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Estratégias de operação de reatores de cultivo de microalgas em esgoto doméstico: performance técnica, econômica e ambiental

Iara Barbosa Magalhães
Doctor Scientiae

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Thesis submitted to the Civil Engineering Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Maria Lucia Calijuri

Co-adviser: Paula Peixoto Assemany

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Assent:

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Iara Barbosa Magalhães
Author

_____

Maria Lucia Calijuri
Adviser

À vó Maria
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“Sozinhos podemos chegar mais rápido, mas juntos chegamos mais longe”
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ABSTRACT

MAGALHÃES, Iara Barbosa, D.Sc., Universidade Federal de Viçosa, March, 2025. **Operational Strategies for Algal Cultivation Reactors in Domestic Wastewater: Technical, Economic, and Environmental Performance**. Adviser: Maria Lucia Calijuri. Co-adviser: Paula Peixoto Assemany.

This thesis aimed to evaluate different algal cultivation approaches to improve domestic wastewater treatment efficiency and biomass production from a technical, economic, and environmental perspective. Initially, a comprehensive literature review was conducted on the application of High-Rate Algal Ponds (HRAPs), investigating design parameters, operational strategies, and recent enhancements, such as hybrid systems and biomass recirculation (Chapter 1). The potential of HRAPs in removing nutrients and emerging contaminants was highlighted, as well as the relevance of optimization studies. Next, the effect of operating HRAPs in series was assessed (Chapter 2), based on the hypothesis that it could increase nutrient and pathogen removal efficiency, in addition to potentially boosting biomass production. The results showed significantly higher removal of coliforms and E. coli in the series configuration. Nevertheless, biomass composition and total organic carbon removal appeared to benefit from parallel operation, underscoring the importance of nutrient balance. The third chapter examined the effects of organic loading rate on HRAP performance. Although variations in organic matter and nutrient removal efficiency were observed across different loading ranges, no statistically significant differences were detected within the studied interval. Consequently, higher organic loading rates should be investigated to refine design guidelines and evaluate the robustness of HRAPs as a tertiary or secondary treatment option. Regarding tubular photobioreactors, Chapter 4 analyzed the supplementation of artificial light (LEDs) as a means to extend the photoperiod and increase biomass productivity. An increase in algal production, cell density, and enzymatic activity was observed. In addition, a trade-off emerged, reflecting the need to balance cost-effectiveness with impact mitigation. To address these considerations, Chapter 5 presented an integrated assessment, through life cycle and economic viability analyses, of LED-assisted algal systems. Although production increased by up to 34%, the rise in capital and operating costs, coupled with heightened environmental impacts, necessitates strategies such as the use of solar energy, materials optimization, and supportive policies. Thus, this thesis upholds the proposed hypotheses, demonstrating that optimized operational strategies can enhance the technical, economic, and environmental performance of

algal systems, contributing to more sustainable solutions for domestic wastewater treatment.

Keywords: Microalgae; High-Rate Algal Ponds; Photobioreactors; Domestic Wastewater Treatment; Bioremediation; Sustainability; Circular Economy

RESUMO

MAGALHÃES, Iara Barbosa, D.Sc., Universidade Federal de Viçosa, março de 2025. **Estratégias de operação de reatores de cultivo de microalgas em esgoto doméstico: performance técnica, econômica e ambiental**. Orientadora: Maria Lucia Calijuri. Coorientadora: Paula Peixoto Assemany.

A presente tese teve como objetivo avaliar diferentes abordagens de cultivo algal visando melhorar a eficiência do tratamento de esgoto doméstico e a produção de biomassa, sob uma perspectiva técnica, econômica e ambiental. Inicialmente, foi realizada uma revisão bibliográfica abrangente da aplicação das Lagoas de Alta Taxa (LATs), investigando parâmetros de projeto, operação e aprimoramentos recentes, como sistemas híbridos e recirculação de biomassa (Capítulo 1). Destacou-se o potencial das LATs na remoção de nutrientes e contaminantes emergentes, bem como a relevância de estudos de otimização. Em seguida, avaliou-se o efeito da operação em série de LATs (Capítulo 2), hipótese que propõe maior eficiência de remoção de nutrientes e patógenos, além de potencial aumento na produção de biomassa. Os resultados demonstraram remoção significativamente maior de coliformes e E. coli na configuração em série. Ainda assim, a composição da biomassa e a remoção de carbono orgânico total podem ser favorecidas pela operação em paralelo, evidenciando a importância do balanceamento de nutrientes. O terceiro capítulo abordou os efeitos da taxa de aplicação orgânica no desempenho das LATs. Apesar de se observar variação na eficiência de remoção de matéria orgânica e nutrientes em diferentes faixas de carga, não houve diferenças estatisticamente relevantes no intervalo estudado. Assim, indicou-se a necessidade de investigar faixas mais elevadas de carga orgânica para refinar diretrizes de dimensionamento e avaliar a robustez das LATs como opção terciária ou secundária. No âmbito de fotobiorreatores tubulares, o Capítulo 4 analisou a complementação de luz artificial (LEDs) como forma de prolongar o fotoperíodo e aumentar a produtividade de biomassa. Observou-se incremento na produção algal, densidade celular e atividade enzimática. De forma complementar, avaliou-se se o trade-off se reflete na necessidade de balanço entre custo-benefício e mitigação de impactos. Dessa forma, o Capítulo 5 apresentou uma avaliação integrada, por meio de análises de ciclo de vida e de viabilidade econômica, dos sistemas algais assistidos por LEDs. Constatou-se que, apesar do ganho de produção (até 34%), a elevação dos custos de capital e operação, além do aumento de impactos ambientais, requer a adoção de estratégias como uso de energia solar, otimização de materiais e políticas de incentivo.

Assim, esta tese sustenta as hipóteses propostas, demonstrando que a adoção de estratégias de operação otimizadas pode melhorar a eficiência técnica, econômica e ambiental de sistemas algais, contribuindo para soluções mais sustentáveis no tratamento de esgoto doméstico.

Palavras-chave: Microalgas; Lagoas de Alta Taxa; Fotobiorreatores; Tratamento de Esgoto Doméstico; Biorremediação; Sustentabilidade; Economia Circular

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

SUMÁRIO

1. Apresentação

A presente pesquisa teve origem em dois projetos aprovados pela professora Maria Lúcia Calijuri junto à FAPEMIG e ao CNPq, respectivamente: “Tecnologia Inovadora Aplicada ao Tratamento de Efluentes, Produção de Biomassa Algal e Valorização Energética” (FAPEMIG TEC APQ 02527-18 – Edital 001/2018) e “Bioprodutos a partir de biomassa de microalgas: viabilidade energética, econômica e ambiental” (Chamada CNPq Nº 09/2020 - PQ 2020). Ambos os projetos se concentram na otimização da produção de biomassa de microalgas associada ao tratamento de efluentes, uma linha de pesquisa desenvolvida pelo Núcleo de Pesquisas Ambientais Avançadas (nPA), do Departamento de Engenharia Civil da Universidade Federal de Viçosa (UFV), desde 2009.

No decorrer desses projetos, investigaram-se diferentes estratégias operacionais para elevar a produtividade de biomassa, avaliar a tratabilidade do efluente usado como meio de cultivo, analisar os impactos ambientais envolvidos e examinar a viabilidade técnica de diferentes rotas de aproveitamento da biomassa. Nesse contexto, destaca-se a importância de otimizar os parâmetros operacionais na etapa de cultivo, como forma de viabilizar a sustentabilidade técnica, econômica e ambiental dessas tecnologias.

Assim, esta pesquisa teve como objetivo compilar as principais informações sobre a operação de reatores algais e identificar, além de avaliar, parâmetros e estratégias de otimização que se encontram na fronteira do conhecimento. Pretendeu-se, portanto, contribuir para a aplicação e difusão desses reatores como uma tipologia eficiente para o tratamento de esgoto sanitário e a produção de biomassa, no escopo das biorrefinarias de microalgas e das estações de tratamento de efluentes sustentáveis.

2. Introdução Geral

O crescimento populacional e o processo de urbanização têm ampliado a geração de efluentes sanitários, demandando a modernização dos sistemas convencionais de tratamento para que cumpram as exigências legais e evitem impactos negativos na qualidade dos corpos hídricos receptores (DO et al., 2022). Nesse cenário, a biotecnologia de microalgas emerge como alternativa sustentável aos métodos tradicionais de tratamento, ao integrar a biorremediação de efluentes e a recuperação de recursos de maior valor agregado (CHAI et al., 2021).

Quando cultivadas em consórcio com bactérias aeróbias presentes nos efluentes, as microalgas promovem um ciclo simbiótico de troca de O_2 e CO_2, facilitando a assimilação de nutrientes e a degradação da matéria orgânica (GONZÁLEZ-FERNÁNDEZ et al., 2016; SHAHID et al., 2020). Entre os sistemas de cultivo disponíveis, destacam-se as Lagoas de Alta Taxa (LATs), amplamente utilizadas em grande escala graças à simplicidade operacional e ao baixo custo, reconhecidas por sua eficiência na remoção de nutrientes (CRAGGS et al., 2014; SUTHERLAND et al., 2020a; YOUNG; TAYLOR; FALLOWFIELD, 2017). No entanto, um dos desafios das LATs é a produtividade de biomassa relativamente menor em comparação a sistemas fechados, como os reatores de coluna de bolha (MAGALHÃES et al., 2022).

Para superar essas limitações, diversas estratégias de otimização operacional têm sido propostas em reatores algais, como é o caso suplementação de CO_2 (ASSIS et al., 2019), operação em série (SUTHERLAND et al., 2020b), variação de profundidade (COUTO et al., 2018) e a pré-desinfecção ultravioleta (UV) do efluente (ASSEMANY et al., 2015). Diversas dessas abordagens demonstraram melhorias significativas na produtividade de biomassa e na eficiência de tratamento. Contudo, apesar dos avanços, os sistemas abertos permanecem vulneráveis a oscilações das condições ambientais e a eventuais contaminações externas (WAN MAHARI et al., 2022).

Paralelamente, os reatores fechados oferecem maior controle das condições de cultivo, permitindo maior produtividade de biomassa, especialmente ao empregar luz artificial para ampliar o fotoperíodo e intensificar a fotossíntese (GONZÁLEZ-CAMEJO et al., 2019). Embora esses

sistemas mostrem viabilidade para cultivos específicos em condições otimizadas, ainda enfrentam desafios quanto à escala de aplicação e aos custos econômicos em larga escala (SINGH; MISHRA, 2023).

Nesta pesquisa, procurou-se avaliar criticamente os parâmetros operacionais e as estratégias de otimização de reatores algais aplicados ao tratamento de efluente doméstico, considerando aspectos técnicos, econômicos e ambientais. Foram aplicadas ferramentas como a Avaliação de Ciclo de Vida (ACV) e análises econômicas para identificar gargalos tecnológicos e propor soluções que incentivem a adoção desses sistemas em escala real (ARASHIRO et al., 2018; MAGALHÃES et al., 2021). O diferencial desta tese consiste em integrar avaliações técnico-científicas e econômicas, apoiadas pela ACV, para propor soluções factíveis para reatores algais de larga escala, contribuindo de maneira pioneira para a implementação sustentável dessa tecnologia em estações de tratamento sustentáveis.

Com esse propósito, o estudo foi estruturado em cinco capítulos, os quais abordam diferentes, mas interligados, aspectos da operação e otimização de reatores algais:

- **Capítulo I**: *Advancements in High-Rate Algal Pond Technology for Enhanced Wastewater Treatment and Biomass Production: A Review*
- **Capítulo II**: *Effects of series operation of high-rate algal ponds treating sanitary sewage: hydrodynamics, remediation potential, and biomass production*
- **Capítulo III**: *Effects of organic loading rate in high-rate algal ponds: bioremediation potential, implications to design and operation*
- **Capítulo IV**: *Outdoor microalgae cultivation with LED light enhancement: wastewater treatment, biomass yield and potential valorization routes*
- **Capitulo V**: *Shining a light on outdoor algal systems for wastewater treatment: How artificial light enhancement impacts biomass costs and life cycle*

Os dois últimos capítulos dizem respeito a parte do estudo realizada no Laboratório de Energia e Geologia de Portugal (LNEG), mediante bolsa de doutorado sanduíche pleiteada frente a CAPES (PDSE - Edital nº 44/2022 -

Seleção 2023) pela autora do trabalho (Processo 88881.846108/2023-01). De forma integrada, estes capítulos visaram ampliar o conhecimento acerca das potencialidades e limitações dos reatores algais, reforçando a importância das microalgas para o avanço no tratamento sustentável de águas residuárias e para a transição rumo a uma economia circular.

3. Hipóteses

- A operação em série de reatores algais aumenta a eficiência do tratamento de esgoto doméstico e a produção de biomassa algal em comparação às LATs operadas individualmente;
- A taxa de aplicação orgânica influencia a eficiência de remoção de matéria orgânica (DBO) e de nutrientes (nitrogênio e fósforo) nas LATs;
- A complementação de luz artificial em fotobiorreatores tubulares aumenta a produtividade de biomassa e a eficiência de remoção de poluentes, quando comparada ao cultivo exposto exclusivamente à luz natural;
- Embora a complementação de luz artificial gere maior consumo energético, tais efeitos podem ser compensados pelo incremento de produtividade.

4. Objetivos

4.1. Objetivo Geral

Avaliar estratégias de operação de sistemas algais durante o tratamento de esgoto doméstico, com foco na melhoria do desempenho técnico, econômico e ambiental da tecnologia de cultivo.

4.2. Objetivos Específicos

- Consolidar o conhecimento sobre LATs aplicadas ao tratamento de esgoto doméstico, identificando desafios e oportunidades em projeto, operação, eficiência de tratamento e estratégias adotadas para melhoria do desempenho.
- Avaliar a eficiência de tratamento e a produção de biomassa algal em LATs operadas em série, utilizando esgoto doméstico como meio de cultivo.
- Investigar os efeitos da taxa de aplicação orgânica no desempenho de LATs para o tratamento de esgoto doméstico integrado ao cultivo de microalgas.

- Avaliar o efeito da complementação de luz artificial na biorremediação de efluentes domésticos e no crescimento de microalgas em fotobiorreatores tubulares.
- Avaliar os impactos ambientais e econômicos associados à operação de fotobiorreatores com complementação de luz artificial.

5. Chapter 1: Literature Review

Artigo 1: Advancements in high-rate algal pond technology for enhanced wastewater treatment and biomass production: A review[1]

Abstract

This review examines recent advances in high-rate algal pond (HRAP) technology for wastewater treatment and the production of microalgae-rich biomass. It highlights the latest methods developed to enhance the efficiency and sustainability of these systems. Notably, these methods include two-stage cultivation, biomass recirculation, and strategies that collectively improve algal productivity and treatment efficacy, such as the implementation of hybrid systems. Hybrid systems combine HRAPs with other wastewater treatment technologies, such as biofilm reactors. By optimizing these configurations, HRAPs demonstrate effectiveness in nutrient removal, biomass production, and harvesting, enhancing their potential as a scalable and adaptable solution for effluent treatment and biomass production. Additionally, this study reviews HRAP performance in removing emerging contaminants, an important aspect in the context of current effluent treatment. This review establishes a significant advancement in HRAP technology, promoting its adoption as a sustainable and real-scale wastewater treatment option.

Keywords: raceway, wastewater treatment, biological treatment, design, operation.

5.1. Introduction

The growing concerns over water pollution and the need for sustainable wastewater treatment technologies have driven research efforts towards

[1]Attachment 1: Paper published at Journal of Water Process Engineering (ISSN: 2214-7144): MAGALHÃES, Iara Barbosa; PEREIRA, Alexia Saleme Aona de Paula; SILVA, Thiago Abrantes; FERREIRA, Jéssica; BRAGA, Matheus Quintão; COUTO, Eduardo Aguiar; ASSEMANY, Paula Peixoto; CALIJURI, Maria Lúcia. Advancements in high-rate algal pond technology for enhanced wastewater treatment and biomass production: A review. Journal of Water Process Engineering. Elsevier BV, Sep. 2024. DOI 10.1016/j.jwpe.2024.105929

innovative and environmentally friendly solutions [1]. High-rate algal ponds (HRAPs) emerged as a promising alternative for wastewater treatment due to their potential to remove pollutants while simultaneously producing valuable biomass [2,3]. HRAPs are usually shallow open ponds, with a continuous circulation of culture media enforced by a rotating paddlewheel [4]. This design favors the growth and accumulation of algal biomass, given that the stirring of the liquid subjects the cells to varying radiation intensities, effectively minimizing the photoinhibition effect [5].

The first application of HRAPs to wastewater treatment dates to the late 50s and early 60s, developed at the University of California [[6], [7], [8], [9]]. Since then, researchers and industries have applied the technology to treat wastewater from domestic and industrial sources [10], also testing the biomass produced to several conversion routes [11,12]. Since their first application, researchers have proposed strategies to address technical challenges in HRAP technology applied to wastewater treatment, such as the low productivity of biomass and target biocompounds [1], overcoming seasonal variations [13] and reconciling these issues with economic and environmental sustainability [14,15].

Each year, 380 billion m^3 of municipal wastewater are generated globally [16]. In the context of increasing globalization, the challenge of municipal wastewater treatment becomes more pronounced as population growth and urbanization intensify the strain on water resources [17]. Its production is expected to increase by 24 % by 2030 and 51 % by 2050 [18]. Thus, this type of wastewater is a serious worldwide concern, especially in low-income countries that lack in wastewater treatment infrastructure. Municipal wastewater treatment covers 70 %, 38 %, and 28 % of wastewater generated in high-income, upper middle-income, and lower middle-income countries, respectively. In low-income countries, only 8 % of wastewater generated undergoes treatment [19]. Thus, the bioremediation and resource recovery potential of coupling HRAP technology to municipal wastewater treatment can contribute to increasing sanitation universalization and presents several advantages. Firstly, it provides a nutrient-rich and cost-effective source for algal growth, eliminating the need for expensive synthetic nutrients [20]. The wastewater's organic matter and dissolved nutrients serve as valuable resources that microalgae can assimilate, promoting rapid biomass production [1]. Secondly, using wastewater as a culture medium contributes to sustainable

development by repurposing and recycling wastewater, transforming it into a valuable resource in the bioeconomy [21]. This arrangement helps efficient wastewater treatment and reduces the environmental burden of conventional wastewater disposal methods. Additionally, the HRAPs' ability to effectively remove nutrients and contaminants from the wastewater helps mitigate water pollution, protecting aquatic ecosystems and public health [22]. Still, there is a low uptake of the technology, with few real-scale units in operation [23].

One of the pointed bottlenecks is the HRAP design and operation standards, hindering the diffusion potential of the technology for sustainable wastewater treatment [24]. While several reviews and standards are consolidated for different pond treatment technologies [25,26], HRAPs still lack comprehensive and updated guides to practices and results of the technology, especially considering innovative and alternative operation strategies, besides design modifications recently proposed. Bridging this gap is vital to advancing HRAP technology dissemination, streamlining design processes, and optimizing system performance. This review aims to consolidate knowledge on HRAPs applied to sustainable municipal wastewater treatment, providing insights into design, operation, treatment efficiency, and challenges. For each topic, technical implications and critical assessment of future directions are also presented, aimed at consolidating current knowledge and potential and encouraging further cutting-edge developments.

5.2. Municipal wastewater treatment in high-rate algal ponds

5.2.1. Mapping

Research shows that HRAPs are promising for municipal wastewater treatment and resource recovery. However, this treatment approach is not widely spread, and there are few examples of large-scale operational systems [27]. Therefore, a review of functioning full-scale ponds was conducted in published literature, as summarized in Table 5.1.

Table 5.1. Location, size, and performance of large-scale high-rate algal ponds for sanitary sewage treatment.

Location	First-year operational report	Surface area (m^2)	Operational flow (m^3 day^{-1})	Removal efficiency	Reference
Lawrence - United States	1956	–	97.7	$N\text{-}NH_4$ – 61.4 % P – 90.6 %	[14]
Rabat – Morocco	1991	3023	259.2	$N\text{-}NH_4$ – 23-78 % P – 40- 72 %	[175]
St. Helena – United States	1970	20,000	–	–	[29,176]
Hollister – United States		50,000	–	–	[176]
Christchurch - New Zealand	2009	14,000	175[a]	BOD – 47-52 % $N\text{-}NH_4$ – 64-67 % P – 14-24 %	[31]
Chiclana de la Frontera - Spain	2014	10,000	4000	–	[27,29]
Kingston on Murray - Australia	2008	192-226	12	BOD – 91-94 %[b] $N\text{-}NH_4$ – 52-78 % P – (-2)-16 % BOD – 78-86 %[c] $N\text{-}NH_4$ – (-2)-94 % P – (-31) - 10 %	[40]
Cambridge- New Zealand	–	10,000	362.5	$N\text{-}NH_4$ – 32 - 46 % P – 32 - 42 %	[30]
Peterborough - Australia	2019	2 ponds of 5000m^2 each	475	–	[62]

[a]500 m^2/d and 0.35 depth reported.

[b] Septic tank treated domestic wastewater

[c] Facultative pond-treated domestic wastewater

The evaluation of constructed HRAPs for municipal wastewater treatment reveals a geographical distinction, primarily in developed countries like New Zealand and the United States. This trend correlates with Goswami et al.'s [14] study, which found intensive microalgae cultivation research in these countries for valuable products and sanitation. In real-scale studies, naturally growing wild algae were observed [28]. However, the main challenges in treatment lie in removal efficiency due to the operational control complexities [29], which may result in lower algal productivity and nutrient removal rates [30], coupled with heightened climate seasonality [31]. Moreover, being an outdoor open system, controlled and stable results at large scale and throughout continuous operation are not expected.

As reported by Sutherland et al. [30], the size of the HRAP can influence daily areal nutrient removal and biomass production. The study compared nutrient removal and microalgal productivity over three seasons for HRAPs of 5m^2, 330m^2, and 1 ha. The study highlights the implications of daily wastewater treatment efficiency and biomass productivity per land area for scale-up predictions, impacting biomass yield and costs. Thus, further research is recommended to optimize microalgal performance in full-scale HRAP.

However, to elucidate HRAP technology, the current review will address the abovementioned studies along with pilot and bench-scale findings. Still, where pertinent, the aim is to consolidate potential considerations for scale-up in large-scale applications. This approach addresses the scarcity of data in large-scale implementations. The overview of the 32 reviewed studies, comprising 96 experimental results, operational parameters, and treatment efficiencies (see Appendix A).

5.2.2. Application

HRAPs are stabilization ponds that strongly depend on climatic conditions, particularly well-suited for use in warm and tropical climate regions. Their relatively high area requirement makes them an alternative for application in small communities, such as rural areas and remote locations, where decentralized sanitation units are needed. Notably, HRAPs offer the advantage of biomass recovery, which can transform conventional wastewater treatment plants into sustainable systems [32].

In Australia, HRAPs (Table 5.1) are primarily situated in rural areas, with limited access to experienced operators and low energy availability [33]. An interesting case is the HRAP located in Kingston on Murray, South Australia, which outperformed a waste stabilization pond in Lyndoch, resulting in reduced treatment time, construction costs, and increased water availability for reuse, particularly crucial in water-scarce regions like rural Australia [6,33].

In this context, HRAPs have found applications in various stages of wastewater treatment. Among the 32 reviewed studies (see Appendix A), 72 % utilized HRAPs as tertiary treatment units, while 28 % investigated their application as secondary treatment. There are three configurations from tertiary treatment: HRAPs following an Upflow Anaerobic Sludge Blanket (UASB) reactor, an anaerobic pond, and a septic tank. Overall, HRAPs integrated with UASB treatment is the primary configuration [32,[34], [35], [36], [37]]. The UASB reactor handles organic matter removal in this combined approach, while the HRAP focuses on nutrient and pathogen mitigation [37]. Besides, Vassalle et al. [36] demonstrate the promising potential of the UASB+HRAP system for pharmaceutical, estrogen, and xenoestrogen removal from municipal wastewater.

Still, studies also report the comparison in the efficiency of HRAPs both as secondary (HRAP-dissolved air flotation) and tertiary (UASB-HRAP-dissolved air flotation) treatment systems are reported [4]. Both configurations exhibited significant nutrient (74 % nitrogen and 92 % phosphorus) and pharmaceutical (94 % vs. 92 %) removal efficiency.

One particular study by Arashiro et al. [38] investigated HRAP performance with and without primary treatment in municipal wastewater treatment. While a primary settler did not affect treatment efficiency, biomass productivity was up to 30 % higher without it. HRAP studies following septic tank treatment have also been explored [[39], [40], [41]], particularly for their suitability in simple layouts and economic-environmental performance for small communities [42]. However, results from pilot scale studies demonstrated low efficiency for organic matter removal when applying HRAP at the secondary level. Authors reported approximately 30% [39] and 60% [41] of chemical oxygen demand (COD) removal after treating primary wastewater from a septic tank. These results

indicate that HRAPs may be best suited as tertiary units since organic matter removal would not be the main goal.

Still, the studies highlight the adaptability of HRAPs, as they are seamlessly integrated with various treatment technologies and settings, serving diverse treatment objectives based on the specific implementation stage. Overall, removal efficiencies and mechanisms will be further discussed in the specific topic.

5.3. Design parameters and methods

The design of HRAPs is considered an upgrade from conventional facultative ponds [43]. Although HRAPs and waste stabilization ponds depend upon the same mechanism for treatment (favoring the microalgal-bacteria consortia), HRAPs operate at shallow depths, which overcomes vertical stratification [44]. Also, the shallow depth and intentional mixing by the paddlewheel increase the potential for pathogen inactivation by sunlight-mediated mechanisms. Reduced depth also impacts construction costs since less earthwork is required than conventional stabilization ponds (which also results in lower evaporative loss than other ponds) [44].

Table 5.2 provides an overview of operational parameters documented for HRAP used in municipal wastewater treatment (see Appendix A for detailed sources).

Table 5.2. Summary of design and operational parameters for high-rate algal ponds in sanitary wastewater treatment.

Parameter	Unit	Mean	Minimum	Maximum	Median	n
Area	m^2	2299.78	1.50	12500.00	85.00	79
Depth	m	0.35	0.20	0.60	0.30	90
HRT	day	6.29	2.00	10.00	7.00	81
Organic load	kg BOD ha^{-1} day^{-1}	216.74	18.46	550.00	137.06	32
Mixing speed	m s^{-1}	0.18	0.002	0.35	0.20	62

5.3.1. Hydraulic retention time (HRT)

HRAP systems exhibit faster treatment rates than non-mixed pond systems, enabling them to operate at shorter hydraulic retention times (HRT) or accommodate higher organic loading rates [6]. This enhanced efficiency in

treatment is significant due to the reduced time required by HRAPs, necessitating at least 80 % lower HRT. This advantageous design allows for continuous operation at 3–10 days HRT [45], in contrast to the 10–40 days required in traditional systems [46]. The lower HRT also leads to a smaller standing volume in HRAPs than in waste stabilization ponds, resulting in a substantial size reduction of approximately 40 % [6]. This is a key factor when discussing feasibility, with HRT playing a major role in area demand, often pointed out as a downside of HRAP systems compared to closed photobioreactors.

