org/10.1016/j.watres.2018.09.039>.
- Park, J. B. K. and Craggs, R. J. (2010) Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Science & Technology*, **5**, 633–640.
- Pompei, C. M. E., Ruas, G., Bolzani, H. R., Fernandes, L. M., and Silva, G. H. R. da (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*, **349**(January), 123935. [online] <https://doi.org/10.1016/j.envpol.2024.123935>.
- Pompei, C. M. E., Ruas, G., Bolzani, H. R., and Silva, G. H. R. da (2023) Assessment of total coliforms and *E. coli* removal in algae-based pond under tropical temperature in addition of carbon dioxide (CO₂) and shading. *Ecological Engineering*, **196**(August), 107102. [online] <https://doi.org/10.1016/j.ecoleng.2023.107102>.
- Rogers, J. N., Rosenberg, J. N., Guzman, B. J., Oh, V. H., Mimbela, L. E., Ghassemi, A., Betenbaugh, M. J., Oyler, G. A., and Donohue, M. D. (2014) A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. *Algal Research*, **4**(1), 76–88. [online] <http://dx.doi.org/10.1016/j.algal.2013.11.007>.
- Ruas, G., Farias, S. L., Nantes, M. A., Serejo, M. L., Henrique, G., and Boncz, M. Á. (2022) CO₂ Addition and Semicontinuous Feed Regime in Shaded HRAP — Pathogen Removal Performance. *Water*, **14**(24), 4047.
- Ruas, G., Farias, S. L., Scarcelli, P. G., Serejo, M. L., and Boncz, M. A. (2020) The effect of CO₂ addition and hydraulic retention time on pathogens removal in HRAPs. *Water Science & Technology*.
- Sánchez Zurano, A., Garrido Cárdenas, J. A., Gómez Serrano, C., Morales Amaral, M., Acién-Fernández, F. G., Fernández Sevilla, J. M., and Molina Grima, E. (2020) Year-long assessment of a pilot-scale thin-layer reactor for microalgae wastewater treatment. Variation in the microalgae-bacteria consortium and the impact of environmental conditions. *Algal Research*, **50**(July), 101983. [online] <https://doi.org/10.1016/j.algal.2020.101983>.
- Serejo, M. L., Farias, S. L., Ruas, G., Paulo, P. L., and Boncz, M. A. (2020) Surfactant removal and biomass production in a microalgal-bacterial process: effect of feeding regime. *Water Science and Technology*, **82**(6), 1176–1183.
- Shahid, A., Malik, S., Zhu, H., Xu, J., Nawaz, M. Z., Nawaz, S., Asraful Alam, M., and Mehmood, M. A. (2020) Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Science of the Total Environment*, **704**, 135303. [online] <https://doi.org/10.1016/j.scitotenv.2019.135303>.

- Sivakaminathan, S., Wolf, J., Yarnold, J., Roles, J., Ross, I. L., Stephens, E., Henderson, G., and Hankamer, B. (2020) Light guide systems enhance microalgae production efficiency in outdoor high rate ponds. *Algal Research*, **47**(September 2019), 101846. [online] <https://doi.org/10.1016/j.algal.2020.101846>.
- Sutherland, D. L. and Ralph, P. J. (2020) 15 years of research on wastewater treatment high rate algal ponds in New Zealand: discoveries and future directions. *New Zealand Journal of Botany*, **0**(0), 1–24. [online] <https://doi.org/10.1080/0028825X.2020.1756860>.
- Sutherland, D. L., Turnbull, M. H., Broady, P. A., and Craggs, R. J. (2014) Wastewater microalgal production , nutrient removal and physiological adaptation in response to changes in mixing frequency. *Water Research*, **61**, 130–140. [online] <http://dx.doi.org/10.1016/j.watres.2014.05.011>.
- Valdés, F. J., Hernández, M. R., Catalá, L., and Marcilla, A. (2012) Estimation of CO₂ stripping/CO₂ microalgae consumption ratios in a bubble column photobioreactor using the analysis of the pH profiles. Application to *Nannochloropsis oculata* microalgae culture. *Bioresource Technology*, **119**, 1–6
- Young, P., Fallowfield, M., and J, T. H. (2017) Mini-review : high rate algal ponds , flexible systems for sustainable wastewater treatment. *World Journal of Microbiology and Biotechnology*, **33**(117), 0.