Among the operational parameters crucial to effluent treatment in HRAPs, the HRT is decisive for treatment efficiency and microalgal biomass production [21,47]. Higher HRT values are recommended when the HRAP receives raw effluent (after primary treatment), which still carries a high organic matter load. However, this approach may compromise biomass productivity. Alemu et al. [45] studied the effects of depth (0.25 m and 0.3 m), organic load (333-65 kg BOD ha^{-1} day^{-1}), and HRT (2-8 days) on HRAPs applied to wastewater treatment. The study concluded that a 6-day HRT at 0.3 m depth and 109.3 kg BOD ha^{-1} day^{-1} were the most promising nutrient removal and biomass production approaches. It is important to note that when volume and depth are fixed in HRAPs, HRT directly regulates organic and nutrient application rates. Still, overly high HRTs can limit nutrients in the media, hindering productivity and biomass composition. Managing HRT throughout the year is also recommended, with shorter HRTs being preferable during summer when algae growth is at its peak, aiming to achieve higher biomass productivity [48]. Sutherland et al. [49] used an HRT of 7 days for autumn and spring, 9 days for winter, and 5.5 days during summer. Biomass productivity was up to 703 mg m^{-3} day^{-1} in summer, compared to 287 mg m^{-3} day^{-1} in winter. The reduced HRT also did not impact the nitrogen removal rates, up to 77 % in the summer compared to 53 % in winter.

5.3.2. Mixing Speed

Mixing is a pivotal factor in HRAPs, as it prevents thermal stratification and ensures consistent and intermittent light exposure, thereby optimizing algal biomass growth [49]. Moreover, the mixing speed is essential to keep the light-

dark cycles within the pond, favoring the flashing light effect on algae while they travel from the surface to the bottom of the pond as a result of the paddles mixing; this effect favors higher photosynthetic and growth rates, besides favoring the production of secondary metabolites such as pigments [50].

To prevent the settling of algae cells in ponds, a minimum fluid velocity of 0.1 m/s is necessary [51]. Studies suggest that the ideal operational conditions for the most favorable algal-bacterial growth in HRAPs occur in a low water level (0.1 m) and a moderate paddle rotational speed (11.6 rpm) [52]. Rotating paddlewheels induces rotational speed, and design aspects also play a role. Although larger pedal diameters enhance mixing efficiency and reduce backflow risk, they come with higher construction and operational costs [53]. A higher number of blades per paddle improves efficiency and minimizes motor overload, but more than eight blades become impractical from a construction perspective, with marginal efficiency gains [54].

Additionally, a smaller distance between the blade and the bottom enhances the assembly's efficiency, usually 2 cm [53]. Vertical mixing enhances microalgal growth by ensuring frequent light exposure for cells, preventing microalgal/bacterial flock settling, and facilitating nutrient diffusion across the boundary layer surrounding cells. Nonetheless, large-scale operations often face laminar flows and “dead zones,” particularly in extended channels, with the severity of these phenomena dependent on channel length [43].

5.3.3. Surface organic loading rate

As mentioned, HRAP systems were specifically engineered to function at higher treatment rates than non-mixed pond systems. Fig. 5.1 presents the literature's analysis of organic matter removal efficiencies, contrasted with the applied surface organic loading rate (SOLR).

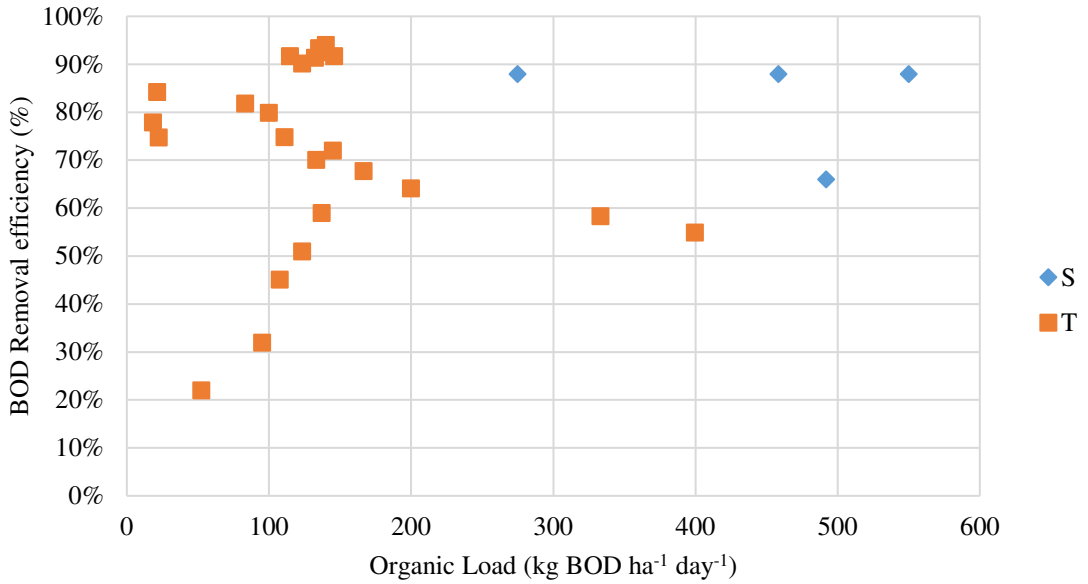

Fig. 5.1. Removal efficiencies and organic loads from literature for high-rate algal ponds applied as secondary (S) and tertiary (T) sewage treatment units.

Overall, the scatter does not present a specific behavior within the applications reported in the literature. The data scarcity should also be highlighted, with few studies reporting on biochemical oxygen demand (BOD). However, from the studies reviewed in Table 5.2, the reported organic loads are usually within the range reported by Craggs et al. (2014), within 100-150 kg BOD ha^{-1} day^{-1}, dependent on the local climate. Still, it is important to note that such loads are lower than those reported by von Sperling (2002) for facultative ponds in warm winters and high solar incidence (240-350 kg BOD ha^{-1} day^{-1}).

In a tropical context, Alemu et al. (2018) investigated the interplay among depth, SOLR, and HRT on organic matter removal in two HRAPs. They observed an inversely proportional relationship between SOLR (kg BOD ha^{-1} day) and percentage removal (% BOD removal) across studied depths and HRTs, highlighting an interrelation between SOLR and treatment efficiency. This connection enhanced removal efficiency and biomass productivity at lower loading rates (≈ 100 kg BOD ha^{-1} day^{-1}). Authors discussed that in overly high load rates, the pond can shift to an anaerobic system, suppressing algae and limiting oxygen for organic matter degradation by bacteria.

Concerning the design of facultative ponds, the SOLR (given in kg BOD ha^{-1} day^{-1}) is a common parameter used (von Sperling, 2002). It can be calculated from the relationship in Equation 5.1:

$$A = \frac{L}{L_s} \qquad \text{Equation (5.1)}$$

Here, A represents the required pond area (ha), L stands for the influent total BOD load (soluble + particulate) (kg BOD day^{-1}), and Ls is the surface loading rate (kg BOD ha^{-1} day^{-1}). The dimensioning criterion based on loading rate is grounded in the oxygen generated by algal photosynthesis, addressing the oxygen demand for organic matter stabilization [26]. According to von Sperling [26], this rate may vary due to temperature, latitude, solar exposure, altitude, and other environmental variables (see Section 5.4). As seen in Eq. (5.1), SLOR reflects the system's areal demand, given that sufficient superficial area should be provided for solar exposition and efficient photosynthesis. The HRT, in turn, expresses sufficient time for bacteria respiration and organic matter removal.

Moreover, if we consider the main applicability of HRAPs as tertiary units, removing organic matter is not the main goal to be achieved. In this sense, the study of El Hafiane and Hamouri [56] tested the use of HRAP at two different points in the treatment line: (i) Configuration I consisted of a two-step up-flow anaerobic reactor (TSUAR) followed by a horizontal subsurface flow constructed wetland (HSFCW), then an HRAP, and finally two maturation ponds (MP1 and MP2); (ii) Configuration II was obtained by introducing a gravel filter (GF) behind the HSFCW and bypassing MP2. Configuration II consisted of a TSUAR followed by a GF, then an HRAP, and finally MP1. These configurations changed the operational parameters of the HRAP, functioning under an SLOR based on chemical oxygen demand (COD) of 233 kg CODt ha^{-1} in Configuration I and 83 kg CODt ha^{-1} in Configuration II. The results showed that the HRAP functioned as a secondary/tertiary unit in Configuration I, while in II, it was solely a tertiary step. This improved nitrogen, phosphorus, and pathogen removal when working solely as a tertiary treatment. This suggests that even when working on low organic loads, HRAPs are still efficient treatment options. The objective of HRAPs within the treatment system may vary, and when working as tertiary units, the applied organic load is of secondary importance.

Studies investigating the potential integration of SOLR in HRAP projects, aiming to establish benchmarks for technological implementation, are strongly recommended. However, none of the reviewed studies linked operational parameters to treatment efficiency through statistical models that could replicate HRAP sizing results. The challenge lies in obtaining removal ranges tied to effluent application rates and diverse operational configurations to define sizing criteria. The focus on HRAP design and dimensioning should establish suitable operational guidelines across varying conditions, ensuring technology applicability.

5.3.4. Depth

According to the reviewed studies, depth usually ranges from 0.2 to 0.6 m, with a mean value of 0.35 m (Table 5.2). Depth affects the availability of light for photosynthesis, but excessive solar radiation can also lead to photooxidation and hinder microalgae growth [57]. Lundquist et al. [58] highlighted that higher depths offer advantages in nutrient removal due to extended CO_2 retention underwater before outgassing, a point supported by Sutherland et al. [49]. Authors achieved enhanced NH_4–N uptake and areal productivity (134 to 200 %) in a 0.4 m deep HRAP compared to a 0.2 m deep one. Still, the authors could not conclude the cause of the improved performance of the deeper pond. Moreover, Couto et al. [57] studied the effects of depth along with CO_2 supply and UV disinfection of the effluent. Results indicated that coupling such technologies to the HRAP led to extended depth ranges for operation (0.4 m), which could lead to surface area savings.

The role of depth in pathogen removal is pivotal, as deeper ponds can attenuate solar radiation and slow bacterial die-offs [59]. However, lower depths can lead to larger surface areas, posing challenges for HRAP diffusion and economic feasibility [60]. Hence, balancing efficient pathogen removal and footprint to match treatment capacity warrants further exploration.

Furthermore, Buchanan et al. [40] suggested conducting studies that maintain a consistent HRT for HRAPs at varying depths year-round to clarify the relationship between HRT and depth and to establish long-term operational strategies at a real scale. Moreover, approaches like LED enhancement integrated with HRAPs

have been successfully proposed for increasing depth when treating digestate [61]. However, their feasibility for wastewater treatment and addressing economic and environmental concerns should be investigated.

5.3.5. Designing methods

While waste stabilization ponds and other treatment methods are established and have well-diffused design parameters [25], HRAPs need a consensus. The only government-approved standard found was an Australian reference [62]. The guidelines were proposed and validated by the Local Government Association (LGA) Community Wastewater Management System (CWMS) Committee in partnership with Professor Howard Fallowfield of Flinders University in South Australia. The criteria were based on the assumption that the HRAP should achieve a minimum of 1 log reduction of viral pathogens. In a novel approach, this document presents the validated results for a commissioned HRAP built in Peterborough, South Australia (see data from Table 5.1). The project guidelines require that HRAPs receive effluent from a septic tank, with BOD and suspended solids (SS) loads of 40 g per person per day (g/p/d) and 25 g/p/d, respectively. Design criteria include overall channel length to total channel width shall not be <6:1 for single channel designs; depth between 30 and 50 cm; at least 30 cm freeboard; for the HRT, the volume of the HRAP (combined if in series) shall be not <10 days; 8 bladed paddlewheels, with rotation of approximately 12 rpm and mixing speed at least 0.2 m/s. The application and validation of the proposed model to other regions have yet to be reported.

In comparison, Santiago [53] proposed and adapted two models for HRAP dimensioning methodology. Both proposed methods need the appropriate HRT based on the BOD removal coefficient (k_{BOD}) and desired removal efficiency. The first method assesses flow speed and Manning's n roughness coefficient based on defining a limit area, as Borowitzka [54] defined. Depth, width, and length are then determined, followed by calculating the installed power. The second method, however, is based on optimizing the aerial requirements and installed power. Numerical methods for nonlinear optimization are proposed to minimize the function “FO = Power + Area”. Some designer constraints are proposed as designer criteria, namely, the velocity (v) should range from 0.1 to 0.35 m/s; the

depth (d) should range from 0.1 to 0.5 m; and the width (w) must be at most 75 times smaller than L. Using such method, the author ran simulations for different Manning's n and wastewater influent, reaching lower aerial requirements for higher n values [53]. Further reports must be made in the literature on applying such methods for large-scale facilities. This also highlights the need for comprehensive standards for HRAP design.

Still, it is important to highlight the ample variety of HRAP designs and structure modifications, such as those proposed by Sompech et al. [63] for seawater cultivation. The authors highlight that the usual pond design presents large dead zones, which can increase energy consumption and lower biomass productivity. Baffles and flow deflectors were simulated using computational fluid dynamics, proposing improved designs. HRAPs for wastewater treatment also rely on such methods, as is the case for design modifications implemented in Cambridge, New Zealand [23]. Area availability was an issue, and wider earthen central baffles were used in a teardrop shape to replace semi-circular deflectors from a previous design. Moreover, shorter channel circuits from 1100 to 700 m were used, besides repositioning the paddlewheel downstream in the HRAP corner and employing a serpentine channel design instead of a straight channel [23]. All the design choices of the Cambridge system were based on experience with the previous system, which once again highlights the need for HRAP operation on a full scale for technological advances and diffusion.

5.4. Operational and Environmental Parameters

5.4.1. pH and Dissolved Oxygen Concentration

The optimal pH range for microalgae cultivation typically lies between 7 and 7.6. However, species-specific variations can exist [64], and a broader range of 7 to 9 has been observed [65]. The higher the elevation of pH, the higher the carbon limitation in the culture media (Hueback, 2007). Elevated pH levels have implications: they intensify photorespiration and impact wastewater's unionized free ammonia (NH_3) ratio, thus heightening toxicity and constraining metabolism and productivity in HRAPs [66]. Also, daily pH fluctuations occur due to photosynthetic CO_2 and HCO_{3^-} uptake by microalgae, elevating pH during the

daytime in outdoor HRAPs and increasing ammonia stripping and phosphorus precipitation [67].

Moreover, dissolved oxygen (DO) levels in ponds can also rise during the day if atmospheric CO_2 and O_2 transfer rates and production and consumption rates are inadequate to balance phototrophic processes. Regardless, DO levels will rise during the daytime; if such balance is inadequate, it can reach supersaturation levels. Heterotrophic activity, including that of microalgae, also plays a role at night in reducing pH and DO levels after photosynthesis ceases [68]. However, HRAP can reach higher DO concentrations from other ponds due to their enhanced photosynthetic rates. Still, despite the higher photosynthetic rate, the movement of the blades helps release DO into the atmosphere, which does not happen in facultative ponds, for example.

Consequently, pH and DO monitoring are common in pilot-scale experiments and large-scale facilities. A strategy for regulating such parameters involves external CO_2 supplementation into the culture medium [65], which will be further discussed below (Section 5.9.1).

5.4.2. Climate and Light availability

Outdoor cultivations in HRAPs benefit from the abundance and cost-effectiveness of solar light to facilitate algal growth [69]. Nevertheless, they are susceptible to productivity losses due to the day/night cycle and changes in climatic and seasonal conditions [70]. Algal growth is favored with temperatures from 15 to 30 °C; however, optimal conditions are typically observed within 20 to 25 °C [71]. Additionally, microalgae exclusively tap into the 400-700 nm Photosynthetically Active Radiation (PAR) spectrum, utilizing roughly 50 % of sunlight, while the remainder dissipates as heat or fluorescence [72]. PAR exhibits considerable diurnal and spatial variability, which can limit HRAP application in some locations.

Moreover, excessive light can lead to the photoinhibition of photosynthetic organisms [73]. Outdoor pond cultivation is particularly exposed to such an issue, and photoinhibition can reportedly lower area productivity by up to 19 % [74]. However, photoinhibition may be less concerning when using wastewater, i.e., domestic wastewater, as a microalgae cultivation medium due to light attenuation

by suspended solids and other compounds (and microorganisms) that act as light barriers.

Nevertheless, HRAPs have been applied successfully in cold climates, addressing challenging scenarios and demonstrating nutrient and pharmaceutical removal from domestic and hospital wastewater in batch pilot-scale studies [75]. Nonetheless, coping with harsh winter conditions in outdoor HRAPs poses a significant concern [76], and the need for continuous year-long performance data remains unprecedented.

5.4.3. Nutrient Balance

Diverse wastewater compositions significantly influence microalgae growth and impact the achievement of high nutrient recovery and biomass productivity [77]. In wastewater-based microalgae cultivation, the carbon/nitrogen (C/N) and carbon/phosphorus (C/P) ratios serve as indicators for nutrient imbalances, with a suitable C/N ratio of approximately 6 and a C/P ratio of 48 to maximize biomass growth [78]. Notably, typical wastewater values also exhibit notable variation, with reported average C/N/P values of 20:8:1 [79]. This emphasizes the necessity of carbon supplementation, and opportunities for overcoming such issues are addressed below in specific topics on CO_2 supply (Section 5.9.1) and mixing wastewater (Section 5.9.5). Moreover, nutrient balance is important in the final biomass composition. Inducing stress in the media, which naturally occurs on effluent sources, can enhance metabolite production, mainly lipids, in the final biomass [64]. Further discussion can be found in Section 6.6.

5.5. Removal mechanisms and treatment efficiency

Fig. 5.2 reports an overview of studies on using HRAPs as municipal wastewater units and achieving removal efficiencies (see Appendix A for complete sources).

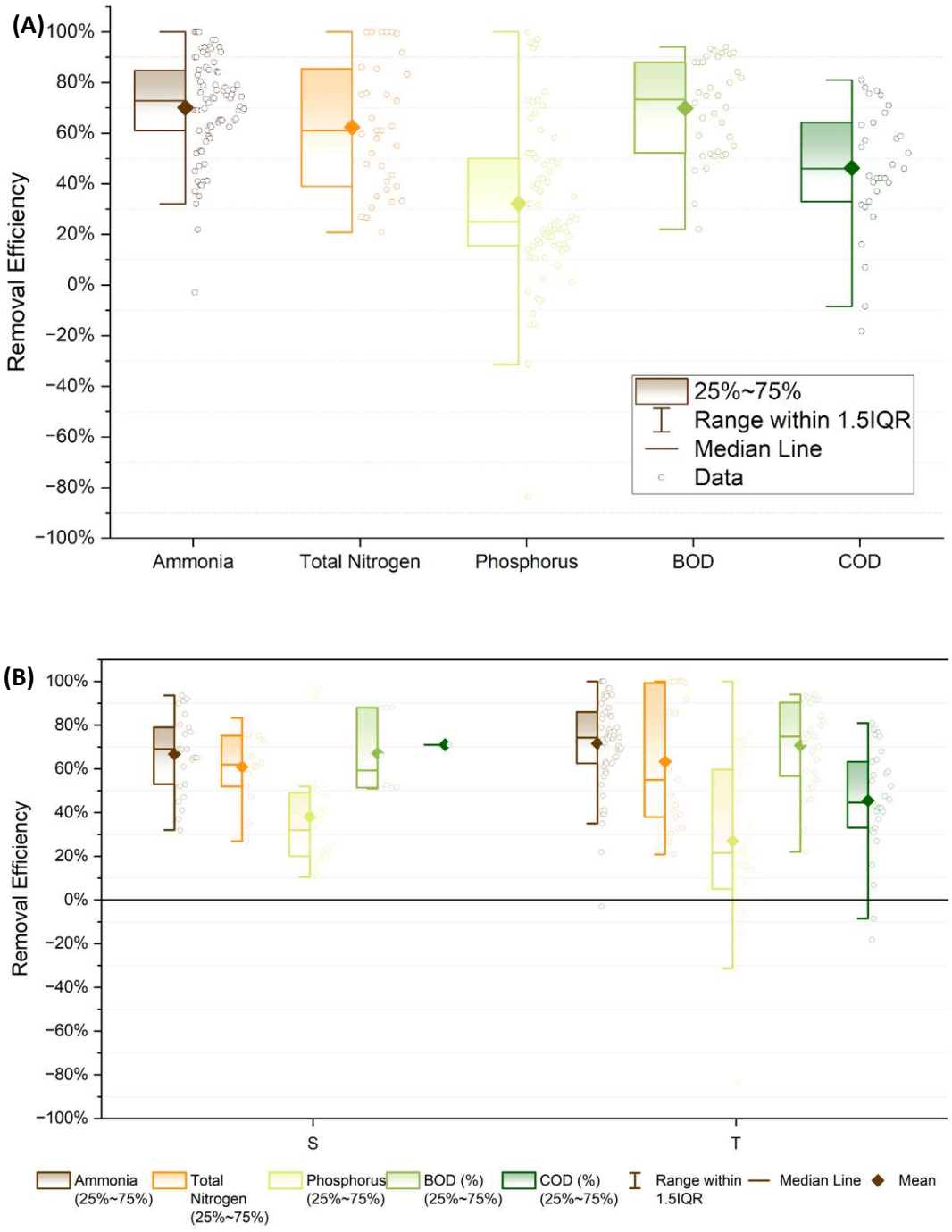

Fig. 5.2. Removal Efficiencies of High-Rate Ponds in Municipal Wastewater Treatment: (A) Comprehensive Removal Efficiencies Across All Studies; (B) Removal Efficiencies Sub grouped by Application as Secondary (S) or Tertiary (T) Units.

The high dispersion of removal efficiencies should be highlighted in the figure. As several operational strategies are approached, each parameter becomes complex with single mechanisms or causes. Still, the differences between the efficiencies of applying HRAPs as secondary units are also presented.

5.5.1. Nutrients

Picot et al. [80] highlighted key nutrient removal pathways for HRAPs: photosynthetic assimilation and nitrogen volatilization with phosphorus precipitation. Both mechanisms are highly pH-dependent, especially ammonia volatilization and phosphorus precipitation. The increase in pH resulting from the carbon limitation in the wastewater promotes the volatilization of NH_3, contributing to nitrogen removal, albeit without nitrogen recovery. Furthermore, the increase in pH favors the chemical precipitation of phosphorus, rendering it unavailable to microalgae. Studies in HRAPs have demonstrated that the environment influences nutrient removal mechanisms, with pH and DO playing pivotal roles [81]. As Zhou et al. [82] highlighted, in HRAPs, NH_4+-N removal primarily occurs through nitrification and algae assimilation, while total nitrogen (TN) is predominantly removed via algae sedimentation. In the case of such a study, authors pointed out that ammonia volatilization played a minor role in NH_4+-N and TN removal. The study also highlights that total phosphorus removal is mainly attributed to algal assimilation in the form of organic phosphate and chemical precipitation in the form of calcium- and magnesium-bound phosphate. However, other studies suggest that ammonia volatilization can be the favored path for the removal of HRAPs, given the daytime rise in pH (>9.0) due to photosynthetic activity [83]. Although this is an efficient treatment path, it may not be the most interesting for nutrient recovery since the nitrogen is then released into the atmosphere instead of assimilated by the biomass [57]. Finally, nitrification, although not representing direct removal, transforms one form of nitrogen into another and occurs in HRAPs due to DO availability.

5.5.2. Organic Matter

HRAPs establish an oxygen/carbon dioxide utilization and production cycle within an algae-bacteria consortium in wastewater treatment. Microalgae assimilate nutrients, while bacteria degrade organic matter [84]). Soluble and particulate organic matter is oxidized by heterotrophic bacteria, aided by algal photosynthesis supplying oxygen [53]. Oswald [85] proposed the maximum BOD removal in an HRAP (mg O_2/L) as a function of solar irradiation (S, [W h/m^2 d]), microalgae photosynthetic efficiency (F, [%]), heat combustion of algae (h, [cal/mg]), HRAP depth (d, [cm]), and residence time (θ, [d]), as indicated in Equation (6.2).

$$BOD = \frac{85.93 \times \theta \times F \times S}{d \times h} \qquad \text{Equation (6.2)}$$

Successful organic matter degradation in HRAPs hinges on effluent's organic attributes and pH control. Municipal wastewater typically has BOD values of 300 mg/L [86], yet HRAPs have been applied to agro-industrial wastewater with substantially higher organic loads [87]. Reported biological oxygen demand (BOD5) rates range from 22% to 93.4%, with an average value of 59% [6]. Craggs et al. [31] achieved 47-52% BOD5 removal on hectare-scale HRAPs without CO_2 addition treating effluent following primary treatment (solids removal). Still, Fig. 6.2 also presents studies reporting increased COD following HRAP treatment [88]. This limitation also suggests the appropriate use of HRAPs as tertiary units, where organic matter removal would not be the goal. Still, enhanced BOD and COD removal can result from HRAP depth and HRT management [45].

5.5.3. Pathogens

The major mechanisms for pathogen removal in wastewater through microalgae occur via inactivation and degradation (mainly through environmental parameters conditioning the culture media, such as DO and pH fluctuations), adsorption, and precipitation [89]. The $\log_{10}$ reduction values for *Escherichia coli* in HRAPs vary from 1 to 4 log10 *E. coli* MPN 100 mL^{-1} (See Appendix A). Large-scale, continuously operated studies are still incipient, however. When reviewing pathogen removal compared to waste stabilization ponds, Chambonniere et al. [90] review and discuss sunlight-mediated and non-sunlight-mediated mechanisms in HRAPs compared to other waste stabilization systems such as

maturation ponds and facultative ponds. The authors concluded that while optimized for high algal activity, HRAPs' light supply optimization could compromise sunlight-mediated disinfection mechanisms. However, mixing in HRAPs might counteract this light attenuation, enabling intermittent exposure to intense sunlight near the surface. The resulting high algal activity increases pH and DO fluctuations, boosting sunlight-mediated mechanisms and potentially aiding pathogen removal through high pH toxicity [89].

Additionally, toxic algae metabolites produced as a response to algae cultivation conditions can also contribute to the inactivation of pathogens. Under high pH conditions, toxins from long-chain fatty acids produced by *C. vulgaris* were found harmful to pathogens and fecal bacteria, while green algae also reportedly mitigate fecal coliforms by increasing chlorophyll-a secretion [89].

Furthermore, the secretion of extracellular polymeric substances (EPSs) by algae, bacteria, and other microorganisms plays a significant role in this process. EPSs are capable of keeping cells adhered/aggregated in a matrix rich in phytohormones, amino acids, vitamins, and polysaccharides [91]. This EPS matrix not only contributes to improving sedimentation properties and facilitating harvesting [92,93] but also acts as a means of communication between microorganisms and their surroundings [92].

Studies have demonstrated that these extracellular metabolites can contribute to the interaction between algae and bacteria cooperatively or competitively/antagonistically. Antibacterial substances can inhibit growth or affect quorum sensing [[94], [95], [96]]. The possibility of algae releasing substances that inactivate fecal coliforms has been reported [97]. Furthermore, several microalgae species produce metabolites with antiviral properties, such as sulfated polysaccharides (mainly xylose, glucose, and galactose) [98].

In an in vitro activity study, Najdenski et al. [99] demonstrated that extracellular compounds in *Gloeocapsa* culture medium sp. and *Synechocystis sp*. and the EPS of *Gloeocapsa sp*. had inhibition capacity against Gram-positive *Staphylococcus aureus, Streptococcus pyogenes* and *Bacillus cereus*, as well as antifungal activity against the fungus *Candida albicans*. Chlorelin (mixture of fatty acids) obtained from *Chlorella* cultures had antibacterial properties against Gram positive and Gram negative bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* [100].

Natrah et al. [101] summarized studies where different microalgae were reported to produce substances with antibacterial properties, such as, for example, *Chlorella vulgaris, Chlorella pyrenoidosa, Isochrysis galbana, Tetraselmis, Chlorococcus, Dunaliella Primolecta, Skeletonema costatum, Nannochloropsis sp.* and *Phaeodactylum tricornutum*.

However, it is noteworthy that investigations into the interactions between extracellular substances from bacteria and algae still need to be further explored [92], including studies from wastewater treatment's perspective and the potential for removing pathogens through extracellular metabolites. Thus, pathogen removal should be further studied to account for the different ecological and environmental conditions of HRAPs, especially in real-scale plants that grow long-term. Still, given the impact of depth on pond volume's solar radiation exposure and the influence of algal photosynthesis on pH, which is itself affected by solar radiation exposure, one can posit that these studies collectively indicate depth as the primary factor influencing disinfection in HRAPs [6]. However, future studies should also examine the trade-off between *E. coli* inactivation and the addition of CO_2 to the media. Since wastewater imposes a carbon limitation on algae growth, CO_2 addition is a viable strategy (see also Section 5.9.1). However, it hinders pH elevation, reducing the potential for *E. coli* inactivation. Thus, reconciling these conflicting parameters requires further investigation to guarantee successful biomass growth and pathogen removal.

Regarding other pathogen organisms, such as helminths and protozoa, literature data on HRAP efficiency removal are scarce. In conventional ponds, they are usually removed by gravitational sedimentation in the sludge. However, in HRAPs, the mixing velocity promoted by the paddlewheels compromises their removal.

5.5.4. Emerging Contaminants

Emerging contaminants (EC) are an increasing concern for public health, especially considering conventional treatment methods are not designed to treat or remove them [22]. The ability of microalgae to treat EC has been reported for the most common aquatic contaminants, such as pharmaceuticals and personal care products [102] and pesticides [89]. Only some studies investigated HRAP efficiency in EC removal with real wastewater [103]. Still, up to 90 % removal of

EC was reported for pilot scale HRAP, highlighting the major roles of biodegradation and photodegradation in the removal [104]. The study by Vassalle et al. [36] used a system composed of a UASB reactor followed by an HRAP to monitor the treatment of five pharmaceuticals (ibuprofen, diclofenac, naproxen, paracetamol, and gemfibrozil), four estrogens (estrone (E1), 17β-estradiol (E2), 17α-ethinynestradiol (EE2) and estriol (E3)) and two xenoestrogens (nonylphenol and bisphenol A). The results showed that the UASB reactor was inefficient for removing most contaminants. At the same time, the HRAP achieved 90 %–95 % estrogens removal and 64 %–70 % pharmaceuticals removal (except for a lower 39 % removal of gemfibrozil). Still, lower efficiencies were reported for xenoestrogens (40 % and 70 % for bisphenol-A and nonylphenol). Irradiance was highlighted as the main cause of the results: direct path for photodegradation and indirect path through algae growth, promoting bioabsorption and biodegradation. Moreover, the potential of microalgae-based treatment has also been explored in light of the increasing concern over detecting microplastics (MPs) in wastewater treatment units. Reviews on the effects of freshwater and seawater cultures concluded that microplastics at low ppm concentrations negatively impact microalgae through growth inhibition, chlorophyll reduction, oxidative stress induction, and morphology alterations. However, the responses can depend on MP properties such as polymer type, size, and surface charge [105]. Current research also highlights that the production of exopolymer substances (EPS) by microalgae facilitates the removal of MPs via hetero-aggregation, which, however, can come at the price of decreased biomass production, reportedly up to 47 % loss [106]. With such results, several opportunities and research gaps are needed to fully understand and regulate the impacts of MPs in microalgae-based treatments.

Overall, a major gap still exists in regulatory standards for EC removal [22], and further research on the use of HRAP is still needed to confirm long-term and real-scale efficiency for most emerging contaminants.

5.6. Biomass Production and Characterization

Municipal wastewater-grown algal biomass has found applications in various conversion routes, including biofertilizers [107], biofuels [5], and other value-added biocompounds such as carotenoids [108]. Microalgae biomass has a high

variability in composition since the production of target compounds is highly dependent on species, environmental conditions, and nutrient balance, especially when grown in wastewater. Fig. 5.3 presents the assessment from the reviewed studies (see Appendix A for databases).

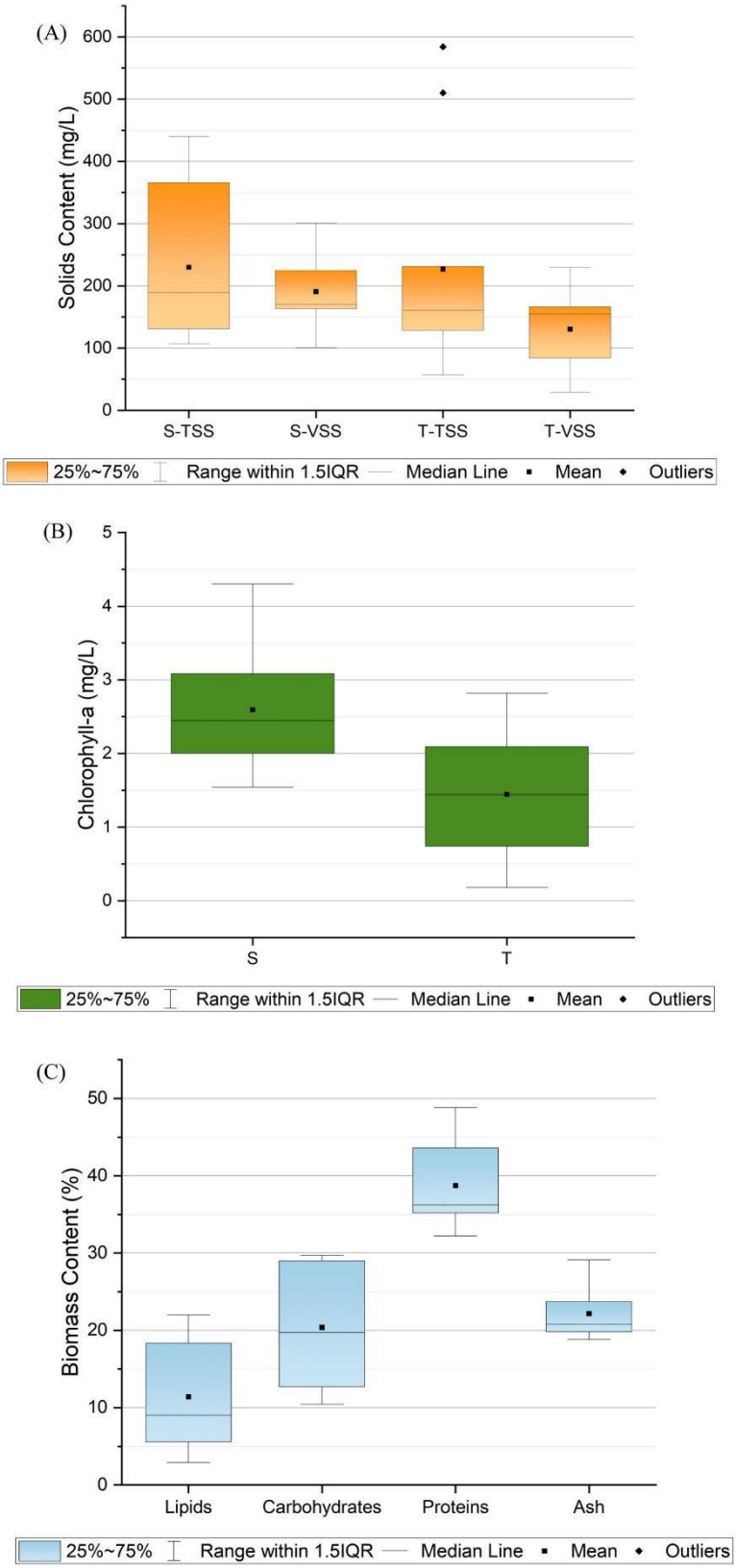

Fig. 5.3. Total (TSS) and volatile (VSS) solids content (A), chlorophyll-a content (B) and biomass composition (C) distribution for secondary (S) and tertiary (T) wastewater.

From the graphs in Fig. 5.3, it is possible to see that HRAPs applied to secondary treatment generally offer a higher biomass production, both in total biomass content (A) and algal production (B), as highlighted by the mean chlorophyll-a values. Still, the previous discussion on treatment efficiencies highlighted that when the goal is wastewater treatment, the secondary application can result in lower removal rates (see Section 5.5). Thus, reconciling the treatment efficiency and feasible biomass production is still a major challenge, even today. Closed systems for algal cultivation, like photobioreactors, often offer controlled conditions, resulting in lower CO_2 losses and higher productivity than open systems such as HRAPs [109]. The productivity challenges are even more pronounced in full-scale ponds [30]. Operational modifications have been identified as the most cost-effective means to enhance biomass productivity and nutrient removal [21]. However, they still present a knowledge gap [110]. HRAPs with wastewater typically exhibit algal productivity ranging from 5 to 15 g m^{-2} day^{-1} without CO_2 addition but can rise to 30 g m^{-2} day^{-1} with optimal CO_2 supplementation [67]. Other strategies, like biomass recycling [111] and HRT management [21], can also enhance productivity while maintaining operational balance, as discussed in Section 5.9. To target specific metabolites and overall biomass composition, two-stage cultivation under nutrient stress [112] and wastewater blending are strategies reported in the literature with promising results, as detailed in subsequent sections.

Also, from Fig. 5.3(C), lipids can range from 2.9 to 22 %, carbohydrates from 10.43 to 29.7 %, proteins from 32.21 to 48.8 %, and ash content from 18.8 to 29.1 %. Overall, challenges such as the limitation of applying wastewater-grown biomass to human consumption [20] and lower lipid content compared to freshwater cultivation [67] are important to note. However, it is possible to see that proteins are generally the predominant metabolite in microalgae biomass from HRAPs treating domestic wastewater. Calijuri et al. [12] highlighted that protein content is a key parameter when assessing the use of microalgae biomass for bioplastic production. Although promising, studies on this route are still sparse. Biopolymer production combines the advantages of high protein content and increased value for the final product while overcoming challenges such as the limitation of using wastewater-grown biomass for products intended for human consumption, which typically offer higher value-added opportunities

(such as pigments, nutraceuticals, and pharmaceuticals). As such, further studies in conversion technologies, productivity, and economic and environmental feasibility are highly desirable. As for the ash content, Couto et al. [113] suggest that ash primarily comprises inorganic substances, including metals, originating from biomass sources such as microalgae, bacteria, and other microorganisms. However, a substantial portion of the ash is attributed to the sand content originating from domestic wastewater. Few studies have reported on these parameters, underscoring the need for further investigation and inclusion of biochemical composition in future research to enhance our understanding of the combined roles of treatment efficiency and biomass production.

Still, a complete review of the biomass composition from HRAP applied to wastewater treatment, including the effects of downstream processes (harvesting, dewatering, and processing), the major potential recovery routes, and operation logistics to achieve feasible production, is highly desirable. However, most cases can be very complex and hard to conclude for broad application. Still, further details on sustainable and innovative harvesting approaches are discussed on Section 5.9.7.

5.7. Microbiological Community

The achievement of consistent production of microalgae biomass with high nutrient absorption and high yields of biochemical products remains a challenge to be addressed, especially when using wastewater as a cultivation medium. Microalgae, along with other microbial communities (bacteria, fungi, viruses, zooplankton grazers, parasites, among others), play a fundamental role in the phytoremediation of wastewater. However, this diversity of microorganisms can hinder the growth and accumulation of metabolites [48,114]. Still, mixed cultures are more robust when aiming for large-scale wastewater treatment, providing more stable biomass production and effective treatment efficiency [115]. Moreover, the presence of co-cultures of algae and bacteria diversifies contaminant removal pathways and promotes microalgal flocculation, potentially reducing harvesting costs [115].

In the bacteria-microalgae consortium, pollutants and nutrients are degraded through bacteria's action, transforming complex substrates into simple organic compounds that are more easily assimilated by microalgae [100,103]. This

consortium can favor the increase in the growth rate of microalgae, influence the generation of products in wastewater treatment, and diversify the contaminant removal routes [102].

To develop a functional microalgae-bacteria consortium for the treatment of dairy manure wastewater, Chang et al. [116] used *Chlorella sorokiniana* AK-1 and added a mixed bacterial culture. The initial bacterial initiation phase caused the maximum removal of nutrients, followed by the inoculation of microalgae, reaching 84.3 % and 90.2 % removal of COD and TN, respectively.

The mixed culture of microalgae is also a strategy for accumulating biocompounds, such as lipids, in addition to promoting treatment. *Chlorella vulgaris* microalgae culture (NRMC-F 0128) and *Scenedesmus dimorphus* (NRMC-F 0178) in the open air produced high amounts of lipids, around 30.40 % and 28.50 %, in textile mill effluent and industrial tannery effluent, respectively [117]. The treatment and nutrient removal potential of several monocultures (containing species of *Chlorophyta, Cyanophyta* and *Eustigmatophyta*) was compared with mixed cultures (mixing two or three species) of microalgae in research carried out by Luo et al. [115]. The mixed culture provided more stable biomass growth and more efficient removal of phosphate and nitrate, around 20 %, compared to monoculture. However, the interaction of different microalgae species caused greater production of biopolymers, which made the biomass dehydration process more difficult and could directly impact the harvesting cost.

Recent research has also directed its research towards the development of genetically modified microalgae, either to improve genetic transformation techniques [118] or to improve the characteristics of the microalgae biomass itself by modifying the expression of key enzymes to increase, for example, lipid content and growth rate [119,120]. Intending to improve the photosynthetic fixation of CO_2 in microalgae, Yang et al. [121] demonstrated the feasibility of improving photosynthetic capacity through genetic manipulation of the Calvin Cycle of the microalgae *Chlorella vulgaris*. The mutant strain *Chlorella sorokiniana* MB-12 was evaluated for Chen et al. [122] lutein yield and was metabolically superior to the wild-type strain. In this way, advances in genetic engineering can also contribute to the application of microalgae biotechnology in the treatment of wastewater and the valorization of sanitation resources.

As addressed before, bacteria are responsible for providing CO_2 for photosynthesis and for degrading pollutants and nutrients by transforming complex substrates into simple organic compounds that are more easily consumed by microalgae [48,123]. Establishing zooplankton grazers can either benefit or hinder the performance of HRAPs, as they can quickly consume dominant microalgae species and affect productivity and nutrient removal. Also, there are herbivorous species that feed through filtration (e.g., the rotifer B. calyciflorus) and consume easily ingestible smaller microalgal species with low sedimentation, leading to poor harvests [124].

The interaction between microalgae and fungi is also being studied as an emerging, environmentally friendly, and economically viable flocculation method [125]. Chen et al. [126] observed a cell harvesting efficiency of 98.62 % within 2.5 h using fungal pellet-assisted *(Penicillium sp.*) microalgal (*Chlorella sp.*) harvesting methods.

Microbiological diversity is influenced by various environmental factors such as temperature, light availability, and nutrients [23,88], as well as biological interactions due to competition for resources or predation by herbivores, which can vary seasonally throughout the year [123,127], besides reactor operation dynamics [111].

To understand the influence of various factors on the diversity of a consortium of microalgae produced in HRAPs, Cho et al. [128] evaluated environmental, biological, and chemical parameters over a year. The dominant microalgal species that persisted throughout the period were *Scenedesmus sp.*, *Microcystis sp.*, and *Chlorella sp*. Sutherland et al. [129] investigated the composition of the microalgal community in HRAPs over 23 months and identified 33 microalgal species. Furthermore, they concluded that 80 % of the changes in dominant species were associated with alterations in the number of microalgae grazers.

Collao et al. [123] assessed the microbial community of biomass during urban wastewater treatment in HRAPs and identified the family *Rhodobacteraceae* (heterotrophic and phototrophic) as the most dominant. Sánchez Zurano et al. [127] observed *Proteobacteria* and *Bacteroidetes* as the most abundant bacterial phyla, with *Rhodobacterales* and *Sphingomonadales* as the most abundant orders, and noted that environmental variations and nutrient availability directly affected this microbiological diversity. Galès et al. [130] observed ecological

interactions during wastewater treatment in HRAPs in different temperate climates. They concluded that the rapid growth of *Chlorella sp*., followed by the species *Scenedesmus sp.,* coupled with nitrifying and denitrifying bacterial activity, removed most of the ammonia. Gutiérrez et al. [111] investigated the recirculation of harvested biomass. They found that higher recirculation rates promoted the dominance of sediment-prone species (*Stigeoclonium sp.* or diatoms) while observing microalgae grazers like ciliate and flagellate protozoa.
Overall, the diversity of microalgae and other organisms is crucial for stable biomass productivity in wastewater treatment, as selective species enrichment weakens the consortium's adaptive capacity, increasing system vulnerability [131]. Thus, understanding the microbiological community that develops in HRAPs is a crucial step for the optimization and feasibility (both technically and economically) of wastewater treatment via microalgae biotechnology.
It is also important to highlight that monitoring and controlling the microbiological community in HRAPs can prevent operational problems such as pond crashes, which severely impact the economics of the algal processes. Favoring polycultures instead of a single strain can increase the resistance to crashes, which reportedly results in a more stable system [132]. Still, further research is needed on intervention strategies for crashes, but more importantly, on monitoring parameters and biomarkers to prevent them from happening in the first place [133].

5.8. Modelling

Kinetic modeling of HRAPs is a crucial tool for understanding and optimizing their performance [24]. Factors such as light intensity, nutrient concentrations, temperature, and flow dynamics influence biomass production and wastewater treatment in these systems. Understanding and optimizing these factors within the context of kinetic and hydrodynamic models enhances system performance.
In this context, Ortiz et al. [24] conducted an analysis using Computational Fluid Dynamics (CFD) to study velocity fields, flow patterns, and the presence of dead zones in HRAPs. Their research led to optimizing HRAP design by incorporating tear-shaped central separation walls and deflector baffles, enhancing mixing and reducing low-velocity zones. This design modification prevented biomass settling and improved overall efficiency. Additionally, Ortiz et al. [24] integrated diurnal

and seasonal variations of PAR into their biokinetic model. They observed that maximum PAR values, ranging from 921 to 1302 $\mu E \cdot m^{-2} \cdot s^{-1}$, significantly impact biomass production. Seasonal temperature fluctuations, from 7.1 °C in January to 40.1 °C in May, were also incorporated into their model to predict system performance throughout the year. The authors noted that higher temperatures generally enhance growth rates but can also increase the risk of overheating and oxygen depletion, necessitating adaptive management strategies. An optimal HRT of 4 days was found to be sufficient for nutrient removal and biomass production under typical climatic conditions, while a 5-day HRT was recommended during winter for consistent phosphorus removal.

In contrast, Chambonniere et al. [90] demonstrated that a median HRT of 7.5 days effectively supports COD and N-NH_4+ removal efficiencies of 74.8 % and 97.9 %, respectively. This highlights the variability in optimal HRT depending on specific operational goals and environmental conditions. The ratio between the length and width (L/W) of the HRAP is another important parameter that affects flow uniformity and the presence of dead zones. Higher L/W ratios, preferably >10, minimize dead zones and promote uniform velocity distribution, thereby benefiting algae productivity [51].

Integrating kinetic and hydrodynamic models is essential to optimize biomass production and wastewater treatment in HRAPs. Careful management of key parameters such as light intensity, nutrient availability, HRT, temperature, and hydrodynamic conditions is necessary to enhance system performance and achieve sustainable operational outcomes. By considering the key biological and environmental factors that affect algal growth, kinetic models can predict biomass production, nutrient removal, and other important process parameters [90].

Several kinetic models have been developed for HRAPs. Common models are based on Monod kinetics, which assumes that algal growth is limited by the availability of a single nutrient [134]. However, depending on the specific application, a model that considers multiple factors may be more appropriate. For example, Solimeno et al. [135] developed a mechanistic model named BIO_ALGAE to understand interactions in mixed algal-bacterial systems for wastewater treatment. This model integrates microalgae and bacterial processes, considering light, temperature, and pH. The authors found that adjusting organic matter in influent altered microalgae production. In another work, Solimeno and

García [136] validated the BIO_ALGAE model using data from a pilot HRAP across seasons and different HRTs. They found that optimized HRAP operation, with shorter HRT in warm months and longer in the cold, increased annual microalgae production to 14.1 g TSS m^{-2} d^{-1} compared to constant HRT operation (4 and 8 days, respectively), in which production was 10.2 and 9.2 g TSS m^{-2} d^{-1}, respectively.

In addition to kinetic modeling, hydrodynamic modeling is crucial for understanding HRAP performance. The hydrodynamics of HRAPs have been characterized by Mendoza et al. [137] using tracer experiments expressed by the Bodestein Number (Bo). This parameter assesses mixing in different reactor sections of the HRAP, with Bo ≤20 indicating complete mixing and Bo ≥100 indicating plug flow. Plug flow was identified as the dominant fluid model in the HRAP, especially in the straight channels (Bo in the 200-540 range). Similar findings were reported by Pham [138] for pilot scale systems, using stimulus-response methods to assess the flow mode of the HRAP. The authors observed higher Bo values when increasing the paddle rotation speed and decreasing the water depth. However, few studies report on such parameters, highlighting the need for further hydrodynamic characterization of HRAP units.

Hydrodynamic models can predict the mixing and flow patterns in the HRAP, which significantly impact algal growth and nutrient removal [51]. For example, a study by Ortiz et al. [24] aimed to optimize the design of a secondary HRAP using computational fluid dynamics. The study found that the optimal HRT for nutrient removal and biomass production was 4 days. Additionally, the most efficient configuration of the HRAP included two baffles and tear-shapes with a diameter equal to 1/4 of the channel width.

Combining kinetic and hydrodynamic modeling provides a comprehensive understanding of HRAP performance. This approach can optimize the design and operation of these systems, leading to improved wastewater treatment and biomass production.

5.9. Alternative and Innovative Technologies applied to HRAPs

Given the overview, the authors emphasize strategies that promise to tackle HRAP wastewater treatment technology challenges. As discussed earlier, these

approaches target issues linked to HRAP applications, including nutrient constraints, disinfection needs, and biomass collection. While not all strategies have been implemented in HRAPs or at full scale, the authors explore each strategy's potential implications, opportunities, and challenges for HRAP technology. Fig. 5.4 presents an overview of the strategies addressed.

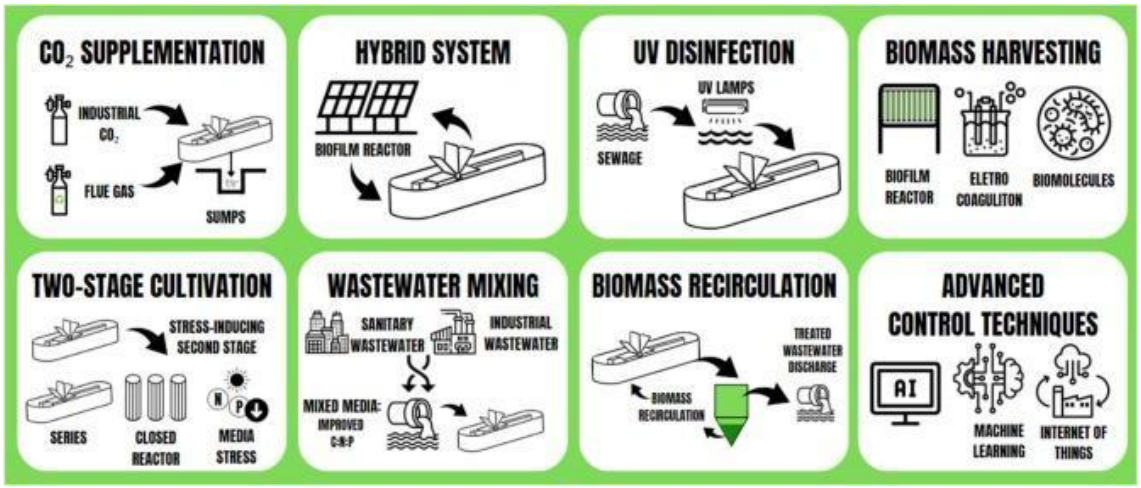

Fig. 5.4. Innovative operational strategies for wastewater treatment and biomass production in high-rate algal ponds.

5.9.1. CO_2 supply

While HRAPs serve as established wastewater treatment technology, microalgal productivity, and nutrient removal are often limited, primarily due to dissolved inorganic carbon shortage during peak photosynthesis [66]. Generating 1 kg of algal biomass requires 1.83 kg of CO_2 [139], and carbon insufficiency significantly reduces microalgae growth rate.

Carbon scarcity within wastewater becomes a pivotal factor impacting biomass productivity and nutrient removal, as Park and Craggs [83] observed. This issue was addressed as early as 1960 [140] with the proposition of CO_2 addition via carbonation columns to alleviate the constraint. Such CO_2 supplementation can lead to a twofold increase in wastewater treatment productivity, reaching 16–20 g m^{-2} day [141]. The concept introduced by Putt et al. [142] exhibited enhanced productivity efficiency within HRAPs and was subsequently adopted by studies such as Assis et al. [39] and Assis et al. [143]. Various carbon sources can be harnessed for supplementation, including commercial pure CO_2, as Park and Craggs [83] demonstrated, resulting in a relative rise of algal biomass.

Also, CO_2 sumps are another method reportedly used in full-scale HRAPs [144]. Using a vertical divider baffle, the CO_2 addition sump is divided into two sections, where the media flows upward and water flows downward. The gas is introduced into the downward-flowing section of the sump by releasing it through diffusers located at the bottom of the divider baffle. This setup allows for controlled and efficient CO_2 distribution within the system. This system's high frequency of CO_2 addition reportedly increased organic biomass by 120% and total microalgal biovolume by 157%. Still, adding such a system can reach 25% of total capital costs, and adding more than one sump per pond to reach the necessary carbon requirements seems unfeasible [144].

However, the cost of procuring CO_2 could impede the broad application of this strategy. Environmentally, industrial exhaust gases are explored as an alternative CO_2 source, yet their composition—containing NOx and SOx [145]—can detrimentally impact microalgae growth. Assis et al. [39] conducted a study investigating the impact of CO_2 supplementation on microalgae productivity, comparing pure gas to combustion-derived gas. The findings advocate using combustion gases, as they did not lead to reduced productivity upon supplementation. Moreover, this study controlled the addition of CO_2 as a function of pH levels. Thus, the CO_2 added to the HRAP also played the role of controlling the pH of the media and avoiding nutrient loss.

Couto et al. [57] also modeled biomass production and compared the models in a cluster analysis. For HRAPs with CO_2, the 30 cm depth was grouped with the 40 cm depth. Without CO_2, the 30 cm depth was grouped with the 20 cm depth. This result indicated that CO_2 can be a tool to adopt higher depths in HRAPs, culminating in area savings. Overall, the CO_2 supply is a high-potential strategy to make feasible HRAP application, especially if flue gas is used.

5.9.2. Hybrid Systems

The evolution of designs and the need to address the deficiencies of isolated systems led to the proposal of hybrid systems. Combining closed and open reactors has been suggested to increase microalgae biomass productivity and target value-added metabolites from the produced biomass. Liu et al. [146] used open ponds connected to closed photobioreactors, achieving up to 73 % higher biomass yield than the open pond alone and 12.5% higher than the closed

reactor. The validation of such a method for wastewater has yet to be tested, and future studies are encouraged but should account for the cost impacts of such a strategy.