CHAPTER 5 :

CONCLUSIONS AND RECOMMENDATIONS

This research demonstrated that High Rate Algal Ponds (HRAPs) integrating microalgae–bacteria consortia are highly effective in removing priority contaminants from domestic wastewater under tropical conditions, while simultaneously producing biomass with potential value. The study provided new insights into how cultivation strategies, feeding regimes, and mixing operations influence pollutant removal mechanisms, biomass characteristics, and energy requirements.

Batch-scale experiments revealed that the combination of microalgae and bacteria achieved the highest removal efficiencies of anionic surfactants, reaching up to 97% under controlled pH, CO₂ supplementation, and atmospheric aeration. Light availability and the addition of an organic carbon source also played a role in shaping biodegradation pathways, while systems operated with microalgae or bacteria alone exhibited lower and less consistent performance.

Semi-continuous feeding regimes enhanced biomass productivity and the accumulation of total suspended solids, suggesting their potential for biomass valorization strategies. In contrast, continuous regimes improved nutrient removal and provided greater operational stability, though at the expense of reduced *Escherichia coli* inactivation. These findings indicate that feeding strategies must be tailored to the intended objectives of the system, whether prioritizing biomass production or effluent quality.

Intermittent mixing emerged as a promising energy-saving strategy, maintaining high surfactant and pathogen removal efficiencies while reducing power consumption. The preservation of biomass quality under intermittent conditions highlights its relevance for large-scale applications in regions where energy costs are a limiting factor.

Overall, the results confirm that integrated operational adjustments are essential to optimize HRAP performance, linking pollutant removal to cultivation dynamics and energy demand. This study provides a technical basis for adapting and scaling HRAPs in tropical settings, highlighting their significant potential as sustainable wastewater management solutions.

Potentials and Prospection

The results highlight the promising potential of HRAPs as sustainable wastewater management solutions. In addition to improving effluent quality, these systems enable the co-production of biomass that can be valorized as biofertilizers, bioenergy feedstock, or other

high-value bioproducts, contributing to resource recovery and circular economy strategies. HRAPs offer operational flexibility through adjustable feeding regimes, mixing patterns, and aeration, allowing systems to be tailored to specific treatment objectives, from maximizing pollutant removal to enhancing biomass productivity. Future work should focus on evaluating energy-efficient operations, scaling strategies, and techno-economic feasibility to support broader adoption of HRAP technology.

Application Scenarios

HRAPs can be implemented across a variety of settings depending on treatment goals and available resources. In rural and peri-urban communities, they provide decentralized treatment with safe effluent discharge and local biomass reuse. In urban contexts, HRAPs can serve as secondary treatment alternatives to reduce reliance on energy-intensive activated sludge systems or as tertiary polishing steps to enhance nutrient removal and pathogen reduction before reuse or discharge. Integration with downstream processes, such as anaerobic digestion or nutrient recovery, can further improve system efficiency and resource recovery. In energy-limited scenarios, strategies such as intermittent mixing and optimized feeding enable effective operation with reduced power demand. Pilot projects in industrial or municipal facilities can help refine design parameters and guide scale-up strategies for wider implementation.

Recommendations

1. **Adopt microalgae–bacteria consortia** as the standard configuration to maximize surfactant and pathogen removal.
2. **Use semi-continuous feeding regimes** to enhance biomass productivity. Alternatively, use a continuous regime when nutrient removal and system stability are the main priorities.
3. **Implement pH control and CO₂ supplementation** to promote surfactant biodegradation and improve overall treatment efficiency.
4. **Employ intermittent mixing** as an energy-saving strategy without compromising treatment performance or biomass quality.
5. **Conduct pilot-scale studies** integrating hydrodynamic parameters (water depth, hydraulic retention time) to refine design criteria for tropical regions.

6. **Advance techno-economic assessments and regulatory frameworks** to support the incorporation of HRAPs as nature-based solutions for domestic wastewater treatment.