The hybrid system combining attached and suspended growth has been reported for wastewater treatment. A hybrid biofilm reactor coupled to the HRAP was studied by Assis et al. [143], comparing HRAP with CO_2 supply and hybrid systems with and without CO_2 supply. Total biomass productivity was 6.79 g m^{-2} day^{-1} in the hybrid systems, which was higher than the treatments. More importantly, using the hybrid system facilitated the harvesting step, indicating cost minimization of the overall process since this step is a major bottleneck in algae systems. Following this, Assis et al. [147] performed the life cycle assessment of hybrid systems. They concluded that the biofilm reactor reduced the environmental impacts of a conventional HRAP by up to 28 % in all impact categories. The large-scale applicability of such a strategy still pends research, and further insight into the long-term use of the fabrics for attached growth is critical for technological feasibility [148].

5.9.3. Ultraviolet Pre-Disinfection

Pre-disinfection of wastewater before HRAPs can enhance microalgae growth by reducing competition for space and nutrients [34]. Assemany et al. [149] investigated the pre-treatment of the wastewater with UV disinfection before cultivation in HRAPs. The study concluded that UV disinfection did not notably affect fatty acid profile and total lipid productivities but enhanced algal biomass concentration, productivity, and lipid content. Also, Couto et al. [57] experimented with 40 cm HRAPs, with pre-disinfection, with and without CO_2 supply. The study concluded that pre-disinfection in deeper ponds (40 cm) yielded similar biomass growth to shallower ponds (30 cm). This resulted in an alternative to reduce area demand without compromising biomass production and treatment. However, more research is still needed to explore the correlation between effluent pre-disinfection and biomass productivity.

5.9.4. Series operation and two-stage cultivation

Sutherland et al. [30] demonstrated that achieving high-quality effluent can be accomplished by operating HRAPs with reduced HRTs and compensating for this reduction by employing multiple HRAPs in series. The study investigated biomass productivity in secondary wastewater (biological treatment). It monitored the treatment quality for two HRAPs operated in series (HRT = 4 days each) compared to two parallel sets of conventional HRAPs (HRT = 8 days). The series of HRAPs were included in an Enhanced Pond System, comprising an anaerobic pond and two sets of identical HRAPs followed by harvesting ponds and maturation ponds, totaling six maturation ponds in the system. The series worked by using effluent after the first set of maturation ponds led to the second HRAP. The authors reported a significant increase of approximately 50 % in biomass productivity and a 35% improvement in nitrogen removal during the series operation. In contrast, no statistically significant difference was observed in phosphorus removal. These findings emphasize the effectiveness of simple HRAP modifications in achieving cost-effective wastewater treatment and resource recovery.

Two-stage microalgae cultivation enhances target compound productivity by separating biomass growth and product accumulation into distinct steps [112]. The initial stage focuses on high biomass productivity under optimal conditions, while the subsequent stage induces stress for target metabolite accumulation. The transition from the first stage to the second can involve combining different growth modes, operation modes, physiochemical conditions, or cultivation systems. This alteration includes combining multiple stages to enforce changes in photoautotrophy, heterotrophy, and mixotrophy; batch, semi-batch, fed-batch, or continuous operation; nutrient levels, light intensity, salinity, temperature, and pH; and closed or open cultivation systems.

However, full-scale studies and life cycle assessments are highly desirable at the current stage. Still, this strategy is one of the most promising and versatile approaches to sustainable HRAP treatment.

5.9.5. Wastewater blends

In addition to municipal wastewater, HRAPs have been employed to treat various wastewater sources, including meat processing [109], piggery [150], paper mill [151], among others. One approach to address the individual limitations of algal

productivity in each media and enhance growth at minimal costs involves blending different wastewaters. By achieving the correct blend, it becomes possible to improve the nutritional balance of the medium, dilute toxic agents (such as heavy metals and ammonia), and control eventual rotifers or grazers [152], consequently boosting algal growth. Nevertheless, the feasibility of such combinations requires further investigation on a case-by-case basis, given wastewater's high variability and complexity. Literature highlights studies exploring blending wastewater in HRAPs with effluent from piggery [153], paint booth [154], and fruit-based juice industry [159], for example. Notably, the viability of this approach hinges on the availability of suitable wastewater sources within the specific industrial context and geographical location, which necessitates careful consideration. However, this strategy merits its own comprehensive review, elucidating its potential benefits and associated challenges.

5.9.6. Biomass Recirculation

Biomass recycling is a strategy that involves reintroducing a portion of harvested algal biomass back into the HRAP, aiming to enhance algae concentration to the optimal level for improved biomass production and to foster the dominance of specific algae species, facilitating biomass harvesting by gravitational sedimentation. This can result in benefits such as higher biomass and metabolite productivity and simplified harvesting procedures [48]. Moreover, biomass recirculation can be an operational strategy to decrease the HRT during the summer without raising nutrient loading, as it lowers influent concentration while maintaining the same nutrient loading as a longer HRT, enabling shorter HRT operation [66]. However, at least near-complete biomass harvesting is required before recirculation, or issues of decreased light availability can lower biomass productivity.

The recirculation of settled biomass might prove more effective than pre-disinfection in controlling dominant species [5]. Moreover, as Park et al. [155] demonstrated, biomass recirculation can promote species growth with superior settling characteristics, thus enhancing subsequent steps in the downstream process. Although some studies did not reach higher biomass production, improved harvesting efficiency from 5 to 89 % to 92–94 % was reported by adopting biomass recycling [111].

5.9.7. Biomass Harvesting

The harvesting and dehydration stage can also influence the characteristics of the biomass [156,2] and impact its environmental and economic viability [[157], [158], [159]]. Therefore, the search for more efficient and sustainable technologies, from both an environmental and economic perspective, is the focus of several research efforts [[160], [161], [162]].

Ferreira et al. [157] evaluated different algal biomass harvesting systems and found that the highest concentration efficiency (99%) and greatest amount of harvested biomass (121.13 g day^{-1}) were achieved with a system composed of coagulation with tannin followed by gravitational sedimentation. In comparison, the system using a biofilm reactor had the highest carbohydrate content (36.83 %). Besides the type of reactor used, the operation of the harvesting system also impacts harvest efficiency. Silva et al. [159] evaluated different scraping frequencies of a hybrid system (composed of HRAPs and biofilm reactors) and found that a lower scraping frequency (every 2 days) resulted in greater microalgae harvest (18.75 g TVS m^{-2} d^{-1}) and increased lipid content (15.45 %).

Another emerging technique for harvesting microalgae involves the use of nanoparticles. Harvesting *Haematococcus pluvialis* with Fe_3O_4 nanoparticles (200 mg/L) achieved 99% efficiency [163]. The authors found that the nanoparticles could be reused, with only a 5.41% reduction in efficiency per cycle, which can positively impact the cost-benefit ratio of the harvesting strategy. Electrocoagulation is also a promising technology for harvesting microalgae. Several studies aim to improve harvesting efficiency and reduce energy consumption. Khatib et al. [164] used integrated aluminum cylinder electrodes, which improved harvesting efficiency by 28 % and reduced electrode passivation, as this new electrode configuration increased the electric field intensity in the reactor. Advancing the harvesting stage is a fundamental step towards achieving economic and environmental viability in wastewater treatment and enhancing biomass value.

5.9.8. Advanced control techniques

Integrating Machine Learning (ML) and Artificial Intelligence (AI) into microalgae cultivation within wastewater treatment systems is an important step in environmental biotechnology. These technologies introduce innovative methods to optimize complex biological processes, enhancing system efficiency and promoting sustainable bioremediation solutions. Previous studies [165,166] highlight the benefits of AI and ML in optimizing operational parameters and predicting system behavior. By analyzing real-time data on environmental conditions, nutrient levels, and microbial activity, these technologies enable dynamic changes in culture conditions to maximize biomass production and pollutant removal. AI-based sensors monitor critical parameters such as pH, DO, and nutrient levels, feeding this data into ML models. These models predict optimal conditions for co-culturing processes, boosting system performance and energy efficiency.

A standout feature of AI and ML is their capability for real-time monitoring and control of wastewater treatment systems. Intelligent sensors and Internet of Things (IoT) devices collect continuous data, allowing AI algorithms to analyze and optimize interactions between microalgae and bacteria. This reduces the need for manual interventions and enhances system stability, which is important for maintaining efficiency under varying environmental conditions [167].

Artificial Neural Networks (ANNs) excel in modeling non-linear relationships and interactions in biological systems. For instance, previous research utilized ANNs to fine-tune growth conditions for microalgae and bacteria, predict biomass productivity, and control light intensity in photobioreactors [168]. [169] demonstrated the superior accuracy and robustness of ANN models in predicting system performance and optimizing operational parameters compared to traditional methods. Their study employed a three-layer feed-forward back-propagation ANN model with a 6-10-1 architecture to predict microalgae dry cell weight, achieving a high predictive performance (R^2 = 0.983). The ANN model used six inputs: temperature, pH, dissolved oxygen, electrical conductivity (EC), nitrate, and phosphate. The study revealed significant nutrient removal efficiencies, with BG11-supplemented wastewater achieving a high algal biomass concentration of 0.79 ± 0.04 g/L and removal efficiencies for NO_3-, NH_4+, and $PO_4$3- of 83.20 ± 2.90 %, ≈100 %, and 93.50 ± 3.28 %, respectively. The biochemical composition of the microalgae showed lipid, protein, and

carbohydrate contents of 25.60 ± 0.80 %, 29.00 ± 0.88 %, and 18.40 ± 1.00 % dry cell weight basis, respectively. Sensitivity analysis of the ANN model indicated the relative importance of the environmental factors in the following order: NO_3- (21.7 %), $PO_4$3- (19.2 %), pH (17.1 %), DO (17.1 %), temperature (13.6 %), and EC (11.3 %).

Additionally, Convolutional Neural Networks (CNNs) have been applied to automate the detection and identification of contaminants in wastewater, enhancing the efficiency and accuracy of water quality monitoring systems. Integrating AI techniques like ANN and CNN into wastewater treatment systems significantly advances sustainable and efficient wastewater management solutions. This technology allows precise classification and targeted removal strategies, enhancing bioremediation processes' efficiency by saving time and resources typically spent on physical experiments [170].

However, challenges remain in integrating AI and ML into wastewater treatment. One significant hurdle is data scarcity, as accurate predictive models require extensive datasets, which are often limited. Collaborative efforts are essential to gather and share data, strengthening the robustness of ML models [168]. Another challenge is model complexity and interpretability, especially in systems with intricate biological interactions. Explainable AI techniques can address this by providing clear understandings into model predictions and decision-making processes, thus improving the transparency and usability of AI models in practical applications. For instance, Meenatchisundaram et al. [171] compared seven ML models, including Decision Trees, Random Forest, K-Nearest Neighbours, Gradient Boosting Regressor, Multi-Layer Perceptron Regression, Support Vector Regression, and ANN, for optimizing biomass yield in microalgae-based wastewater treatment. The ANN outperformed the others, with an R^2 value of 0.98, indicating high prediction accuracy. The study highlighted the importance of input variables such as COD, phosphate, nitrate, nitrite, pH, and HRT. The optimal biomass yield of 948 mg/L was achieved under specific conditions: COD at 350 mg/L, phosphate at 50 mg/L, nitrate at 60 mg/L, nitrite at 140 mg/L, pH at 7.1, and a HRT of 9 days.

Considering the potential of integrating AI and ML in wastewater treatment, future research should focus on developing scalable and economically viable strategies.

It is also important to simplify models to reduce computational requirements, standardize protocols for model development, and create user-friendly interfaces.

5.10. Sustainability

In the context of a circular bioeconomy, wastewater treatment plants focused on resource recovery and compliant with sustainability principles are strongly encouraged. In pursuit of this, reactors and systems' environmental, economic, and social performance is evaluated. Tua et al. [172] assessed the addition of an HRAP to a municipal wastewater treatment plant through a life cycle assessment (LCA). The unit received feedstock as supernatant from digestate dewatering (centrate) and flue gas from combined heat and power units, with the produced biomass directed towards anaerobic digestion. The authors noted that despite introducing an extra stage to the system, the additional electricity generation, partly from the energy valorization of algal biomass, was twice the electricity needed for the HRAP operation [172]. However, the NH_3 volatilization associated with this new process led to higher environmental impacts in Particulate Matter Formation, Acidification, and Terrestrial and Marine Eutrophication Categories, highlighting the significance of pH control with CO2 supplementation [172].

Compared to the more commonly employed activated sludge process, HRAP exhibited lower environmental impacts due to its lower energy consumption for operation [42,173]. Thus, environmental benefits were observed in Climate Change, Ozone Depletion, Freshwater and Marine Eutrophication, Photochemical Oxidant Formation, and Fossil Depletion categories [173]. On the other hand, the construction of the HRAP and, once again, NH_3 volatilization were highlighted as areas that needed improvement. The former had implications in the Depletion of Metals category [42,173], while the latter caused environmental issues related to toxicity and terrestrial acidification [173]. From an economic standpoint, capital and operation/maintenance costs were observed at 164.14 € e.p.−1 and 0.42 € million−3 for an HRAP serving small communities, which were 3.3× and 2× lower than those observed for activated sludge, respectively [42]. Still, future LCA studies could address strategies to reduce NH_3 volatilization, such as comparing the addition of CO_2 for pH control and mixing effluents with higher carbon content.

Moreover, the LCA is a promising tool for assessing the trade-off between the gains in productivity and the environmental burdens associated with coupling enhancement technologies to HRAPs. Magalhães et al. [148] performed the LCA for some of the alternatives presented here, namely using wastewater pre-treated with UV disinfection, with supplementation from industrial CO_2 and with exhaust gas from gasoline combustion, coupled with a biofilm reactor for biomass attached growth. Results show that, compared to the base HRAP cultivation scenario, the gains in productivity did not offset the environmental burdens of coupling such technologies. Using the recovered gas to supplement the carbon from the HRAP was the most promising scenario, reaching the lowest environmental impact. Thus, the paths for sustainable HRAP systems should consider LCA an important tool for evaluating innovative operational strategies and technologies.

Assessments from a social perspective are still incipient. In a recent study, Josa and Garfí [174] demonstrated that urban wastewater treatment has a slightly lower social impact than wastewater treatment from the food industry, given more regulations and standards for the former. Compared to constructed wetlands, the operating and maintenance cost was around 5 % higher, but due to the reduced material requirement in constructing the HRAP, the capital cost was 1.3× lower [42].

5.11. Challenges, opportunities and future prospects

In light of the topics explored throughout this research article, Table 5.3 provides a comprehensive overview of HRAP technology. It outlines the key challenges identified, along with future prospects and research opportunities related to the application of HRAP in municipal wastewater treatment.

Table 5.3. Summary of the advancements of high-rate algal pond technology applied to municipal wastewater treatment.

Category	Current challenges and limitations	Research opportunities
Design	Lack of standardized design parameters	Develop comprehensive HRAP design standards.
		Research full-scale, long term HRAP operations
	Scarce validated design methods, with a wide range of performances	Explore innovative designs to reduce dead zones, increase light and hydrodynamic efficiency
		Validated regional studies with a comprehensive correlation of operational parameters and treatment performance
Pathogen Removal	Significantly variable *E. coli* removal in HRAPs significantly (1 to 4 log_{10} reduction)	Effects of CO_2 addition on *E. coli* inactivation and pH
	More research is needed on algae-bacteria interactions for pathogen removal	Explore extracellular metabolites for pathogen removal
		Correlate environmental factors affecting pathogen inactivation.
	Limited data on HRAP efficiency for removing virus, helminths and protozoa	Expand studies on virus, helminth and protozoa removal in HRAPs
Emerging Contaminants (EC) Removal	Lack of regulatory standards for EC removal.	Confirm and correlate the conditions for HRAP to efficiently remove several contaminants.
		Investigate biodegradation and photodegradation roles in EC removal.
	Diverse range of ECs in wastewater	Understand the impacts of microplastics on microalgae and optimize removal.
		Develop regulatory standards for emerging contaminant removal.
Microbiological Community	Microbial diversity can hinder microalgae growth and increase harvesting costs.	Advance genetic engineering.
	Variability in microbial communities and pond crashes impact HRAP stability.	Favor polycultures to increase system resistance and stability.

Category	Current challenges and limitations	Research opportunities
	Microbiological diversity is influenced by environmental factors and seasonal changes.	Develop monitoring and intervention strategies to prevent pond crashes.
CO_2 Supply	High capital and operation costs of CO2 supply systems.	Develop alternative low-cost CO_2 supplementation systems Investigate recovered gas from other industrial processes
	Toxic compounds from recovered gas have to be studied case-by-case	Explore CO_2 supplementation for deeper ponds and improved productivity. Develop models to predict biomass production with CO_2 supplementation.
Hybrid Systems	Lack full-scale hybrid systems	Study long-term, full-scale attached growth coupled to HRAP, with cost assessment
	Cost and environmental impacts of fabrics for attached growth	Study different support materials with reduced environmental impacts
UV Pre-disinfection	High environmental impacts from UV lamps	Compare pre-disinfection methods besides UV
		Assess the costs and scalability of the technology
Series Operation and Two-Stage Cultivation	Lack of studies with cost and environmental analysis	Investigate the effectiveness of operating multiple (>2) HRAPs in series. Develop a correlation between series operation and increased treatment performance for nutrients and pathogens There are several paths to study the effects of combining growth modes and conditions. Conduct life cycle assessments and cost assessment to evaluate sustainability.
Wastewater Blends	High variability and complexity of wastewater	Explore wastewater from different sources for nutritional balance and toxin dilution.

Category	Current challenges and limitations	Research opportunities
	Availability of suitable wastewater sources	Investigate methods to achieve correct wastewater blends in full-scale.
	Controlling rotifers or grazers in blended wastewater	Perform comprehensive reviews of the blending strategy. Case studies on how to integrate municipal wastewater treatment with local industries
Biomass Recirculation	Requires nearly complete harvesting, which can be costly Some studies did not achieve higher biomass production with recycling.	Comprehensive correlation studies with limits to the applicability of the technology
Biomass Harvesting	Different impacts of harvesting methods on biomass characteristics. High energy consumption and costs Maintaining efficiency in sustainable harvesting methods	Studies on integrating emerging techniques like nanoparticle-based harvesting, biofilm reactors and electrocoagulation
Advanced Control Techniques	Accurate predictive models require extensive datasets. Complex systems make models hard to interpret. Need for explainable AI for practical applications. Various ML models need a comparison to identify the best for optimizing biomass yield.	Collaborate on data collection to strengthen ML models. Develop explainable AI techniques for model predictions. Simplify models to reduce computational requirements. Create standardized protocols for model development. Develop user-friendly interfaces for AI models. Focus on scalable and economically viable strategies.
Economic and Environmental Sustainability	Limited assessments of social impact compared to other wastewater treatment methods. Use of secondary data on inventories	Diffuse life cycle assessment studies and comprehensive economic analyses Compile standardized datasets for environmental and economic evaluations, facilitating comparison

Category	Current challenges and limitations	Research opportunities
		Include social life cycle assessment

5.12. Conclusions

Using municipal wastewater as a microalgae culture medium in HRAPs represents a cost-efficient, sustainable, and environmentally friendly approach to wastewater treatment and nutrient recovery. Despite extensive research, real-scale applications remain limited. Key conclusions highlight the necessity of prioritizing HRAPs as tertiary treatment units due to inconsistent organic matter removal. Additionally, there is an urgent need for standardized guidelines to improve design parameters and the hydraulic and kinetic functioning of HRAPs. Further research is required to address emerging contaminants, especially microplastics. Comprehensive analyses of innovative HRAP strategies are crucial for sustainability. Moreover, expanding HRAP applications in developing countries and rural areas is vital for medium-to-large-scale implementation. Overall, this study underscores the diverse applications and continual development of HRAPs, emphasizing their potential to promote sustainable development and support bioeconomic and biorefinery initiatives.

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6. Chapter 2. Series Operation

Artigo 2: Effects of series operation of high-rate algal ponds treating sanitary sewage: hydrodynamics, remediation potential and biomass production[2]

Abstract

Operational modifications are a promising, cost-effective strategy to increase the efficiency of algal systems applied to sewage treatment. In this context, this study investigates the effectiveness of operating High-rate Algal Ponds (HRAPs) in series for sanitary sewage treatment, focusing on improving technical performance by assessing pollutant removal efficiencies and biomass production. A hydrodynamic analysis determined Bodenstein's value of 9.7 for HRAPs, indicating a complete mixing regime and suggesting potential benefits to series operation. Subsequently, two systems were operated, each comprising two HRAPs: one operated in series (with a hydraulic retention time (HRT) of 5 days in each HRAP) and one operated in parallel (with an HRT of 10 days in each HRAP). The series operation achieved higher coliforms and E. coli removal (97.79 % and 98.33 % compared to 84.09 % and 89.56 % in parallel), while no statistically significant differences were observed in nutrient removal or biomass production. However, the parallel system exhibited 14 % higher TOC removal and biomass with greater lipid (19 %), protein (76 %), and carbohydrate (44 %) content, while the series had higher ash content (65 % higher). Discussions approach the roles of hydrodynamics, nutrient balance, and senescence to explain the performance of each system. Future research should focus on reaching a plug flow regime by optimizing mixing, besides testing more than two ponds in series.

[2] Attachment 2: Paper published at Algal Research (ISSN: 2211-9264): MAGALHÃES, Iara Barbosa; REIS, Miriam Costa Fateixa; PEREIRA, Alexia Saleme Aona de Paula; BRAGA, Matheus Quintão; ASSEMANY, Paula Peixoto; CALIJURI, Maria Lúcia. Effects of series operation of high-rate algal ponds treating sanitary sewage: hydrodynamics, remediation potential, and biomass production. Algal Research, [s. l.], vol. 89, p. 104091, jul. 2025. DOI 10.1016/j.algal.2025.104091. Disponível em: http://dx.doi.org/10.1016/j.algal.2025.104091.

Keywords: raceway ponds, Bodenstein number, wastewater treatment, microalgae biomass; hydraulic retention time, operational parameters

6.1. Introduction

High-Rate Algal Ponds (HRAPs) have garnered increasing attention as a cost-effective and sustainable technology for wastewater treatment, owing to their relatively low construction costs [1], scalability [2], and capacity to integrate conventional wastewater treatment plants [3]. By removing nutrients and organic matter from wastewater, HRAPs also facilitate the production of algal biomass, offering additional resource recovery potential [4]. Despite these benefits, achieving economically viable full-scale HRAPs still necessitates further optimization of treatment processes and resource recovery strategies [5,6], leading researchers to explore operational modifications as a promising approach for low-cost improvements [7].

Several operational enhancements—including the implementation of hybrid systems [[8], [9], [10]], the addition of carbonation columns [11], and modifications to operational depth [12] —have been proposed to boost HRAP performance. One especially notable strategy is the arrangement of ponds in series, a concept established in the 1960s for improving the performance of maturation ponds through enhanced hydraulic regimes [13]. This configuration has also demonstrated potential for improving nutrient removal and promoting biomass production in HRAPs [7,14]. For example, Sutherland et al. [7] reported significantly higher total chlorophyll-a productivity when HRAPs were operated in series (2.9 ± 0.7 kg/day) compared to parallel operations (1.2 ± 0.1 kg/day), attributing these gains to shorter hydraulic retention times (HRT) that supported higher daily biomass productivity and nutrient removal efficiency. Notably, however, these series-configured HRAPs employed settling tanks between the ponds, introducing additional infrastructure and masking the effects of a purely direct, uninterrupted flow path on pathogen removal.

Recent modeling work suggests that an HRAP series arrangement could optimize pathogen removal—particularly for Escherichia coli (E. coli)—when plug-flow conditions are approximated [15]. Operations that approach plug flow, widely recognized for favoring processes governed by first-order decay kinetics,

can be cultivated by lengthening the flow path or employing baffles and multiple pond stages [16,17]. To quantify axial dispersion in such systems, researchers frequently use the Bodenstein number (Bo). Low Bo values (≤20) signify complete mixing, whereas higher values (≥100) align with near-plug-flow conditions [18,19]. While HRAPs often exhibit lower Bo values at impeller blades, pond outlets, and bends (<7), the elongated straight sections—comprising most of the reactor volume—can achieve Bo values between 200 and 540 [20].

Building on this foundation, the present study introduces a direct series configuration for HRAPs—one that bypasses intermediate settling stages. This uninterrupted flow path is hypothesized to accentuate plug-flow behavior, and thereby enhance the removal of contaminants governed by first-order decay kinetics. Notably, while prior research has recognized improved nutrient and organic matter removal under series operation, the full implications for pathogen removal under practical operational conditions remain underexplored.

Thus, we compare HRAPs operated in parallel to those directly linked in series, examining their respective performances in pathogen removal (coliforms and *E. coli*), nutrient and organic matter removal, biomass productivity and characterization. We also integrate hydrodynamic insights via the Bodenstein and Reynolds numbers to contextualize observed performance within flow regimes. Ultimately, this study aims to highlight how targeted operational configurations, specifically direct series operation, can bolster cost-effective wastewater treatment and resource recovery—achieving these gains without the added complexity of auxiliary settling infrastructure.

6.2. Material and Methods

The research was conducted at the Sanitary and Environmental Engineering Laboratory (LESA), Department of Civil Engineering, Federal University of Viçosa (UFV). The experiments were carried out from June to September 2022, covering autumn and winter. Sanitary sewage was used as a culture medium, collected weekly after treatment in a septic tank at the Sewage Treatment Station located in the Romão dos Reis neighborhood in Viçosa - Minas Gerais, operated by the Autonomous Water and Sewage Service (SAAE - Viçosa).

The experiments were conducted in pilot-scale HRAPs (1000 L each), with the following characteristics: width: 1.28 m; length: 2.86 m; total depth: 0.5 m; useful depth: 0.3 m; and surface area: 3.3 m2. These HRAPs are made of fiberglass with steel blades and six blades. During operation, the blades are driven by a 1 hp. electric motor. The rotation was reduced by a reducer coupled to the motor and is controlled by a frequency inverter (WEG, CFW-08 series).

6.2.1. Hydrodynamic assessment: Bodenstein Number (Bo)

An evaluation of the Bo of the HRAPs was carried out. The methodology was adapted from NaCl tracer experiments in a stimulus-response approach [18,20,21]. The Aqua TROLL 500 Multiparameter probe was fixed in the outlet of HRAP, coupled with a Temperature-Conductivity Sensor. The continuous reading was set to record NaCl concentration data each 1 s. The HRAPs were filled with tap water in the same configurations previously described. A final 0.293 mg NaCl /L solution for the 1000 L HRAPs was used as a reference, following previous studies [18]. Thus, a concentrated 60 mg NaCl/L solution was prepared, and 5 L was added to the HRAP.

The mixing time was stipulated as the time necessary for variations in conductivity to reach <5 % of the final stable value [20]. The Bo is calculated as a function of the decrease in the output signal in relation to the input signal, with both signals, in this case, being experimentally determined by the conductivity values. To investigate the mixing characteristics inside the reactor, the HRAP was operated in a closed condition, with no inlet or outlet flow. The data from the conductivity probe measurements was processed according to the method proposed by [20]. Eq. 1, following the Solver tool from Excel®, was used to calculate the final Bo value.

$$\frac{C}{C_\infty} = \sqrt{\frac{Bo}{4\pi\theta}} \sum_{j=1}^{\infty} exp\ exp\left[-\frac{Bo}{4\theta}(j - \theta)^2\right] \quad (1)$$

Where $\frac{C}{C_\infty}$ is the dimensionless concentration and θ is the dimensionless time $\frac{t}{t_o}$.

A complementary mixing speed experiment was also conducted with a Polystyrene floater to evaluate the Reynolds Number of the pond [22] later. The methodology included conducting the test 30 times (n = 30) and noting the time it took for the floater to traverse the straight channel from the initial point (parallel to the blades). The final speed was determined as the mean value obtained from these repetitions, ensuring that the length remained consistent across all iterations. The Reynolds Number was then calculated following the equation:

$$Re = \frac{\rho\, v\, R_h}{\mu} \quad (2)$$

Where ρ (kg.m^{-3}) is the density of the fluid (algal culture), υ (m.s^{-1}) is the mixing speed in the channel, Rh (in m) is the hydraulic radius of the channel and μ (Pa.s) is the viscosity of the fluid. Also:

$$R_h = \frac{A}{P} = \frac{d\, w_c}{w_c + 2d} \quad (3)$$

Where A (in m^2) is the cross-sectional area for flow, P (in m) is the perimeter in contact with the fluid, wc (in m) is the channel width, and d (in m) is the channel depth.

6.2.2. Operation

Two continuous operations were carried out simultaneously: (i) two HRAPs operating in series, with an HRT of 5 days each (Operation 1, in series); and (ii) two HRAPs operated individually, with a hydraulic retention time (HRT) of 10 days (Operation 2, in parallel). Previous studies from Assis et al. [8] using the same rectors operated successfully with HRT = 10 days, and thus this value was chosen as the basis for the present study. Fig. 6.1 shows the experimental design.

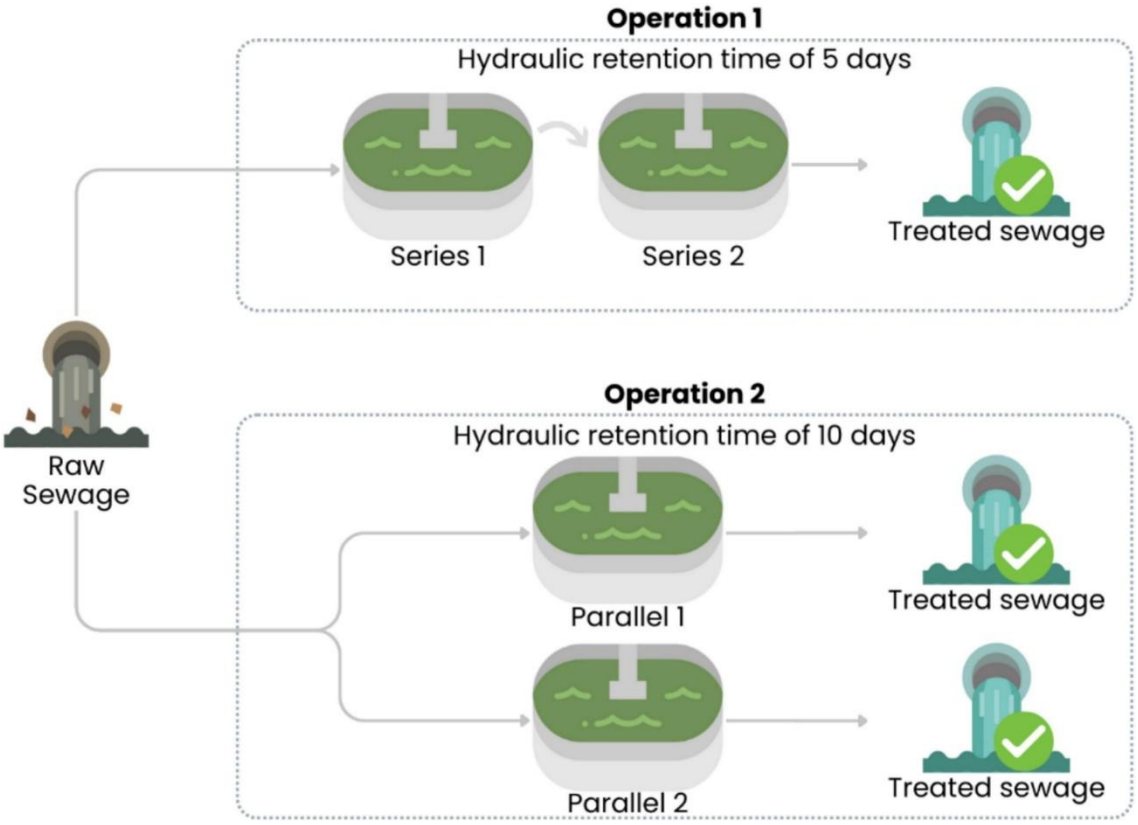

Fig. 6.1. Schematic operation.

Initially, an inoculum was produced by filling the HRAPs with a 1:1 (v/v) sewage and water blend, growing the algae bloom for 15 days. After this time, valves were opened to start the adaptation period to continuous flow (10 days), regulating the inflow of sewage. After these periods, monitoring of the ponds began to assess treatment and biomass production.

6.2.3. Monitoring

Analyses were performed weekly to monitor treatment variables and biomass production from June through September. Considering the fixed area, the HRT variation was induced by regulating the sewage input flow for each operation: series with 200 L/day and parallel with 100 L/day, per HRAP. The systems were fed continuously, and the flow was regulated daily. The HRAPs in the series were connected by an electric pump (Sarlobetter SB 1000 A), which was responsible for conducting the sewage from the first to the second HRAP.

Samples were collected once a week at the exit of each HRAP. The analysis included Total Kjeldahl Nitrogen (TKN), total ammonia nitrogen (N-NH4+), soluble phosphorus (Ps), soluble chemical oxygen demand (CODs), total soluble

organic carbon (TOCs), total and volatile suspended solids (TSS and VSS, respectively), following recommendations from the Standard Methods for the Examination of Water and Wastewater [23]. TOCs were determined using Shimadzu's TOC 5000. E. coli was measured by the chromogenic-fluorogenic method (Colilert ®). Chlorophyll-a was extracted with 80 % ethanol [24] and measured spectrophotometrically [23]; concentrations were determined following the equations provided in the Dutch standard [25].

At noon, a Hach HQ40d probe was used to measure dissolved oxygen (DO), temperature, pH, and electric conductivity of the culture medium in the HRAPs. Furthermore, the average daily temperature and photosynthetically active radiation (PAR) were monitored daily, based on data provided by INMET [26] for station A510, located in Viçosa, Minas Gerais. Data are reported in the Supplementary Material (Appendix B).

Samples to characterize the phytoplankton community were collected in two stages: from the inoculum (before the sewage was fed continuously) and at the end of the operation. Identification, organism density [27], and biovolume were analyzed for each HRAP.

At the end of the experiment, the biomass was collected. Gravitational sedimentation was carried out within the HRAPs, followed by manual harvesting. The collected samples were concentrated by centrifugation (10,000 rpm, for 8 min) and frozen. Subsequently, the frozen biomass underwent lyophilization. Then, the biomass was ground for cell disruption (TE-099, 550–3100 rpm, Tecnal) and stored for carbohydrate, lipid, protein, moisture, and ash content characterization. Carbohydrates were determined by quantitative acid hydrolysis [28], followed by the phenol-sulfuric acid method [29]. The reading was quantified using the glucose standard curve by spectrophotometry (490 nm). Lipid content was determined using the Soxhlet extraction methodology [30]. Neutral lipids were extracted from the ground biomass in the fat determinator (Tecnal TE-044-8/50) for 6 h, with 99 % hexane solvent. Then, membrane lipids were extracted with ethanol. Ash, volatile material, fixed carbon, and moisture contents were determined according to the protocol [31]. Elemental analysis was performed on the Perkin Elmer Series II 2400 Elemental Analyzer.

6.2.4. Data analysis

The results obtained were subjected to analysis of variance and the Kruskal-Wallis test with 5% probability, carried out using the PAST 4.03 software.

6.3. Results and Discussion

6.3.1. Hydrodynamics

In this study, the raceway reactor achieved a mixing velocity of 0.24 m/s, yielding a Reynolds Number (Re) of 36,599—well within the highly turbulent flow range (16,000 < Re < 180,000) reported for full-scale HRAPs [32]. This velocity also aligns with the 0.15–0.30 m/s range observed in commercial systems [33]. Such levels of turbulence can enhance nutrient diffusion by diminishing the boundary layer around cells, reduce cell sedimentation, and increase exposure to light and carbon dioxide, thus supporting algal growth [34]. However, excessive turbulence can subject cells to high shear stress, potentially damaging sensitive algal species and reducing overall productivity. Balancing mixing speeds is therefore critical to optimize turbulence benefits without risking cellular damage.

Tracer experiments (Fig. 6.2) corroborated these hydrodynamic findings, with the measured Bo number of 9.98 indicating a complete-mix flow regime (Bo < 20). Although one might anticipate near plug-flow in a raceway pond, this low Bo suggests that the system behaves more like a continuously stirred tank reactor (CSTR) [35] rather than a plug-flow reactor, which is characterized by Bo > 100 [36]. Retrofitting or modifying existing ponds is often impractical, so operating multiple units in series becomes a viable strategy for approximating plug-flow conditions even under complete-mix hydrodynamics—an approach especially relevant for pathogen inactivation and pollutant removal [37].

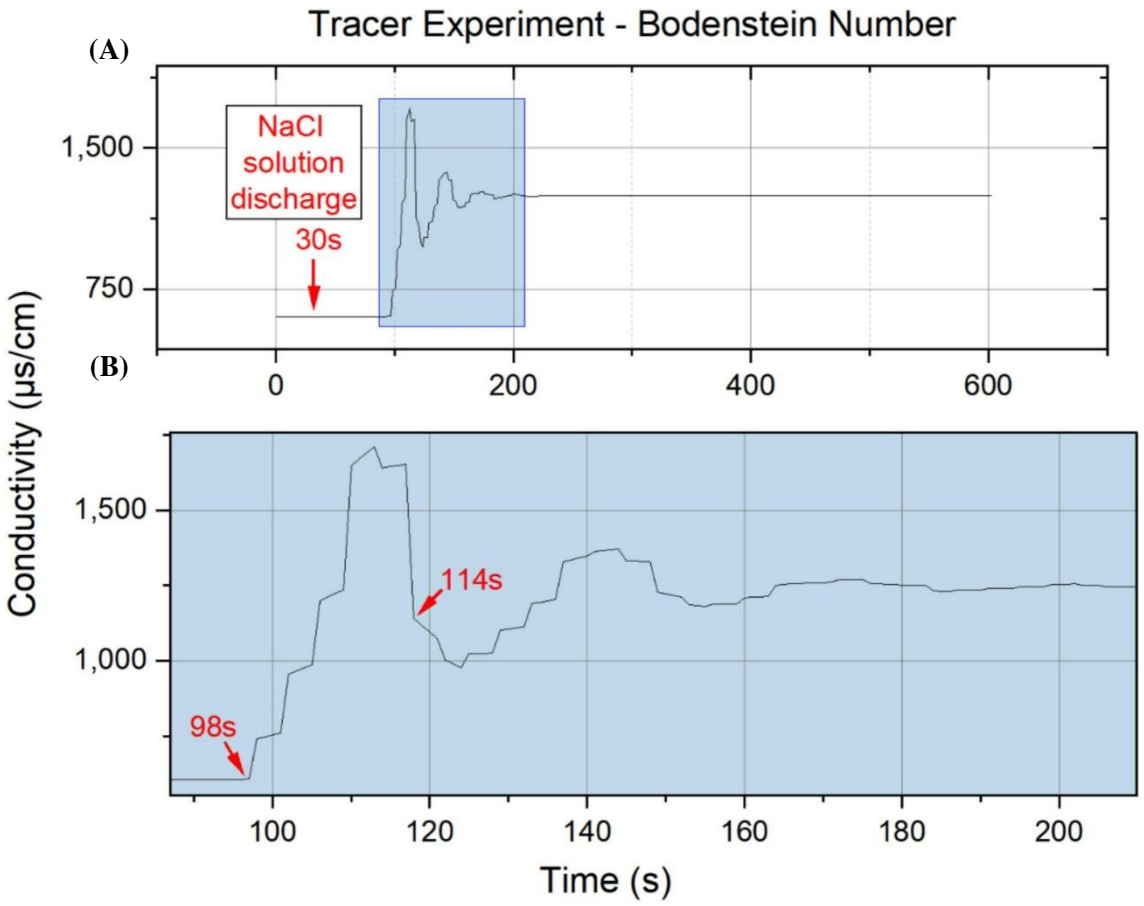

Fig. 6.2. Data collection of conductivity versus time for the HRAP operation. (A) Complete dataset and (B) zoomed data on the peak and stabilization.

Comparisons with other systems underscore the influence of geometry and operating conditions. For instance, Pham et al. [32] documented Bo values reaching 140 at higher paddlewheel speeds (16.8 rpm) and shallower depths (0.1 m) in HRAPs featuring an l/w of 4.5, ultimately achieving near plug-flow conditions (Bo > 100). In contrast, the HRAPs in the present study have the same l/w ratio of 4.5 but experience greater longitudinal dispersion under current operating conditions, which lowers the Bo value. Future work may explore increasing paddlewheel speed, altering reactor depth, or installing baffles to steer the system closer to plug-flow. Another strategy would be to add more units in series, potentially lowering the dispersion number and thus further approximating plug-flow behavior. As Von Sperling et al. [38] observes for wetlands, a perfectly mixed reactor (CSTR) corresponds to a dispersion number $\delta=\infty$ and N = 1, whereas an ideal plug-flow system has $\delta=0$ and $N = \infty$; using multiple reactors (N > 5) can

already substantially reduce dispersion. Applying this concept to HRAPs in series may yield similar benefits.

Additional insights could come from computational fluid dynamics (CFD) studies that use advanced turbulence models such as the Reynolds stress model (RSM) or the k–ω SST model [39], Such simulations can assist in designing channel configurations or baffle placements that promote more uniform flow patterns and reduce dispersion. Ultimately, combining the benefits of vigorous mixing within each unit with a series operation strategy can help these reactors achieve a more plug-flow-like hydraulic pattern overall—improving both pathogen inactivation and pollutant removal while balancing the need for sufficient turbulence to support algal growth.

6.3.2. Treatment Efficiency

Table 6.1 summarizes the influent and effluent concentrations for the HRAPs operated in series versus parallel and Table 6.2 presents the removal efficiencies for each monitored parameter. Fig. 6.3 presents the data distribution for the variables where statistically significant differences were observed. Overall, there was no statistically significant difference in nutrient removal efficiency between the series and parallel modes. Nonetheless, the systems must meet the discharge standard for residual ammonia in domestic sewage in Minas Gerais State, Brazil, which mandates levels below 20 mg/L [40]. The ammonia removal efficiencies were comparable to the 75 % reported by Assis et al. [8], who employed the same raw sewage source and a 10-day HRT. However, those results were obtained during warmer seasons, while the present study was conducted in autumn/winter. The culture media temperature (Fig. S1) hovered near the lower bound of the optimal 20–25 °C range for algal growth [41], potentially contributing to reduced removal performance.

Table 6.1. Concentration (mean ± standard deviation) of parameters in raw sewage (influent) and final effluents from each HRAP system.

Parameter	Unit	Raw Sewage (Influent)	Series 1	Series 2	Parallel	p-value (K–W)a
HRT	day	–	5	5	10	–
Inflow	L/day	–	200	200	100	–

Total coliforms	MPN/100 mL	4.69 × 105	1.57 × 105	1.04 × 104	7.46 × 104	0.01717*
E. coli	MPN/100 mL	1.96 × 105	4.82 × 104	3.28 × 103	2.05 × 104	0.01218*
CODs	mg/L	202.38 (100.88)	103.00 (34.61)	132.58 (38.53)	124.38 (44.36)	0.8206
Ps	mg/L	12.53 (4.53)	10.12 (2.45)	12.62 (3.86)	11.40 (2.03)	0.6744
Ammonia	mg/L	100.99 (45.85)	37.42 (15.71)	31.05 (8.83)	29.18 (12.30)	0.4497
TKN	mg/L	55.74 (37.20)	39.64 (13.80)	38.03 (15.91)	36.79 (11.08)	0.7573
TOC	mg/L	41.14 (12.51)	20.38 (2.56)	26.33 (5.25)	22.93 (9.23)	0.01517*
pH	–	7.47 (0.08)	6.79 (1.01)	5.85 (0.78)	7.14 (0.91)	0.01809*
DO	mg/L	0.77 (0.33)	11.82 (2.64)	10.19 (1.01)	11.89 (2.26)	0.1102
Temperature	°C	22.13 (0.57)	20.50 (3.06)	20.93 (3.12)	20.11 (3.17)	0.4428
Conductivity	µS/cm	736.00 (11.40)	613.86 (35.83)	738.14 (177.08)	657.43 (56.57)	0.4822

[a]*The p-values compare final concentrations from Series 2 vs. Parallel.*
**Statistically significant differences.*

Table 6.2. Removal efficiencies (%) of organic matter, nutrients, and pathogens in each HRAP system.

Parameter	Removal in Series 1 (1st stage)	Removal in Series 2 (2nd stage)	Overall Removal (Series)	Removal in Parallel
Total coliforms	66.59 %	93.39 %	97.79 %	84.09 %
E. coli	75.41 %	93.19 %	98.33 %	89.56 %
CODs	49.10 %	−28.71 %	34.49 %	38.54 %
Ps	19.24 %	−24.73 %	−0.73 %	9.04 %
Ammonia	62.95 %	17.00 %	69.25 %	71.11 %
TKN	28.89 %	4.07 %	31.78 %	34.00 %
TOC	50.46 %	−29.22 %	35.98 %	44.25 %

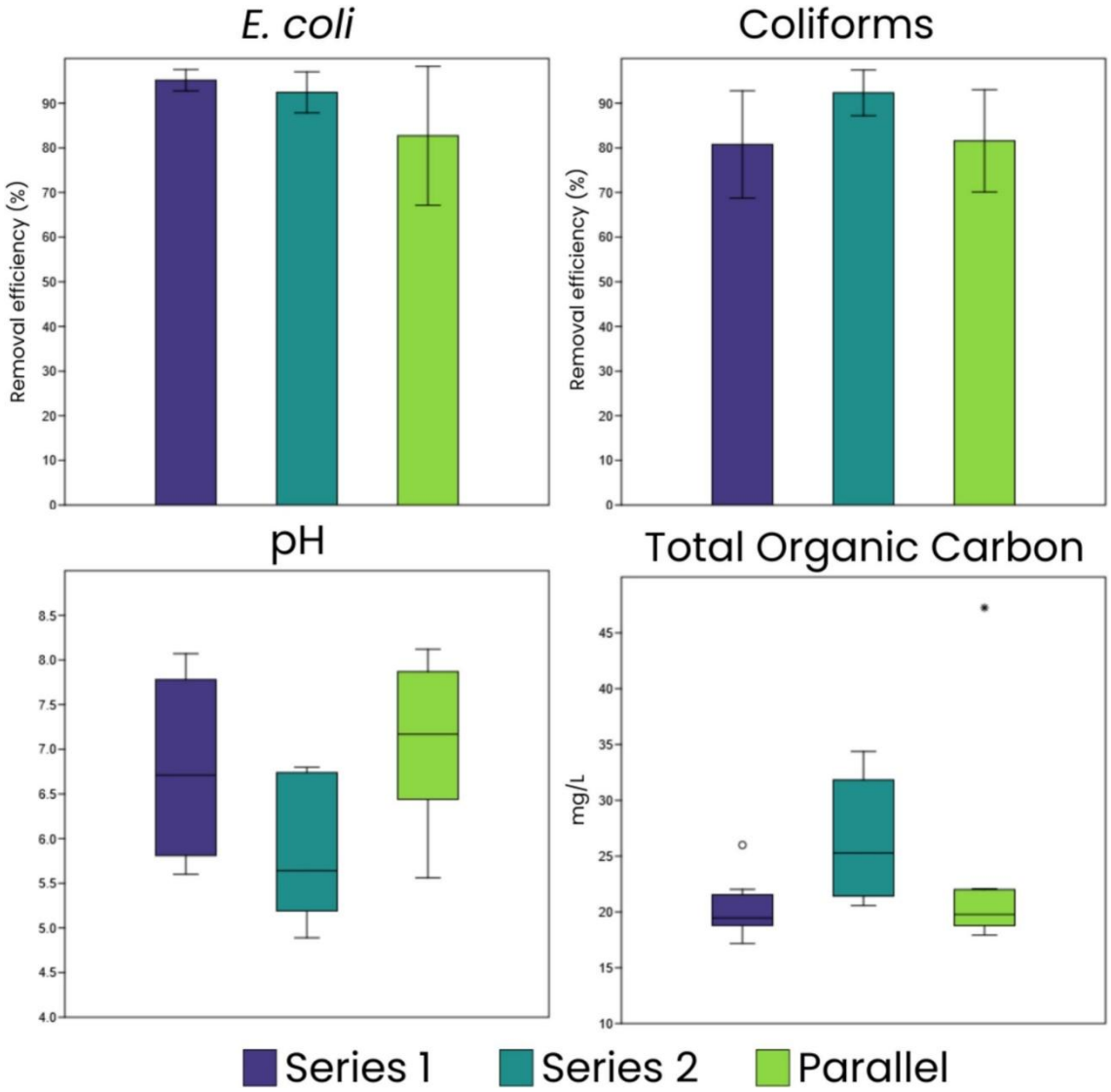

Fig. 6.3. Data distribution for pathogen removal, pH and total organic carbon for each reactor.

Importantly, no statistically significant differences were noted between the first HRAP in series (Series 1) and the parallel HRAP in terms of any monitored parameter. Hence, despite the shorter HRT, the Series 1 reactor maintained comparable treatment efficiency in continuous operation—an encouraging outcome for land-use optimization in future HRAP deployments.

Differences did arise, however, when the second HRAP in series (Series 2) was considered. A lower pH in the second series pond aligns with Sutherland et al. [7], who recorded pH values of 9.0 ± 0.8 in the first pond and 8.8 ± 0.6 in the second. Significant variations were also observed for fecal contamination indicators (coliforms and E. coli), which were higher on average in the series system than in the parallel system. Elevated dissolved oxygen (DO > 30 mg/L) and pH (> 10) have been linked to improved pathogen removal [42,43], yet in this study DO and pH levels were broadly similar between HRAP configurations. These findings are nonetheless supported by Chambonniere et al. [44], who modeled E. coli removal in pilot-scale HRAPs and concluded that first-order decay kinetics become more effective in a plug-flow-like design—precisely what a series configuration can achieve. Classic models, such as Marais [45], also highlight the enhanced disinfection in multiple reactors in series over a single well-mixed pond. Chambonniere et al. [44] further argues that large HRAPs often function as near plug-flow systems with high internal recirculation, improving first-order decay processes. Similarly, Hernández-Crespo et al. [46] demonstrated in wetlands that hydraulic design and mixing patterns play critical roles in E. coli removal via mechanisms such as solar disinfection and predation. Thus, our observation of superior pathogen removal under series operation strongly supports the notion that design approaches favoring plug-flow characteristics are essential for enhanced disinfection in HRAPs. Even if the observed improvements appear modest, as discussed earlier in Section 3.1, adding more reactors in series (N > 5) can shift the overall hydraulic regime further toward plug-flow, thereby enhancing first-order decay processes and bolstering pathogen inactivation in HRAP systems.

By contrast, TOC removal did not follow the anticipated trend; improvements in first-order decay were not observed under series operation. This discrepancy suggests that hydrodynamics alone do not fully account for the results. Instead,

the stronger pathogen removal may be linked to pH shifts upon entering the second HRAP in series. Wide pH swings are detrimental to fecal microorganisms [47], and in this study, the series system registered pH values ranging from 4.89 to 8.07, while the parallel system fluctuated between 5.56 and 8.12.

Temperature and pH are intricately connected in microalgae-based treatments [[48], [49], [50]]. Generally, higher temperatures can decrease pH; however, in open systems, this relationship is moderated by multiple factors such as solar irradiation, nutrient dynamics, algal species composition, and photosynthetic activity [50]. Here, both cultivation modes experienced media temperatures ranging from 15 °C to 25 °C (Fig. S1).

Aside from temperature, cultivation mode can also influence pH. Previous research indicates that pH control arises from operational, hydrodynamic, and biological factors, including nutrient depletion and algal metabolic responses [51,52]. Series systems typically demand more rigorous control measures to manage both microalgal productivity and pH, potentially explaining the larger pH fluctuations observed here. In contrast, the parallel configuration exhibited smaller pH variations (2.56 pH units on average).

Nutrient imbalances may also play a role in TOC accumulation. The influent N/P ratio in raw sewage was approximately 8, while it dropped to around 3 in the second-stage HRAP in the series—a ratio below the optimal range of 5–30 [53]. This nutrient stress might induce microalgae to secrete extracellular polymeric substances (EPS) [54], thereby raising COD and TOC levels (Table 1) and impairing TOC removal in the series system. Although EPS was not measured experimentally, the higher TOC in Series 2 suggests stress-induced organic exudation. Microalgal cells under stressful conditions—including nutrient depletion, suboptimal pH, and light limitations—can produce reactive oxygen species (ROS) [55]. To mitigate ROS damage, these cells may release antioxidant and other organic substances into the surrounding medium [56]. Recirculation inherent in a series setup may further concentrate organic matter (e.g., algal exudates, debris), thus elevating TOC in subsequent ponds [57]. While both recirculation and stress-induced extracellular release are plausible causes, the pronounced TOC differences strongly implicate microalgal stress as a key contributor. Future investigations could measure EPS and ROS production

to more definitively link these mechanisms to TOC accumulation in HRAP systems.

6.3.3. Biomass Production and Characterization

Table 6.3 presents the average biomass production (total and volatile solids, chlorophyll-a) and characterization (primary metabolites composition, proximate and ultimate analysis) obtained in each HRAP at the end of the operation period (see Table 6.3).

Table 6.3. Biomass production and characterization (mean values with standard deviation between parenthesis).

Parameter		Series 1	Series 2	Parallel
Chlorophyll-*a*	**Value (mg/L)**	1.36 (0.54)	1.51 (0.61)	1.85 (0.54)
	Productivity (g/m².day)	0.08 (0.03)	0.05 (0.02)	0.06 (0.02)
	Production (g/m²)	0.41 (0.16)	0.23 (0.09)	0.56 (0.16)
Volatile Suspended Solids	**Value (mg/L)**	126.51 (47.51)	197.67 (54.56)	201 (83.29)
	Productivity (g/m².day)	7.6 (2.85)	5.94 (1.64)	6.04 (2.5)
	Production (g/m²)	40.62 (12.31)	31.51 (6.14)	65.71 (19.54)
Total Suspended Solids	**Value (mg/L)**	156.86 (61.97)	255.09 (75.23)	229.26 (101.89)
	Productivity (g/m².day)	9.42 (3.72)	15.32 (4.52)	6.88 (3.06)
	Production (g/m²)	49.88 (17.4)	82.11 (15.26)	74.9 (25.32)
Characterization	**Lipids (%)**	14.62	14.38	17.84
	Carbohydrates (%)	9.56	6.38	11.46
	Proteins (%)	14.15	4.62	19.52
Proximate Analysis	**Volatile material (%)**	54.67	45.94	63.23
	Fixed carbon (%)	10.35	3.45	11.85
	Ash (%)	26.37	43.45	15.23
	Moisture (%)	8.58	7.04	10.61
Ultimate Analysis	**C (%)**	36.11	26.16	40.46
	H (%)	7.41	5.75	8.02
	N (%)	5.77	2.92	6.66

From the results in Table 3, it is important to highlight that no statistically significant difference was observed in biomass production between the systems (VSS, TSS, and Chlorophyll-a). The values achieved for the Parallel system (HRT = 10) were similar to those reported by Assis et al. [8] using sewage from a septic

tank as HRAP culture media, reporting 0.55 g/m2 for chlorophyll-a production and 36.75 g/m2 for VSS.

Also, de Godos et al. [58] report biomass productivities of 7.5 ± 1.9 g/m^2/d for the winter season in HRAPs, which was lower than the 23.9 ± 4.1 g/m^2/d achieved for summer operation. Thus, seasonal performance most likely limits biomass production, and increased biomass production is expected in warmer seasons. The study, however, was performed in Spain, which exhibits accentuated temperature differences between seasons. Thus, it is important to highlight that in the case of the Brazilian tropical climate, the improvements may not be that high.

Differently from the results by Sutherland et al. [7], no increase in biomass production was observed for the series operation. One of the main differences from the present study was the direct communication between the HRAPs in series. The study referenced used an Advanced Pond System in which the outlet from the first HRAP was directed to harvesting and then maturation ponds. Only then was the media redirected to the second HRAP in the series. When discussing biomass recirculation as an operation strategy, Sutherland and Ralph [59] observed that near-complete biomass harvesting is required for biomass recirculation in HRAPs, with the risk of lower light availability reducing biomass production. This is one of the potential causes for the production results found here. Harvesting steps between the series should be further investigated by future studies, with added potential gains in pathogen removal by favoring the sedimentation removal mechanism [43].

Moreover, higher productivity can also be achieved when considering CO_2 supplementation in the media. Assis et al. [60] reached 6.12 g/m^2/day when supplying the HRAP with CO_2 from the combustion of gasoline. Still, when aligned with the results from treatment efficiency, the pH levels observed that are close to neutral do not indicate a high carbon deficiency from the sewage used. As previously discussed, factors beyond nutrient imbalance, such as temperature, might have played a limiting role in algae growth. Furthermore, the photosynthetic activity failed to outpace the respiration rate, resulting in CO_2 production.

Thus, this strategy should be further studied to determine the effects of different roles of CO_2 supplementation on the first and second stages of the series.

The parallel operation generally achieved an enhanced biochemical biomass composition compared to the series (higher lipids, carbohydrates, and proteins). Nevertheless, a usual range of 30–50 % ash content in the collected biomass is expected when sewage is used as the cultivation media, which can be attributed to the sand present in the sewage itself [61]. Nevertheless, the increase in ash content from Series 1 (26.37 %) to Series 2 (43.45 %) should be highlighted. As previously discussed, the nutrient imbalance observed for Series 2 could explain the biomass composition, with a higher ash percentage than Series 1 or Parallel. The effects of cell aging and nutrient stress in that pond could have led to cell senescence. Extended retention times or reduced loading rates lead to vigorous cell multiplication initially, but further extending the retention time can result in increased cell enlargement and a decrease in multiplication, ultimately leading to cell senescence and death [62]. Thus, nutrient balance and senescence could be the reason for the increased ash content in Series 2. Future studies could benefit from analyzing direct cell lysis and microscopic observation to validate this finding. Moreover, quantification of EPS during the operation should also be included during monitoring in future experiments addressing the impacts of series operation. Nevertheless, potential recovery routes for high-ash biomass can include soil application as a mineral supplier of biofertilizer [63,64].

Also, as can be seen from the ultimate analysis, a higher C content was achieved in the Parallel operation (40.46 %), which is in line with the higher TOC removal results for the system previously presented in Table 6.1, Table 6.2.

Following, Fig. 6.4, Fig. 6.5 present the phytoplankton community characterization of the collected biomass. Using outdoor open systems such as HRAPs can result in differences between the original inoculum and the final biomass. In this study, Chlorella vulgaris was the predominant microalga, consistent with other research employing HRAPs to treat septic tank sewage [8,60]. However, a slight divergence in relative abundance was observed, with the species Botryococcus terribilis and Monoraphidium tortile appearing mainly in the Parallel system. Beyond the previously noted nutrient composition, the HRT may have also influenced the phytoplankton community, as balanced nutrient conditions and longer retention times could foster greater species diversity. The neutral lipid content was 7.61 % for Series 1, 8.61 % for Series 2, and 9.89 % for

the Parallel system, presumably due to the higher abundance of Botryococcus terribilis—a species known for elevated oil productivity but slower growth [65]. While this work centered on the eukaryotic component of the community, the potential role of prokaryotic populations in shaping overall biomass composition should not be overlooked. Future studies that include assessments of prokaryotic microbial abundance, along with the phytoplankton community addressed here, could further elucidate how series operation influences bacteria–microalgae consortia, expanding on the scope of the present research.

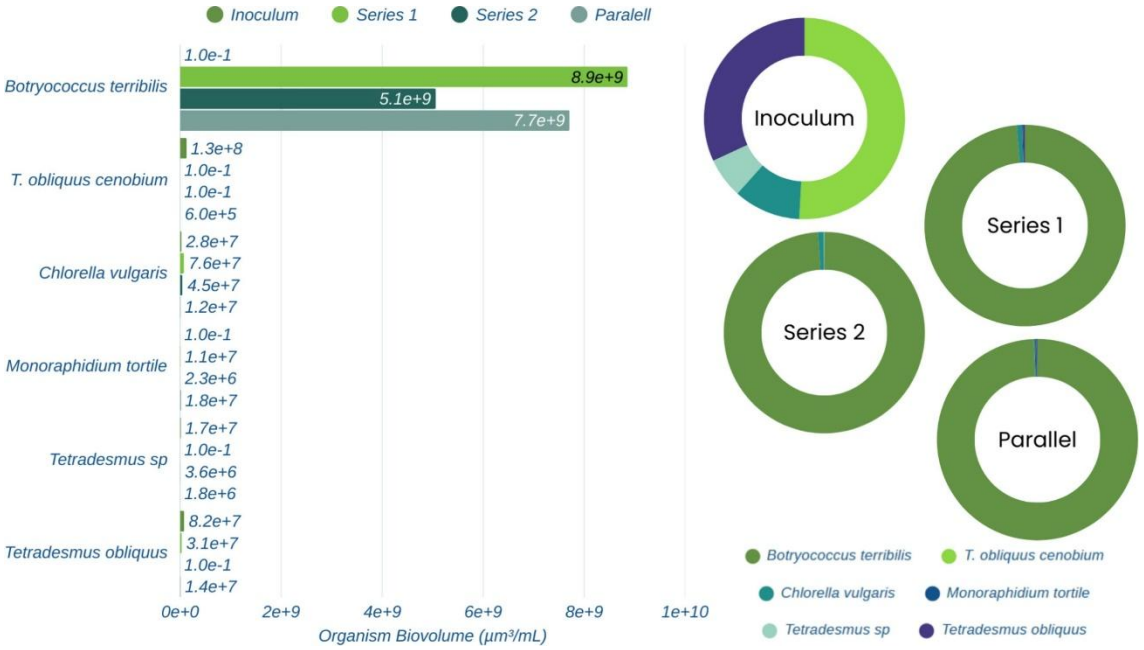

Fig. 6.4. Phytoplankton community: biovolume (µm3/mL) and relative abundance (%).

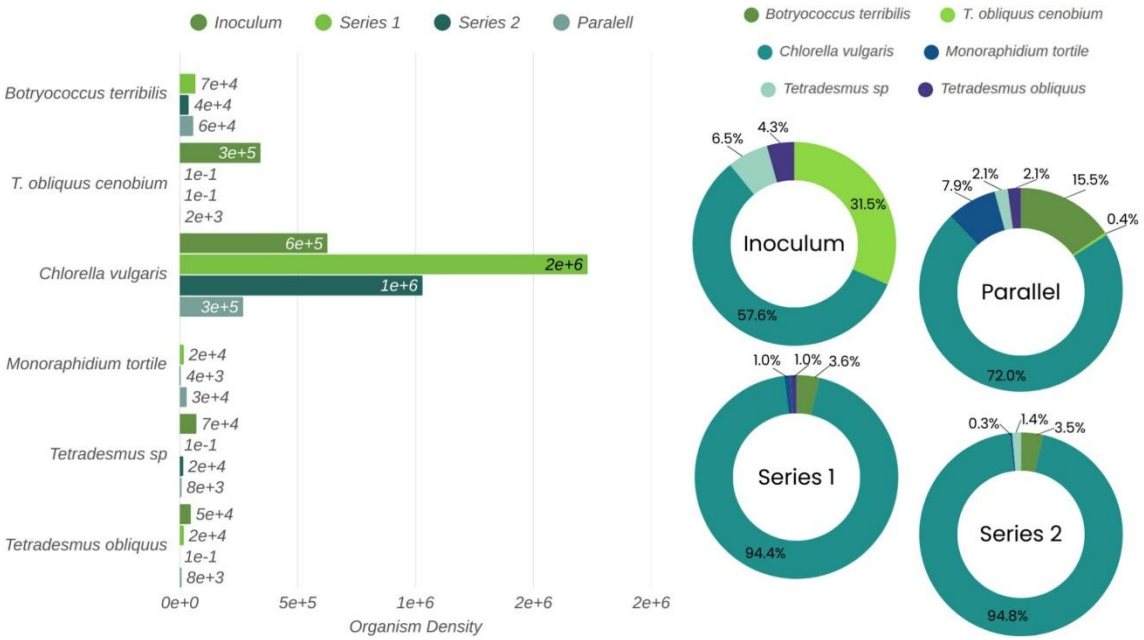

Fig. 6.5. Phytoplankton community: Density of organisms (Org/mL) and relative abundance (%).

6.4. Conclusion

This study investigated HRAP performance in series and parallel configurations, revealing complete mixing (Bodenstein = 9.7) and turbulence (Re = 36,399). Series operation reached higher coliform and E. coli removal (97.79 % and 98.33 %, respectively) than the parallel configuration (84.09 % and 89.56 %), while the parallel setup showed higher total organic carbon removal. No significant differences were observed in biomass production, though the second pond in series presented increased ash content, likely due to nutrient balance and cell senescence. Future research could employ computational models to optimize pond design, conduct extended hydrodynamic evaluations in open conditions, examine settling and biomass harvesting steps in series ponds, and investigate larger-scale operations with multiple ponds in series. Also, additional analysis on prokaryotic microbial abundance, cell lysis, and extracellular polymeric substances could present essential observations to the mechanisms in series operation. These strategies could expand understanding of hydrodynamics and nutrient balance in HRAPs, reinforcing their potential as flexible, sustainable systems for sewage treatment.

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7. Chapter 3: Effects of Organic Loading Rate

Effects of organic loading rate in the performance of high-rate algal ponds: bioremediation potential, implications to design and operation[3]

Abstract

Microalgae play a fundamental role in biotechnology and biomass production, and the organic loading rate (OLR, kg BOD ha^{-1} d^{-1}) is a key parameter in sizing high-rate algal ponds (HRAPs) for domestic wastewater treatment. This study investigated the impact of OLR on the performance of HRAPs fed with domestic wastewater pretreated in a septic tank. Three pilot-scale HRAPs were operated in parallel at influent flow rates designed to achieve OLRs of 64.96, 48.72, and 32.48 kg BOD ha^{-1} d^{-1}. Despite these differences, no statistically significant variations in treatment performance were observed among the systems. Across all HRAPs, biochemical oxygen demand (BOD) removal ranged from 3% to 36%, and ammonia removal was 75%–80%, primarily attributed to nitrification rather than complete nitrogen removal. Phosphorus removal ranged from 8% to 15%. Biomass production (131–185 mg VSS L^{-1}) and composition were not significantly affected by OLR variations, although differences in lipid (14.62%–17.84%) and protein (14.15%–21.59%) contents were noted. These findings suggest that HRAPs can provide moderate treatment and valuable biomass under varying OLR conditions. However, further studies under higher and more representative wastewater loads are recommended to better elucidate the role of OLR in HRAP design and operational strategies.

Keywords: Microalgae biomass; wastewater treatment; raceways; operational strategies

[3] Attachment 3: Published as an expanded abstact
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7.1. Introduction

Increasing urbanization and population growth pose significant challenges to conventional domestic wastewater management (Gouveia et al., 2016). Microalgae-based treatment processes have garnered attention due to their capacity to assimilate nutrients and pollutants while generating valuable biomass (Leong & Chang, 2022). Among these processes, high-rate algal ponds (HRAPs) are widely implemented at full scale, offering a cost-effective and environmentally sustainable approach for domestic wastewater treatment (Sutherland & Ralph, 2020).

The performance of HRAPs is strongly influenced by organic loading, often measured as biochemical oxygen demand (BOD) (Alemu et al., 2018). Understanding the impact of organic loading rate (OLR, kg BOD ha^{-1} d^{-1}) on both nutrient removal and biomass production is crucial for optimizing HRAP design and ensuring reliable treatment performance (Santiago, 2013). In conventional facultative ponds, OLR is a key design parameter (von Sperling, 2002). For HRAPs, typical operational loads range between 100 and 150 kg BOD ha^{-1} d^{-1}, contingent on local climatic conditions (Craggs et al., 2014). However, the determination of suitable removal coefficients—especially under varied operational and environmental conditions—remains a persistent challenge.

In response, the present chapter aimed to assess the influence of OLR on HRAP performance by analyzing three parallel pilot-scale systems treating pre-settled domestic wastewater under different hydraulic retention times (HRTs). Specifically, this work (i) investigates the impact of varying OLR on nutrient and organic matter removal efficiencies, (ii) calculates BOD removal coefficients (K_{BOD}), and (iii) characterizes the produced biomass for its potential valorization. The results provide insights that can refine HRAP modeling, ultimately guiding more precise design and optimization strategies for full-scale deployment.

7.2. Material and Methods

Experiments were conducted between July 2022 and February 2023 at the Laboratory of Sanitary and Environmental Engineering (LESA) in the Civil

Engineering Department of the Federal University of Viçosa (UFV), Brazil (Figure 7.1A). Domestic wastewater, pretreated in a septic tank, was collected weekly from the Romão dos Reis Wastewater Treatment Plant in Viçosa, Minas Gerais, operated by the local water and sewage utility (SAAE–Viçosa). This effluent served as the culture medium for the three pilot-scale HRAPs (Figure 7.1B).

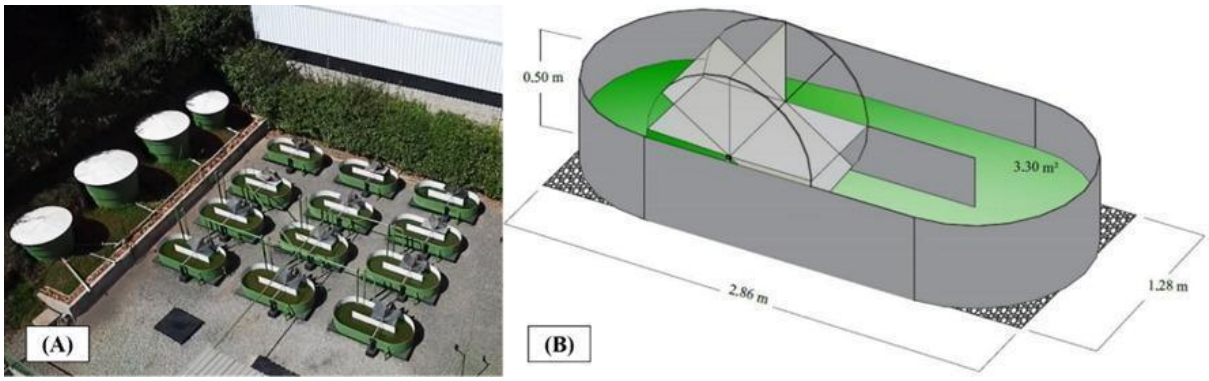


Figure 7.1. Experimental area: (A) Aerial view of the high-rate ponds (HRPs); (B) Schematic diagram of the ponds used

Each fiberglass HRAP had a capacity of 1,000 L, measuring 1.28 m in width, 2.86 m in length, and 0.5 m in total depth, with a working depth of 0.3 m. The resulting surface area was 3.3 m² for each pond. Six-blade steel paddles provided mixing, driven by a motor (connected to a gear reducer) and a frequency inverter (WEG, CFW-08 series). The paddle-wheel velocity was maintained at 0.10–0.15 m s^{-1}.

7.2.1. Operation

Three continuous operations were conducted in parallel. Although the pond surface area remained fixed, the OLR was varied by adjusting the influent flow rate. Consequently, each HRAP was operated under a different HRT:

- HRAP 1: 200 L d^{-1} (HRT = 5 days)
- HRAP 2: 150 L d^{-1} (HRT = 6.7 days)
- HRAP 3: 100 L d^{-1} (HRT = 10 days)

The resulting organic loading rates, calculated from weekly influent BOD measurements, were 64.96, 48.72, and 32.48 kg BOD ha^{-1} d^{-1}, respectively (Figure 7.2). Initially, each HRAP was inoculated in batch mode for 15 days by

mixing wastewater and water at a 1:1 (v/v) ratio, with continuous paddle mixing. After this acclimation period, the system was switched to continuous flow by opening the valves and regulating the influent.

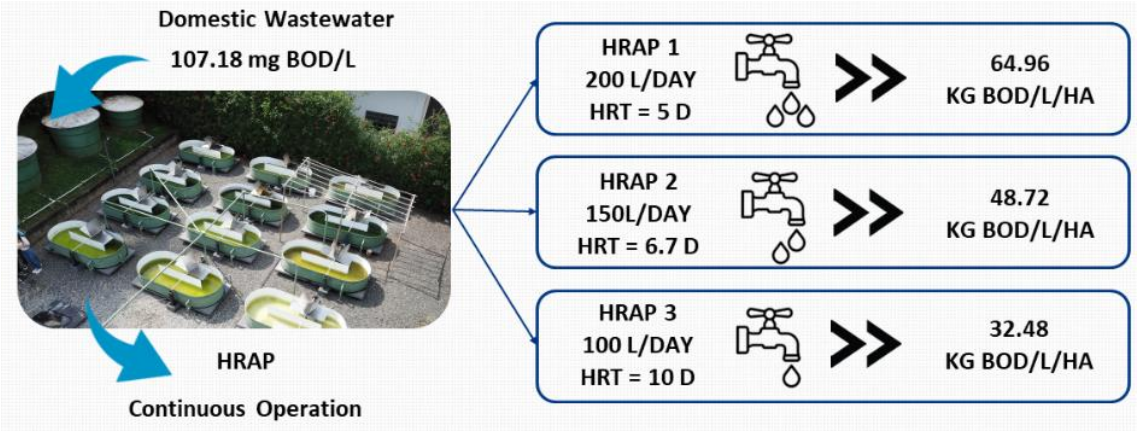


Figure 7.2. Influent BOD, loading rates and Schematic of the continuous operation setup.

7.2.2. Monitoring

Influent flow rates were monitored daily to maintain the target loading rates. Weekly effluent samples from each HRAP were analyzed according to Standard Methods (APHA, 2012) for the following parameters: ammonia nitrogen ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), soluble phosphorus (Ps), soluble chemical oxygen demand (CODs), soluble total organic carbon (TOCs), total suspended solids (TSS), and volatile suspended solids (VSS). TOCs was measured using a Shimadzu TOC 5000 analyzer. Chlorophyll-a (Chl-a) was extracted with 80% ethanol (Nusch, 1980) and quantified by spectrophotometry (APHA, 2012), following Dutch-standard equations (NEN, 1981).

Daily measurements of dissolved oxygen (DO), temperature, pH, and electrical conductivity were taken at midday using a Hach HQ40d probe equipped with luminescent dissolved oxygen (LDO) sensors. Local climatological data, specifically photosynthetically active radiation (PAR) and ambient temperature, were obtained from INMET (2022) for Station A5010 in Viçosa (Appendix C).

Phytoplankton community characterization occurred twice: once before continuous feeding (inoculum stage) and once at the end of each operational period. Species identification, density, and biovolume were determined following Utermöhl (1958). The harvested microalgal biomass underwent gravity

sedimentation within each HRAP, followed by manual collection and concentration via centrifugation (10,000 rpm for 8 min). The concentrated biomass was frozen, lyophilized, ground (TE-099, 550–3100 rpm, Tecnal), and stored for subsequent biochemical characterization. Carbohydrates were analyzed by quantitative acid hydrolysis (Hoebler et al., 1989) and quantified using the phenol–sulfuric acid method (DuBois et al., 1956). Lipid content was determined by Soxhlet extraction (AOAC, 2000), using 99% hexane followed by ethanol extraction for membrane lipids. Ash, volatile matter, fixed carbon, and moisture were measured based on ASTM (2018). Elemental composition (C, H, N) was quantified using a Perkin Elmer Series II 2400 Elemental Analyzer.

7.2.3. Data Analysis

All results were tested for normality, followed by a Kruskal–Wallis test at the 5% probability level using PAST 4.03 software.

BOD removal coefficients (K_{BOD}) were also calculated for each treatment, based on the BOD data and the models proposed by von Sperling (2015). Specifically, the complete-mix model (Equation 1) was used:

(1)

Where S is the BOD concentration (mg/L) at a given time, S_0 is the influent BOD concentration (mg/L), t is the operational time (days), and k is the BOD removal coefficient (d^{-1}). This model was chosen based on the results of a hydrodynamic test that determined the Bodenstein number (Bo) for the HRAPs using a tracer experiment, following the methodology of Pham et al. (2018). The Bo calculated for the reactor, under the current depth and mixing configuration, was 9.98. Since Bo < 20, the reactor exhibited complete mixing behavior.

7.3. Results

7.3.1. Treatment efficiencies and biomass production

Table 7.1 shows the mean and standard deviation of the treatment parameters monitored during the experiment for each system.

Table 7.1. Treatment efficiencies, biomass production and organic matter removal coefficient for each pond (average values and standard deviation between parenthesis).

Variable	Unit	DW	HRAP 1	Remov.	HRAP 2	Remov.	HRAP 3	Remov.	p*
Load	kg BOD/day /ha	-	64.96	-	48.72	-	32.48	-	X
BOD	mg/L	107.18 (50.45)	68.6 (21.13)	35.99%	80.24 (25.9)	25.13%	103.67 (34.69)	3.28%	0.1492
CODs	mg/L	164.75 (81.44)	129.5 (91.88)	21.40%	119.43 (31.44)	27.51%	98.92 (31.36)	12.72%	0.2195
pH	-	7.63 (0.22)	6.13 (1.19)	X	6.72 (1.24)	X	6.48 (1.09)	X	0.5738
DO	mg/L	0.79 (0.36)	10.66 (2.49)	X	10.61 (2.35)	X	10.61 (2.14)	X	0.9475
Temp.	ºC	23.65 (1.77)	23.08 (2.11)	X	23.38 (2.64)	X	22.78 (2.26)	X	X
$N\text{-}NH_4^+$	mg/L	96.12 (57.96)	23.23 (12.21)	75.83%	12.4 (11.02)	87.10%	19.03 (8.07)	80.20%	0.1339
$N\text{-}NO_3^-$	mg/L	11.38 (9.55)	43.56 (24.63)	-282.81%	60.91 (58.53)	-435.33%	58.29 (53.71)	-412.29%	0.9341
Ps	mg/L	10.51 (4.22)	8.88 (2.88)	15.46%	8.13 (3.01)	22.68%	9.62 (3.18)	8.47%	0.357
TOC	mg/L	34.89 (13.47)	36.73 (12.34)	39.77%	23.3 (8.6)	22.66%	20.49 (3.94)	36.45%	0.735
Chl-a	mg/L	-	1.35 (0.73)	X	1.75 (0.87)	X	1.61 (0.94)	X	0.6352
VSS	mg/L	-	100.75 (50.3)	X	157.54 (112.48)	X	119.57 (69.57)	X	0.7254
K_{BOD}	day^{-1}	-	0.147	X	0.049	X	0.024	X	0,4452

**p: p-value for the Kruskal-Wallis test.*

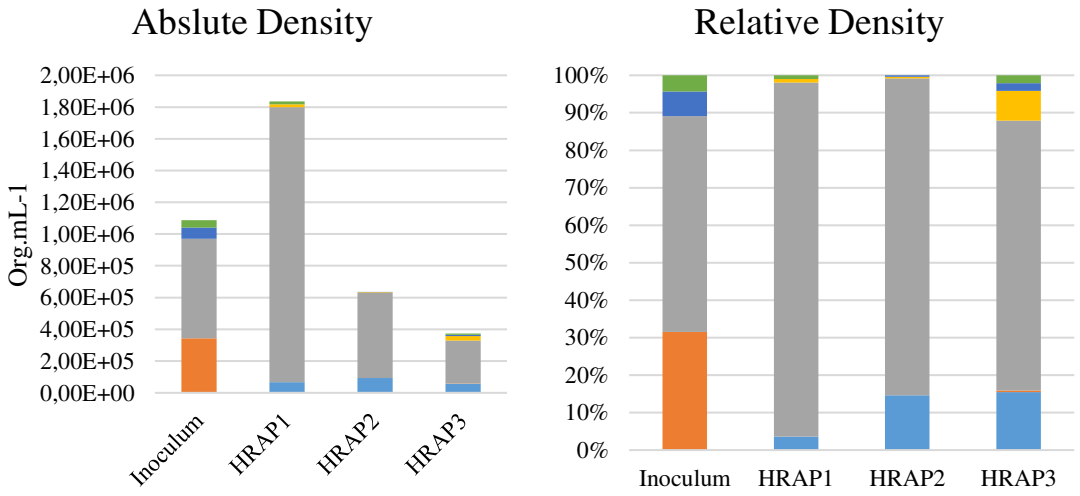
7.3.2. Biomass Characterization

Table 7.2 shows the biochemical characterization of the biomass produced in each system.

Table 7.2. Biochemical composition of the biomass produced under different organic loading rates.

Composition		HRAP 1	HRAP 2	HRAP 3
Characterization	Lipids (%)	14,62	X	17,84
	Carbohydrates (%)	9,56	11,79	11,46
	Proteins (%)	14,15	21,59	19,52
Proximate Analysis	Volatile Matter (%)	54,67	63,82	63,23
	Fixed Carbon (%)	10,35	13,56	11,85
	Ashes (%)	26,37	12,53	15,23
	Moisture (%)	8,58	10,69	10,61
Ultimate Analysis	C (%)	36,11	44,31	40,46
	H (%)	7,41	8,85	8,02
	N (%)	5,77	7,09	6,66

Following, Figure 7.3 shows the results of the phytoplankton community characterization for each operational configuration.



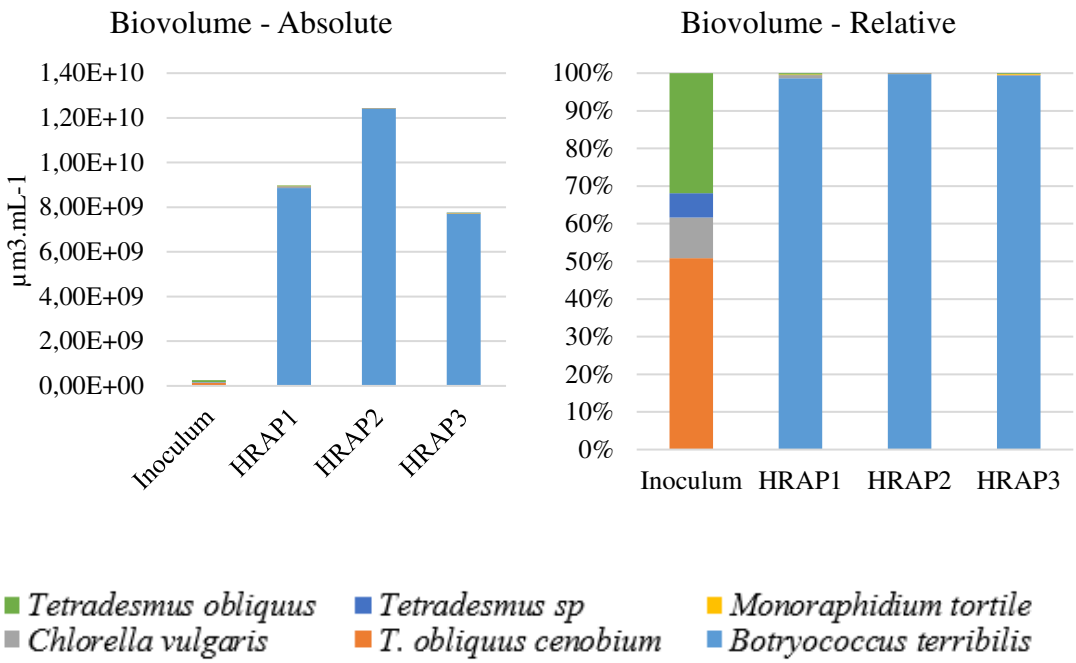


Figure 7.3. Phytoplankton community characterization for each operational configuration.

7.4. Discussion

No significant difference in treatment performance across different flow rates was found, indicating a lack of distinctive treatments. The average BOD removal efficiencies were 35.99%, 25.13%, and 3.28% for HRAPs 1, 2, and 3, respectively. Comparable results were observed by El Hafiane and Hamouri (2005) operating HRAPs with effluent from UASB with and without gravel filters. They applied a rate of 52 kg BOD/day/ha, achieving 22% BOD removal. Inlet BOD concentration was low at 45 mg/L after the gravel filter.

Despite stable pH (6.1–6.7) that minimized ammonia volatilization, ammonia removal reached 75%–80%, driven largely by nitrification (as evidenced by increases in nitrate). Nitrification was incomplete, however, leading to effluent ammonia concentrations above discharge limits (20 mg $N–NH_4^+$ L^{-1}) in some instances (Minas Gerais, 2018). Phosphorus removal (8%–15%) remained modest, consistent with expectations for HRAPs under short HRT and low influent concentrations.

Biomass production ranged from 131-185 mg VSS/L, with chl-a productivity averaging 0.05-0.08 g/m²/day, statistically equal among treatments. Assis et al. (2017) achieved similar chl-a productivity (0.05 g/m²/day) with septic tank sewage. However, the total biomass productivity (3.68 g/m²/day) was lower than the range of the present study (4.44-5.67 g/m²/day).

The primary domestic sewage used as a culture medium had a BOD concentration averaging 107.18 ± 50.45 mg/L, lower than typical values of 250-400 mg/L (Von Sperling, 2016), contributing to lower loading rates. Alemu et al. (2018) operated at higher rates but with higher influent BOD concentrations in the wastewater. The reduced BOD concentration may be due to dilution by rainwater, possibly due to deficiencies in the sewage collection network. Precipitation data support this hypothesis (Appendix C), which poses a challenge for treatment and biomass production in outdoor systems.

Regarding phytoplankton community composition, significant differences were also not observed, with *Chlorella vulgaris* predominating, albeit at slightly different relative densities (3-15%). The k_{BOD} values for each system were 0.147 day^{-1} for HRAP 1, 0.049 day^{-1} for HRAP 2, and 0.024 day^{-1} for HRAP 3, with no statistically significant difference (p-value = 0.4452). While further studies operating at higher organic loads are necessary to compare performance, it can be concluded that HRAPs can effectively serve as tertiary treatment units, focusing on nutrient recovery to improve final effluent quality.

7.5. Conclusions

This study demonstrates that HRAP performance was not significantly affected by variations in organic loading rates in the low range (32.48–64.96 kg BOD ha^{-1} d^{-1}) encountered here. Under these conditions, ammonia removal was driven primarily by nitrification, while phosphorus removal remained moderate. Although lipid and protein contents in the harvested biomass showed slight trends with OLR, differences were not statistically significant. For future research, investigating higher organic loadings—potentially via stronger wastewaters or blended influent streams—would provide a more comprehensive assessment of HRAP capabilities as a secondary or tertiary treatment process. Such studies could refine the estimation of BOD removal coefficients under more

representative domestic wastewater conditions, thereby enhancing design and operational guidelines for large-scale HRAP implementation.

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8. Chapter 4: Light complement strategy

Outdoor microalgae cultivation with LED light enhancement: wastewater treatment, biomass yield and potential valorization routes

Abstract

This study evaluated the effect of LED supplementation on microalgae-based wastewater treatment in outdoor bubble column photobioreactors, focusing on effluent quality, biomass production, and bioproduct recovery. Three lighting regimes were examined: natural sunlight (control), 12 h LED, and 24 h LED. Although no statistically significant differences emerged among treatments for soluble carbon oxygen demand, ammonium, total nitrogen, or phosphate removal, LED supplementation significantly increased biomass production. Volatile suspended solids (VSS) rose from 340 mg L^{-1} (control) to 523 mg L^{-1} (12 h LED) and 555 mg L^{-1} (24 h LED). Flow cytometry also revealed higher cell density and esterase activity under continuous illumination. Biochemical composition shifted with extended LED lighting, notably reducing carbohydrate and ash contents under 24 h LEDs, while fatty acid profiles remained dominated by palmitic acid (C16:0). Overall, LED supplementation effectively enhanced biomass productivity, offering promising pathways for sustainable resource recovery and value-added bioproducts.

Keywords: Photoperiod; pigments; fatty acids; bubble column reactors; flow cytometry.

8.1. Introduction

Microalgae-based biotechnology for wastewater treatment offers a dual benefit of pollutant removal and value-added biomass production, aligning with sustainable development goals (Fernández et al., 2021). In these systems, microalgae can form a synergistic relationship with aerobic bacteria, exchanging O_2 and CO_2 to facilitate both wastewater bioremediation and cellular growth (González-Fernández et al., 2016). However, maintaining high biomass

productivity under real wastewater conditions, although cheaper, can be more challenging than in synthetic media due to variable nutrient composition and potential inhibitory factors (Magalhães et al., 2024).

Light availability is crucial for microalgal growth, as it drives photosynthesis through parameters such as intensity, photoperiod, and spectrum (Iasimone et al., 2018; Peng et al., 2025). Operational strategies that optimize these parameters can cost-effectively boost biomass production and nutrient uptake (Sutherland et al., 2015). In particular, LEDs provide a flexible lighting option, enabling control over light intensity (González-Camejo et al., 2019), spectrum (Ievina and Romagnoli, 2025), and photoperiod (Leong et al., 2022). At the same time, this technology continues to improve in efficiency, durability, and cost-effectiveness (Singh and Mishra, 2023). Although free and abundant, natural sunlight can be inconsistent due to seasonal and diurnal variations (Blanken et al., 2013; Ramanna et al., 2017), whereas artificial illumination—especially using LEDs—offers precise control but at higher operating costs (Singh and Mishra, 2023). Combining natural and artificial light sources may mitigate these trade-offs, as demonstrated in outdoor systems supplemented by LEDs to extend the photoperiod and increase productivity (Carneiro et al., 2022; González-Camejo et al., 2019). For instance, González-Camejo et al. (2019) reported maximum nitrogen (7.7 ± 1.6 mg $N \cdot L^{-1} \cdot d^{-1}$) and phosphorus ($1.03 \pm 0.21$ mg $P \cdot L^{-1} \cdot d^{-1}$) recovery, along with 100 ± 32 mg $VSS \cdot L^{-1} \cdot d^{-1}$ of biomass under continuous artificial illumination in an outdoor photobioreactor. Balbuena-Ortega et al. (2024) further showed that smaller-diameter tubular reactors with green light filters maximized chlorophyll production, while blue filters enhanced pigment accumulation in larger reactors—though these exhibited lower growth rates overall.

Despite these advances, detailed investigations on optimizing LED photoperiods and intensities—particularly under real domestic wastewater conditions—remain limited. Therefore, the present work examines the effects of LED supplementation on microalgae-based treatment of domestic wastewater in closed, vertical tubular outdoor reactors. By analyzing wastewater quality, biomass production, and bioproduct accumulation, we aim to address a key knowledge gap in integrating natural and artificial lighting for sustainable

wastewater management and the valorization of algal biomass. In a novel approach for this cultivation strategy, flow cytometry data are analyzed along with treatment and biomass parameters to understand the effects of light supplement in outdoor reactors.

8.2. Material and Methods

8.2.1. Experimental Setup

The experiments were carried out at the Lumiar Campus of the National Laboratory of Energy and Geology (LNEG) in Lisbon, Portugal, between April and June 2024 (spring season). Secondary domestic wastewater (post activated sludge) from the Quinta do Conde Wastewater Treatment Plant (WWTP) was used as the culture medium.

For inoculum preparation, wastewater was transferred to an Erlenmeyer flask and kept under agitation with illumination for 15 days. Microscopic observation shows that *Chlorella vulgaris* and *Tetradesmus obliquus* were the predominant species. This inoculum was then transferred to a closed reactor before being distributed among the experimental reactors to initiate operation.

Outdoor experiments were conducted in closed vertical tubular photobioreactors with a total operating capacity of 50 L. The reactors had a diameter of 40 cm and were equipped with a lid at the top, with a full height of 2m. Aeration was provided by an air compressor, which supplied air at the bottom of the reactors, ensuring their characterization as bubble column reactors.

Supplemental light for dark periods was provided by waterproof LED strips (LIVARNO HG04149, 22.5W, warm-white) attached to the reactors (Livarno, 2021), as shown in Figure 8.1. Two lighting periods—12 h and 24 h—were evaluated and compared to a control reactor receiving only natural light at ambient temperature. In the 12 h LED reactor, lights were automatically programmed to switch on at 19:00 and off at 07:00.

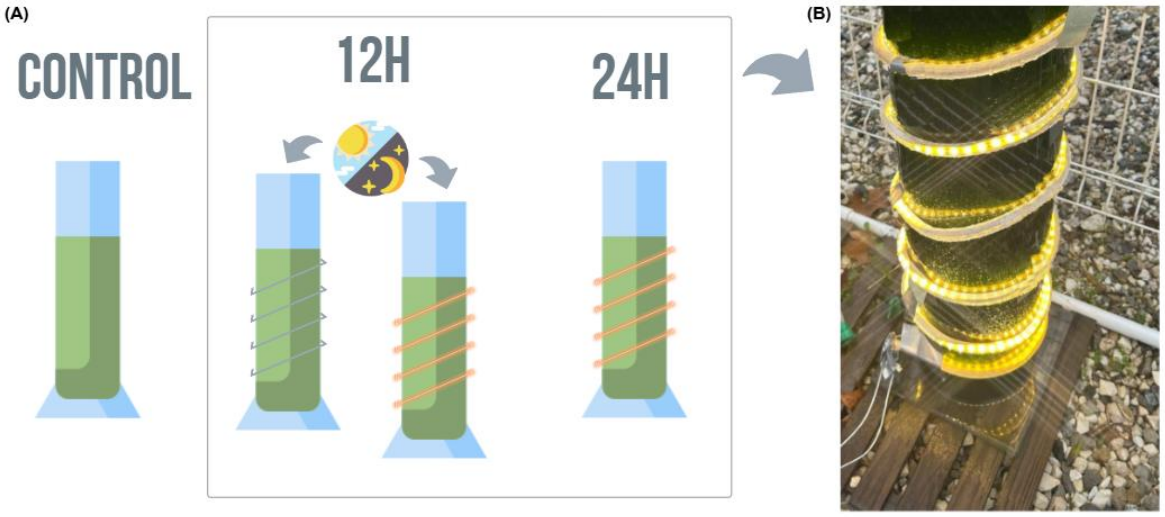

Figure 8.1. Bubble column photobioreactors equipped with LED strips. (a) experimental setup, (b) reactor example with LED illumination.

Each reactor was filled with 20 L of culture medium and operated in consecutive batch cycles with a 7-day duration each, for four weeks. At the beginning of each cycle, the reactors were inoculated at 25% (v/v) and supplemented with wastewater to reach 75% (v/v). Then, monitoring analysis for treatment efficiency performance was performed weekly.

8.2.2. Wastewater Characterization and Biomass Production

The wastewater was characterized before and after treatment in the reactors for all consecutive batches, following APHA (2012) guidelines for soluble chemical oxygen demand (sCOD), total solids (TS), total suspended solids (TSS), volatile suspended solids (VSS) and total ammonia nitrogen (NH_4^+-N). Commercial kits (PhosVer 3 powder pillows, Hach) were used to measure phosphate (PO_4^{3-}-P), with readings performed on a Hach DR/2010 spectrophotometer at 890 nm. Nitrate was asses by the UV Spectrophotometric Method according to Sharma and Kaur (2016) and a probe (InoLab WTW) was used to monitor pH. Results were tested for normality, followed by a Kruskal–Wallis test at the 5% probability level using PAST 4.03 software.

8.2.3. Flow Cytometry

A FACSCalibur flow cytometer (Becton–Dickinson, Franklin Lakes, NJ, USA) was used for multiparametric flow cytometry analyses at the beginning and end of operations. The instrument was routinely calibrated with 3.0–3.4 µm Rainbow calibration beads (BD Biosciences Pharmingen, San Diego, CA, USA). Prior to measurement, samples were withdrawn from the culture and sonicated for 10 seconds to disperse aggregated cells (TranssonicT660/H, Elma, Wetzlar, Germany). The sample was then adjusted (via dilution when necessary) to achieve approximately 1000 events/s. Throughout the experiment, the medium's autofluorescence was monitored.

To assess enzymatic activity, samples were stained with 5-(and-6)-carboxyfluorescein diacetate (CFDA; Molecular Probes, Carlsbad, CA, USA). Living cells with active esterases and intact membranes display fluorescence, whereas dead or metabolically inactive cells do not (Dias et al., 2022). A final CFDA concentration of approximately 0.1 mg/L was prepared by adding 5 µL of a 10 mg/L CFDA stock solution (in acetone) to 495 µL of culture sample. After a 30-minute incubation in the dark, samples were immediately analyzed. CFDA fluorescence was detected with the FITC-A detector, while chlorophyll autofluorescence was monitored in the PC5.5-A channel. All flow cytometry analyses were performed in duplicate.

Data were exported for analysis using the CytoFLEX platform. Subsequently, ANOVA followed by Tukey's test ($p < 0.05$) was conducted to evaluate differences.

8.2.4. Biochemical Composition

Biomass was centrifuged (6–16 KS, Sigma) and freeze-dried (Heto Power Dry LL3000, Thermo Scientific) for biochemical analysis. A composed sample of the biomass collected in each batch was used for characterization. Ash, moisture, protein, carbohydrate, and lipid contents in the produced biomass were quantified. Biomass samples were dried in a freeze dryer (HetoPowerDry LL 3000, Thermo Scientific). Carbohydrate content was determined using the phenol–sulfuric acid method (DuBois et al., 1956). Lipid content was quantified through Soxhlet extraction (Raposo et al., 2011). Before extraction, dried

biomass was pre-treated in a vibrating ball mill (MM400, Retsch). Neutral lipids were extracted with 99% hexane over 6h. The solvent was evaporated using a rotary evaporator (R200, Büchi) with a heated bath, and the lipid content was determined gravimetrically. Protein content was measured according to Lowry et al. (1951). Moisture was determined by weighing the sample before and after drying at 105 °C to constant weight, and ash content was measured by combustion in a muffle furnace at 550 °C for 1 h. Also, ultimate analysis (carbon, hydrogen and nitrogen) was performed using an elemental analyzer (PerkinElmer Series II 2400 Elemental Analyzer).

8.2.5. Fatty acids Profile

The extraction of fatty acids and preparation of their methyl esters followed the approach outlined by Pereira et al. (2024). Subsequent analysis of the methyl esters was performed via gas-liquid chromatography, utilizing a Varian 3800 gas chromatograph (Palo Alto, CA, USA) paired with a flame ionization detector. For separation, a Supelcowax 10 fused silica capillary column (0.32 mm × 30 m, 0.32 µm film thickness; Supelco, Bellefonte, Palo Alto, CA, USA) was employed. Helium served as the carrier gas, flowing at 3.5 mL min^{-1}. The column temperature was initially set at 200 °C for 8 minutes, then ramped up by 4 °C per minute until reaching 240 °C, where it was held for 16 minutes. Injector and detector temperatures were sustained at 250 °C and 280 °C, respectively, with a split ratio adjusted from 1:50 for the first 5 minutes to 1:10 for the remaining duration. The column pressure was maintained at 93 kPa. Identification of chromatographic peaks and determination of response factors were executed using reference standards (Nu-Chek-Prep, Elysian, MN, USA).

8.2.6. Pigments

Pigment quantification (chlorophylls and carotenoids) was performed by adapting the methods described by Lichtenthaler (1987), Ritchie (2008), and Indahsari et al. (2022). To this end, extractions were carried out at low temperatures with minimal light exposure, using 90% acetone as the solvent. The resulting extract was then subjected to spectrophotometric analysis, employing the same solvent as the blank. Absorbance readings were taken at specific wavelengths—630,

647, 664, and 691 nm. From the lyophilized biomass, 2 mg were weighed out. Repeated cycles of vortexing and resting on ice were performed, followed by centrifugation and transfer of the supernatant to a light-protected graduated cylinder, until the residue appeared clear or further absorbance changes were no longer observed. Concentrations of chlorophylls (Chl-a and Chl-b) and carotenoids were calculated according to the equations proposed by each method, taking into account both the extraction volumes and the initial mass or volume of the sample.

8.3. Results and Discussion

8.3.1. Treatment Efficiency

Table 8.1 summarizes the treatment efficiencies achieved for the soluble fraction of wastewater by evaluating key parameters such as pH, sCOD, NH_4^+-N, NO_3^--N, and PO_4^{3-}-P.

Table 8.1. Mean concentration and pollutant removal efficiencies (%) in each reactor.

Variable	Wastewater	Control		12h		24h	
pH	7.72 ± 0.54	9.94 ± 1.10 A		8.85 ± 0.98 A		8.87 ± 0.47 A	
sCOD (mg/L)	49.31	37.59	37.50%	30.08	50.00%	30.08	50.00%
NH_4^+ (mg/L)	43.3 ± 25.5	ND	100.00% A	ND	100.00% A	ND	100.00% A
TKN (mg/L)	66.3± 23.3	14.00 ± 5.60	78.88% A	17.73 ± 4.28	73.26% A	18.67± 8.55	71.84% A
NO_3^- (mg/L)	0.028	0.027	4.49%	0.025	9.27%	0.025	9.27%
PO_4^{3-} (mg/L)	10.22 ± 1.88	0.39 ± 0.29	97.13% A	0.05 ± 0.01	99.67% A	0.03 ± 0.03	99.51% A

** Means followed by the same letter in a line did not differ significantly from each other (n =4); Parameters without letters had insufficient valid data for statistical comparison.* ND = not detected.

The wastewater exhibited an initial pH of 7.72 (Table 8.1). In the control reactor, pH increased to 9.94, likely due to uncontrolled photosynthetic CO_2 uptake under natural sunlight. By contrast, the LED-supplemented reactors maintained a more moderate pH (8.85–8.87) with lower standard deviations, consistent with findings by Chen et al. (2023), who observed that homogenous light distribution in photobioreactors can balance photosynthetic CO_2 consumption with respiration, thus preventing extreme pH shifts.

Overall, Portuguese regulations for treated wastewater align with the broader European Directive (91/271/EEC), which often sets a COD limit of ≤125 mg/L and total phosphorus near ≤2 mg/L for discharges. In that respect, the reactors' final sCOD and PO_4^{3-}-P meet typical limits. The control achieved 37.50% sCOD removal, which rose to 50.00% under both LED regimes. A similar increase under artificial lighting was reported by Nguyen et al. (2022), who observed a 13.2% jump in COD removal when supplementing sunlight with LEDs. Although the statistical test could not always be applied due to data limitations, this trend supports the hypothesis that supplemental lighting could boost microalgal metabolic activity, aiding organic matter degradation. PO_4^{3-}-P removal was also effective across all treatments. The control achieved 97.13% removal, while LED-assisted reactors reached 99.67% (12 h cycle) and 99.51% (24 h cycle), with no significant difference.

All reactors achieved 100% ammonia removal, showing that both natural light and LED-assisted systems effectively support NH_4^+-N removal. Balbuena-Ortega et al. (2024) reported up to 96% total ammonia nitrogen removal in larger-scale vertical tubular reactors, suggesting that both small- and large-scale systems can reach high removal efficiencies under favorable conditions.

The Lisbon metropolitan area is classified as a sensitive zone for wastewater discharge under European and Portuguese regulations, which limit total nitrogen to ≤ 10 mg L^{-1}. In the present study, TKN remained between 14 and 19 mg L^{-1} across all treatments, exceeding this threshold. Part of the discrepancy may stem from the fact that total nitrogen was not recalculated after the settling step performed before sampling; incorporating that reduction would likely improve compliance. Still, Future approaches could address nitrogen reduction by

harnessing the flexibility of LED illumination. For instance, Kang et al. (2018) demonstrated that cultivating algae in domestic wastewater under red light removed 93.9 % of TKN, whereas blue light achieved only 58.8 %. These results suggest that fine-tuning light spectra and photoperiods in LED-assisted photobioreactors could markedly improve nitrogen removal. Although Kang et al. attributed their findings mainly to species-specific responses, maintaining pure cultures in wastewater systems is rarely feasible, underscoring the need to systematically evaluate wavelength effects on mixed algal communities.

8.3.2. Biomass Production

Figure 8.2 illustrates the biomass production, measured as VSS, for each treatment (Control, 12 h LED, and 24 h LED).

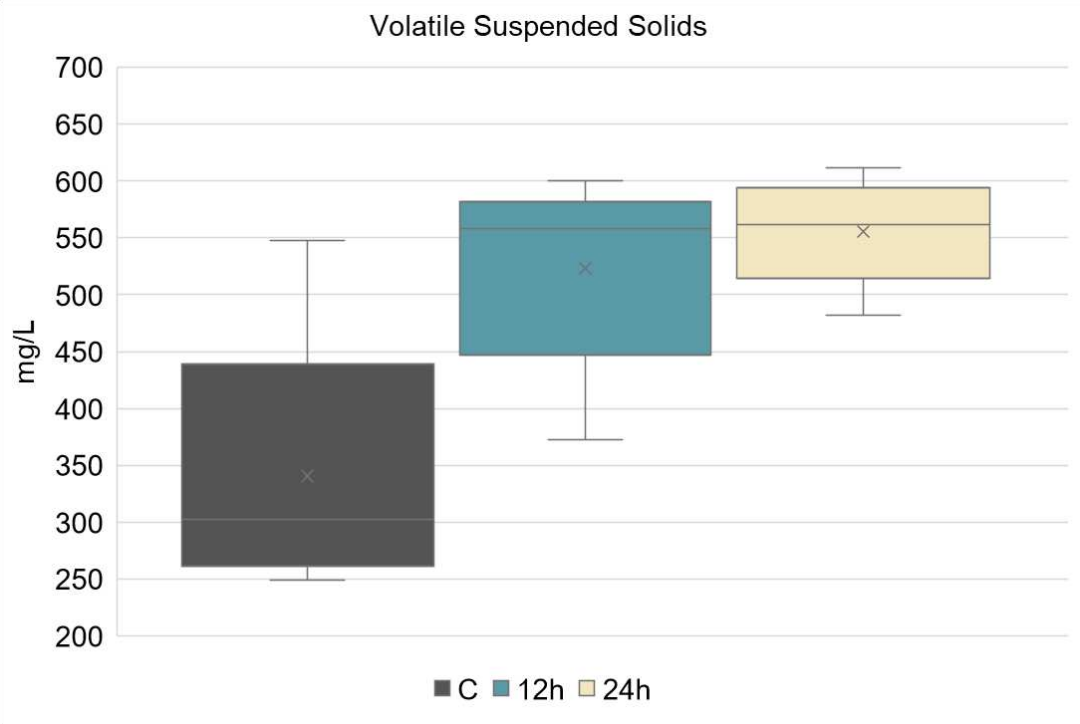

Figure 8.2. Volatile Suspended Solids for each treatment Control, 12h LED, and 24h LED.

LED supplementation significantly increased biomass yield compared to natural light alone ($p = 0.03439$). The control showed the lowest VSS (340.68_A mg/L), while the 12 h LED and 24 h LED treatments reached 523.17_B mg/L and 555.61_B mg/L, respectively. This represents productivities of 48.7 mg/L·d (control), 74.7

mg/L·d (12 h), and 79.4 mg/L·d (24 h). Both LED-assisted reactors generated significantly more biomass than the sunlight-only control; however, biomass yields under the 12 h and 24 h photoperiods did not differ statistically. This plateau indicates that photosynthesis became light-saturated once the 12-h threshold was reached, so the additional electricity required for continuous illumination would raise operating costs without delivering a proportional benefit. A more economical strategy is to optimise when the LEDs are used—shortening the artificial-light window or activating it only during dawn and dusk, when solar irradiance is low—rather than simply extending the total hours of supplementation.

The beneficial effect of LED illumination on biomass growth aligns with several reports. For example, Kvíderová et al. (2024) documented a 2.5-fold biomass increase under targeted nighttime LED use. In contrast, González-Camejo et al. (2019) tested multiple photoperiods (24, 12, and 8 h) in outdoor systems treating urban wastewater and found no substantial difference among treatments, with productivities spanning 23–46 mg VSS/L·d.

Li et al. (2024) similarly observed that 12 h of light provided the best compromise between biomass production and preventing photoinhibition in bacteria–algae system. Furthermore, Wang et al. (2023) showed that optimized photoperiods (14 h) can enhance synergy among different microorganisms, whereas Sirisuk et al. (2018) highlighted the importance of spectral quality by reporting optimal growth under continuous red or red–blue LEDs.

The 24 h LED treatment in this study exhibited a slightly lower standard deviation, suggesting a more uniform growth pattern—an observation also reported by Maia et al. (2025), who noted that continuous lighting can stabilize biomass accumulation.

8.3.3. Flow Cytometry

Figure 8.3 illustrates the distribution of microalgal cells (events) at the beginning (post-inoculation) and the end of the operation for each reactor, plotted by autofluorescence (PC-5.5-A) versus side scatter (SSC-A).

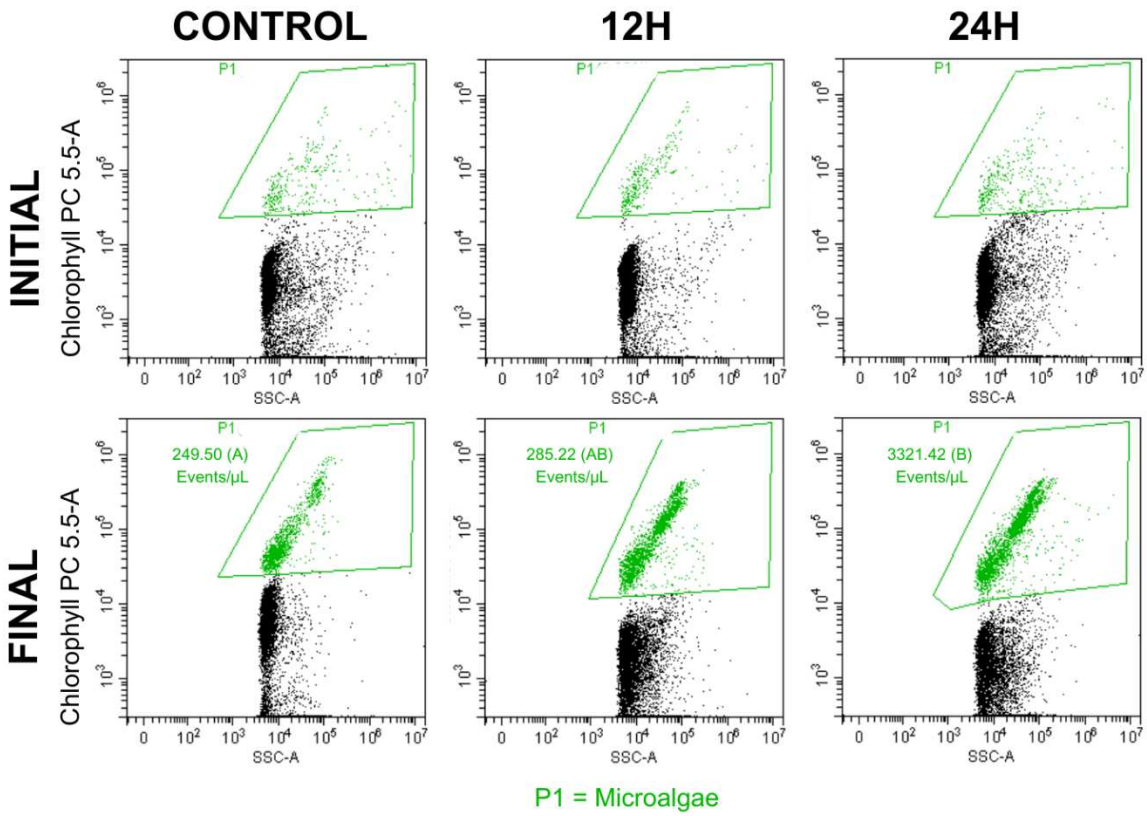

Figure 8.3. Event distribution and density (P1 in Events/µL) by microalgal autofluorescence (PC-5.5-A) vs. side scatter (SSC-A) for control, 12 h LED, and 24 h LED reactors. Letters (A) and (B) represent different means by ANOVA.

The gated region (P1) encompasses chlorophyll-rich cells, distinguishing them from non-algal debris and thus allowing more accurate quantification. Among the treatments, the 24 h LED reactor achieved the highest cell density (321.42_{A} events/µL), significantly exceeding both the control (249.5_{B} events/µL) and the 12 h LED reactor (285.22_{AB} events/µL). Although the 12 h LED condition was numerically higher than the control, it did not differ statistically.

To further evaluate metabolic activity, the cultures were stained with CFDA, which fluoresces upon cleavage by intracellular esterase. In Figure 8.4, CFDA-positive (viable) cells occupy the upper region (H1-UR), while CFDA-negative cells remain in the lower region (H1-LR). Notably, 24 h LED treatment yielded the highest proportion of esterase-active cells (97.47%), surpassing the 12 h LED (96.55%) and control (93.68%) reactors. Similar findings have been documented by other

authors (Dias et al., 2023; Vale et al., 2022), who observed that esterase activity is responsive to operational conditions.

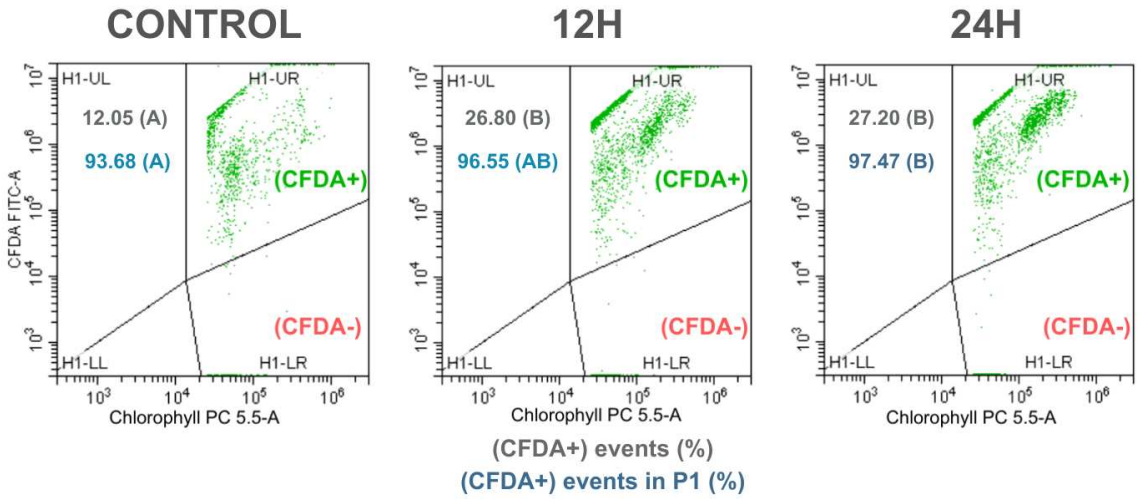

Figure 8.4. CFDA-stained microalgal cells showing esterase activity and percentage of metabolically active microalgae in each reactor. Letters (A) and (B) represent different means by ANOVA.

Moreover, Akimov et al. (2024) indicate that while CFDA fluorescence and the proportion of enzyme-active cells typically decline as cultures mature, they do not fundamentally shift under sublethal abiotic changes. Here, extended illumination appears to bolster metabolic function without inflicting photoinhibition, as the higher light regimes did not negatively impact cell viability. Consequently, optimizing illumination strategies, such as continuous or partial LED supplementation, can be instrumental in maximizing both cell viability and overall productivity. Additionally, flow cytometry offers a near real-time tool for monitoring culture health and metabolism, thereby guiding operational adjustments to enhance wastewater treatment efficacy and algal bioproduct yields.

8.3.4. Biomass Characterization

Table 8.2 outlines the biochemical and elemental composition of the harvested biomass across all treatments.

Table 8.2. Biomass biochemical, proximate and ultimate composition.

Characterization	Control	12h	24h
Neutral Lipids (%)	2.75	2.94	1.81

Biochemical Analysis	Carbohydrates (%)	35.4	25.4	14.2
	Proteins (%)	27.69	31.09	27.58
Proximate Analysis	Volatile material (%)	80.71	91.89	87.72
	Fixed carbon (%)	5.29	1.70	6.62
	Ash (%)	14.00	6.41	5.66
	Moisture (%)	9.81	11.67	11.86
Ultimate Analysis	C (%)	44.5	41.7	42.9
	H (%)	7.7	7.6	7.8
	N (%)	6.4	5.4	4.6

Carbohydrate content declined markedly under continuous illumination (35.4% to 14.2%), indicating a metabolic shift away from carbohydrate storage. Similar trends have been reported by Li et al. (2024) and Silvello et al. (2022), who noted that extended lighting periods can diminish carbohydrate accumulation due to altered photosynthetic electron transport and increased energy dissipation.

Neutral lipids reached 2.94% under 12 h LED but dropped to 1.81% with 24 h LED, suggesting that continuous light supplementation could be inducing photoinhibition or metabolic stress. These findings align with Rani and Ojha (2021), who showed that the balance between light and dark phases can significantly modulate macromolecule accumulation in microalgae. However, it is important to highlight that the lipid contents are low in all treatments compared to other results such as Magalhães et al. (2025), reporting lipids in the range of 14-17% when cultivating algae in high-rate ponds with urban wastewater.

Proximate analysis showed notably lower ash content in LED-assisted biomass (5.66%–6.41%) relative to the control (14.00%), resembling findings by Megía-Hervás et al. (2020), who observed decreased ash content from roughly 20% to 12 % with longer light phases (from 12:12 to 18:6) for the cultivation of diatom *Phaeodactylum tricornutum*. Since there were no differences in the wastewater used as culture media, ash reduction could be connected to the observed increase in biomass production due to increase in photosynthetic efficiency, as was also reported by Megía-Hervás et al. (2020).

In the 12 h LED treatment, the fixed carbon level (1.70%) is substantially lower than in both the control (5.29%) and the 24 h LED condition (6.62%). This difference likely reflects the biochemical composition of the biomass rather than

specific protein or carbohydrate decomposition ranges at lower temperatures. In other words, partial LED supplementation can encourage the production of more labile organic molecules—such as simple carbohydrates and proteins—that are fully volatilized at high temperatures, leaving little residue. In contrast, the control and 24 h LED reactors appear to accumulate more recalcitrant or cell wall–associated structures, which remain as fixed carbon during proximate analysis. However, future research using thermogravimetric analysis could conclusively verify whether partial LED illumination indeed promotes more readily volatilized organic components in the biomass.

Moisture content stayed at roughly 10–12% across all treatments. Ultimate analysis revealed minor shifts in elemental composition, with carbon at 41.7–44.5%, hydrogen at 7.6–7.8%, and nitrogen at 4.6–6.4%. Overall, these results indicate that photoperiod tuning can modulate both the quality and quantity of microalgal biomass, providing pathways for optimizing resource recovery or downstream applications.

8.3.5. Fatty Acids Profile

Figure 8.5 shows the fatty acid (FA) profiles, expressed as a percentage of total FAME (w/w), for the control, 12 h LED, and 24 h LED reactors. Palmitic acid (C16:0) emerged as the dominant type, comprising 65–76% of total FAME, consistent with other reports on *Chlorella* strains (Leong et al., 2022). Accordingly, saturated fatty acids (SFAs) ranged from 77% in the control to 86% in the 12 h LED reactor and 79% in the 24 h LED reactor, with no statistically significant difference among treatments.

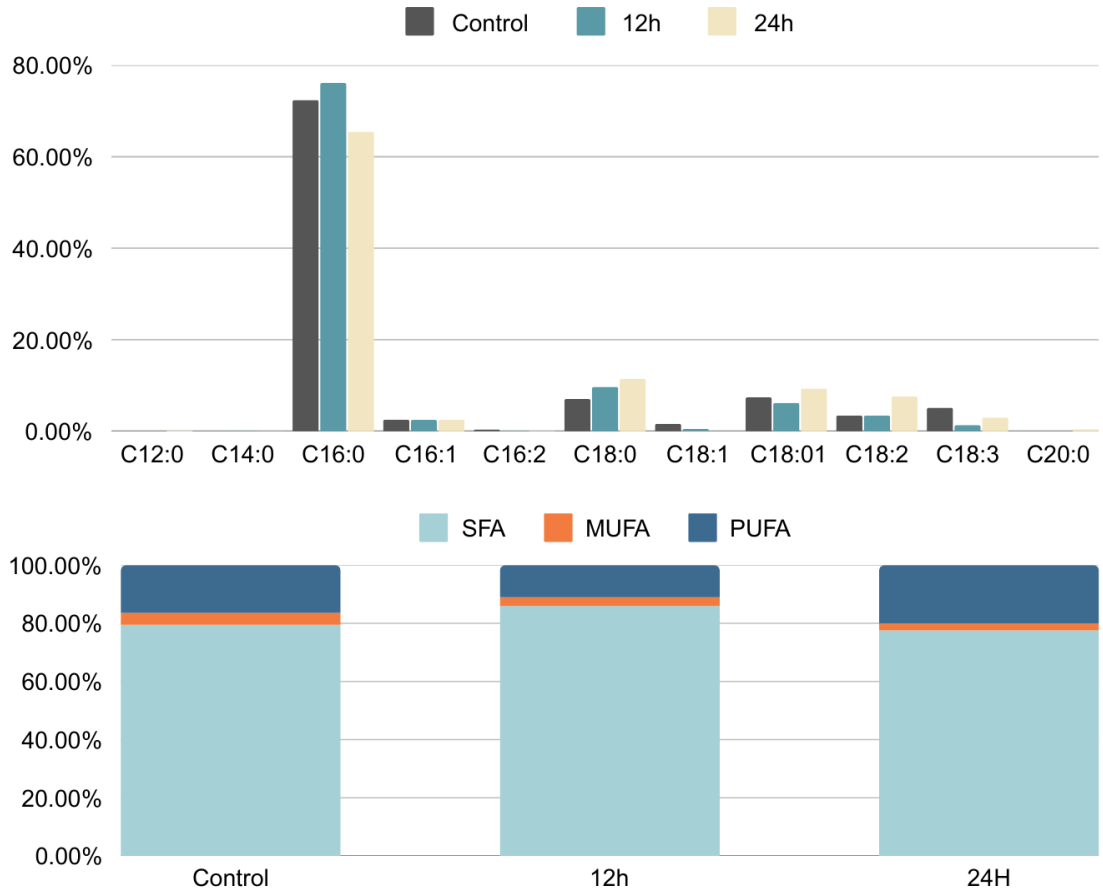

Figure 8.5. Fatty acids profile for each reactor, presented in yield (%, w w^{-1}).

Polyunsaturated fatty acids (PUFAs) varied from 20% (control) to 11% (12 h LED) and 16% (24 h LED), whereas monounsaturated fatty acids (MUFAs) also showed minor fluctuations, but none of these changes were statistically significant. Similar minimal variations in FAME profiles under different photoperiods have been documented by Alharbi et al. (2024) and Leong et al. (2022), underscoring the inherent stability of *Chlorella*'s lipid metabolism across moderate changes in illumination.

Despite the lack of a strong photoperiodic effect on FA composition, LED supplementation did enhance overall biomass production. Moreover, the consistent prevalence of C16–C18 species highlights *Chlorella*'s suitability for biodiesel applications, irrespective of specific lighting regimens.

8.3.6. Pigments

Table 8.3 presents the pigment contents (µg/mg) for each treatment, including chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (a+b), and total carotenoids. Although the control displayed numerically higher values than the LED-assisted reactors, ANOVA revealed no significant differences ($p > 0.05$).

Table 8.3. Pigment concentrations (µg/mg) in harvested biomass. Chlorophyll a, chlorophyll b, total chlorophyll (a + b), and carotenoid contents in each treatment.

Sample		C	12h	24h
Chl-*a*		4.01 ± 1.62	2.00 ± 2.14	1.58 ± 1.48
Chl-*b*		0.38± 0.26	0.15± 0.17	0.12± 0.12
Total Chl (*a*+*b*)		4.38 ± 1.88	2.15 ± 2.32	1.70 ± 1.59
Total Carotenoids	*Lichtenthaler (1987)*	0.54 ± 0.06	0.42 ± 0.20	0.33 ± 0.06
	Indahsari et al. (2022)	0.53 ± 0.06	0.41± 0.02	0.32 ± 0.06

These results imply that variations in sunlight intensity, temperature, and nutrient levels in outdoor conditions may overshadow any incremental gains from LED supplementation. Continuous or prolonged artificial lighting can also lessen microalgae's physiological requirement for pigment synthesis as they acclimate to stable illumination. Nevertheless, other investigations suggest that LED supplementation can be species- and condition-specific. Carneiro et al. (2022), for instance, reported significant pigment productivity gains in *Nannochloropsis oceanica* under 24 h LED illumination in outdoor raceway ponds, highlighting the potential influence of factors such as strain, reactor design, and operational parameters.

Comparisons with additional studies reveal similar nuances. Rani and Ojha (2021) observed comparable carotenoid yields (*ca.* 10–11 mg/L) for *C. sorokiniana* grown indoors under real wastewater conditions with both 24 h and 12 h photoperiods. By contrast, chlorophyll levels increased markedly (≈22 to 60 mg/L) under both continuous and 12:12 photoperiod regimes, underscoring the interplay between photoperiod length, reactor setup, and algal species. Furthermore, the high nitrogen removal reported earlier may have contributed to

low pigment levels, as some strains reduce pigment synthesis when nitrogen becomes a limiting substrate (Li et al., 2018; Song and Pei, 2018). This is also an indicative that controlling pH levels during cultivation is essential for its many roles in nutrient assimilation and, consequently, biocoumpound accumulation.

Overall, while LED supplementation did not markedly influence pigments in the present *Chlorella*-based system, it did enhance biomass production and cellular activity. Future research should expand pigment characterization beyond chlorophyll and total carotenoids. Techniques such as thin-layer or flash chromatography could be used to profile individual carotenoid fractions (e.g., lutein, β-carotene) and determine whether altered light regimes selectively stimulate the production of distinct pigment types. Such detailed analyses may reveal additional benefits of LED supplementation, guiding targeted strategies for bioactive compound enrichment in microalgal cultures.

8.3.7. Valorization Routes and Implications for Process Optimization

Combining LED supplementation with outdoor microalgae cultivation opens promising valorization routes by boosting both treatment performance and biomass yields. Although continuous illumination maximized cell density and esterase activity, partial LED use (12 h) achieved comparable improvements for biomass production in terms of VSS. Tailoring photoperiods, intensities, and spectral compositions can further elevate nutrient uptake, cellular metabolism, and the accumulation of high-value molecules (such as lipids, proteins, pigments). Integrating such strategies in large-scale applications requires balanced approaches to reactor design, operational ease, and techno-economic feasibility. Still, comprehensive life-cycle assessments are essential to validate the sustainability benefits, ensuring that LED-based intensification aligns with net energy savings and minimized environmental impacts. Overall, these results underscore the need for integrated, data-driven optimizations to scale up microalgal wastewater treatment as a viable platform for both resource recovery and industrial bioproduct development.

8.4. Conclusion

LED illumination substantially improved microalgal biomass production in outdoor bubble column photobioreactors, while all treatments performed comparably for sCOD and phosphate removal. Flow cytometry demonstrated that continuous lighting outperformed control cultivation in cell density and esterase activity. Biochemical composition under continuous illumination shifted toward lower ash and carbohydrate contents. Overall, these findings underscore the potential of LED supplementation as a strategy to overcome low biomass yields in outdoor systems, provided that techno-economic and life-cycle assessments are conducted to ensure sustainable scale-up for integrated wastewater remediation and bioproduct generation.

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9. Chapter 5. Economic and Environmental Perspectives

Shining a light on outdoor algal systems for wastewater treatment: How artificial light enhancement impacts biomass costs and life cycle[4]

Abstract

Microalgae-based wastewater treatment is increasingly viewed as a cleaner production strategy, combining nutrient removal and biomass generation for high-value applications. However, productivity constraints remain a critical barrier to broader implementation. This study examines the viability of integrating light-emitting diodes (LEDs) into outdoor bubble column reactors for domestic wastewater treatment and biomass production, focusing on environmental impacts and techno-economic performance. Three lighting regimes—natural light only (control), 12-hour LED cycles, and 24-hour LED cycles—were experimentally evaluated and scaled up using Aspen Plus® simulation. Life cycle assessments (LCA) were conducted to quantify environmental impacts, (ReCiPe 2016 method), and a detailed techno-economic analysis determined minimum biomass selling prices. Compared to the control, LED-assisted systems increased biomass yields by 24–34%, yet capital and operational costs offset productivity gains. Under grid electricity, minimum selling prices considering capital and operational costs ranged from 80.76 to 91.37 USD/kg for LED systems versus 68.85 USD/kg for the control. Photovoltaic (PV) integration reduced operational costs by up to 16.89%, but LED scenarios remained more expensive. LCA findings highlighted substantially higher environmental impacts (78–149 times) for LED systems, partly alleviated by PV-powered operations. Sensitivity analysis identified nutrient availability, process scale, and reactor costs as pivotal factors influencing the feasibility of LED-enhanced wastewater treatment. Overall, while LED technology offers notable productivity benefits, its economic and environmental trade-offs underscore the need for integrated

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approaches—ranging from material innovations to policy incentives—to achieve truly sustainable wastewater-based microalgal production.

Keywords: Microalgae; Process scale-up challenges; Environmental Impacts; Minimum Biomass Selling Price; Life cycle assessment; Photovoltaics

9.1. Introduction

Microalgal biotechnology has emerged as a promising alternative to conventional wastewater treatment, offering high pollutant removal efficiency, carbon capture, and the production of valuable metabolites. This integration of wastewater treatment with resource recovery provides a sustainable framework for waste management (Chen et al., 2023; Pereira et al., 2024). Nevertheless, low biomass yields, and high production costs limit broader implementation (Rajesh Banu et al., 2020).

Key strategy to address these challenges in microalgae cultivation involves leveraging the benefits of light-emitting diodes (LEDs). Schulze et al. (2014) demonstrated that LED quality significantly influences microalgal growth and biochemical profiles, while LEDs also offer spectral flexibility, energy efficiency, and durability (Ievina and Romagnoli, 2025). For instance, González-Camejo et al. (2019) reported a significant increase in biomass production—from 333 ± 86 mg/L to 538 ± 101 mg/L (in Volatile Suspended Solids)—by cultivating a mixed algae-bacteria consortium in outdoor flat-panel reactors equipped with LED lamps and using urban wastewater as the culture medium. Similarly, nighttime white-LED illumination in a 150 L (12 m²) thin-film platform boosted the growth of *Dictyosphaerium chlorelloides* by about 2.5-fold over sunlight alone—reducing the time to stationary phase from 42 to 24 days and increasing biomass production from 3.3 to 8.54 g m^{-2} d^{-1} (Kvíderová et al., 2024). However, integrating LEDs into external reactors poses challenges, such as compatibility with natural lighting and high energy demands, directly affecting the process's economic and environmental performance (Kurniawan et al., 2024).

A complete assessment is thus required to balance productivity gains against associated costs and impacts. Life Cycle Assessment (LCA) is commonly used

to evaluate the environmental performance of optimization technologies in microalgae cultivation, identify critical bottlenecks, and inform technological improvements (Magalhães et al., 2021). It has also been applied to a range of valorization routes—biofertilizers, fatty acids, pigments, and more—quantifying the environmental outcomes of various processes and inputs (Ferreira et al., 2020; Herrera et al., 2021; Magalhães et al., 2021; Schneider et al., 2018). For instance, Magalhães et al. (2021) found that using recycled carbon from gasoline combustion gases reduced environmental impacts by 56% compared to a microalgae base cultivation system. However, higher-productivity systems did not necessarily yield lower impacts. LCA has also proven valuable for assessing broader sustainability issues when algal systems are integrated into biofuel production, biofertilizers, fatty acids, and pigments (Bartek et al., 2021; Castro et al., 2020; Deprá et al., 2020; Souza et al., 2019; Sun et al., 2019).

Techno-economic analysis (TEA) is equally important for evaluating the financial feasibility of microalgae-based processes, helping to quantify risks, guide decision-making, and inform future research. For example, Nobre et al. (2024) demonstrated that integrating microalgae cultivation into municipal wastewater treatment plants could generate annual gains of approximately €619,100 through biofertilizer production. Aspen Plus® and SuperPro Designer® facilitate the simulation of production costs varying operational scales (Castro et al., 2023; Valdovinos-García et al., 2021). Still, few studies address the high costs associated with wastewater-based cultivation. Acién Fernández et al. (2019) identified wastewater as a promising route to reduce microalgae production costs, though achieving values under 5 EUR/kg remained hypothetical. More frequent cost-reduction strategies have been reported for freshwater cultivation; for instance, Ibanez et al. (2020) achieved a significant decrease in production costs (from 1.44 USD/g to 0.14 USD/g) by using a high-density culture medium and reducing equipment size by over 80%.

A comprehensive approach integrating process modeling with environmental and economic assessments can provide deeper insights into optimizing microalgae-based wastewater treatment. Castro et al. (2023) illustrated this by comparing hydrothermal carbonization scenarios for producing solid biofuels and

biofertilizers, showing that biofertilizers required 2.5 times less energy and had lower capital (CAPEX) and operating (OPEX) costs than solid biofuels.

Against this backdrop, the present study examines the environmental performance and techno-economic feasibility of using LEDs in outdoor photobioreactors for microalgae cultivation with domestic wastewater. Specifically, it addresses three key questions: (1) Does LED usage improve biomass production; (2) Can productivity gains justify the associated environmental impacts and costs? (3) What are the cost and environmental hotspots, and which technologies could mitigate them? Three lighting conditions—natural light only, 12-hour LED cycles, and 24-hour LED cycles—were evaluated experimentally, and the results were modeled in Aspen Plus, assessed environmentally in SimaPro, and subjected to a detailed TEA. This evaluation aims to guide future approaches to optimizing resource recovery in wastewater treatment while balancing environmental and economic outputs.

9.2. Material and Methods

9.2.1. Experimental Setup

The experiments were conducted at the Lumiar Campus of the National Laboratory of Energy and Geology (LNEG) in Lisbon, Portugal, between April and June 2024, corresponding to the spring season. Secondary domestic sewage, collected after activated sludge treatment and before disinfection from the Quinta do Conde Wastewater Treatment Plant (WWTP), served as the cultivation medium without nutrient supplementation.

Three bubble column reactors were operated in semi-continuous mode with a hydraulic retention time of seven days. Two lighting regimes were tested: 12 hours and 24 hours of LED illumination, compared to a control reactor exposed only to natural light and ambient temperature. Exterior illumination was supplied by warm-white LED rope lights (LIVARNO® HG04149). Each rope is rated at 22.5 W, operates at 230–240 V AC, carries an IP44 splash-proof rating, and consists of 240 non-replaceable LEDs along a 10 m flexible strip (total length with power lead = 11.5 m). Biomass production was quantified based on total solids, while phosphate (PO_4^{3-}) and ammonia (NH_4^+) concentrations were monitored throughout

the experiments. These nutrient concentrations and solids were analyzed according to the methodology outlined by APHA (2012).

Biomass productivity data were subjected to statistical analysis using the Kruskal-Wallis test at a 5% significance level, performed with PAST 4.03 software. The productivity results were subsequently used for process modeling in Aspen Plus. The software developed and evaluated three models, followed by the analysis of six scenarios for environmental and economic assessments. These scenarios compared energy powered from the country's electricity mix with photovoltaic generation, providing insights into the sustainability and cost-effectiveness of the proposed systems.

9.2.2. Simulation and Process Configuration

Microalgae cultivation using sewage as a nutrient source was simulated in Aspen Plus (version 14.1). The process was based on a wastewater flow rate of 419 L/h, which represents the value needed to produce approximately 10kg of concentrated microalgae biomass per day on the Control system. The ELECNRTL thermodynamic package was used to model the chemical equilibrium of ionic species in solution (Barbera et al., 2022).

Three cultivation scenarios were analyzed: (1) without synthetic light (LED), (2) with synthetic light (12h) at night, and (3) with continuous synthetic light (24h). Experimental data (from Topic 9.2.1) on microalgae yield, ammonium (NH_4^+) and phosphate (PO_4^{3-}) concentrations, and atmospheric air flow (0.1 vvm) was used to solve a system of equations and determine the stoichiometric coefficients of Reaction 9.1 (Barbera et al., 2022). The elemental composition of the microalgal biomass ($C_xH_yN_zP_wO_t$), classified as a non-conventional compound, was determined from PROXANAL and ULTANAL analyses. Aspen Plus models HCOALGEN and DCOALIGT were used to estimate biomass properties such as enthalpy and density. Tables S1 and S2 (Appendix D) provide details on the components and chemical reactions.

$$a\,CO_2 + b\,NH_4^+ + c\,H_2PO_4^- + d\,HPO_4^{2-} + e\,H_2O \rightarrow f\,ALGAE + g\,O_2 + h\,H_3O^+$$

Reaction (9.1)

A vacuum belt filter (S-BELT) with a 5× concentration factor was included as an alternative to energy-intensive drying methods to simulate biomass harvesting. Filtration was selected for its lower energy consumption (Borowitzka and Moheimani, 2013; Marangon et al., 2021).

9.2.3. Life Cycle Assessment

The environmental analysis was conducted following the principles and guidelines of LCA, considering the comprehensive mapping of the process. The methodology for modeling microalgae cultivation was based on Ferreira et al. (2024), accounting for inputs (resources), outputs (waste and products), and avoided products. The goal of the LCA was to compare the environmental impacts across scenarios, considering different biomass yields. The functional unit adopted was the production of 1 kg of biomass (concentrated). The system boundaries were defined from cultivation through biomass concentration (gate-to-gate). Upstream wastewater pre-treatment is identical across scenarios, and downstream biomass processing is outside the present scope, allowing the analysis to focus exclusively on the effects of LED-assisted cultivation. Inventory data were derived from experimental results (Topic 9.2.1), modeling in Aspen Plus (Topic 9.2.2), and specific secondary data from the literature (see Supplementary Files, Appendix D). Fig. 9.1 illustrates the system boundaries and the main processes considered in the scenarios.

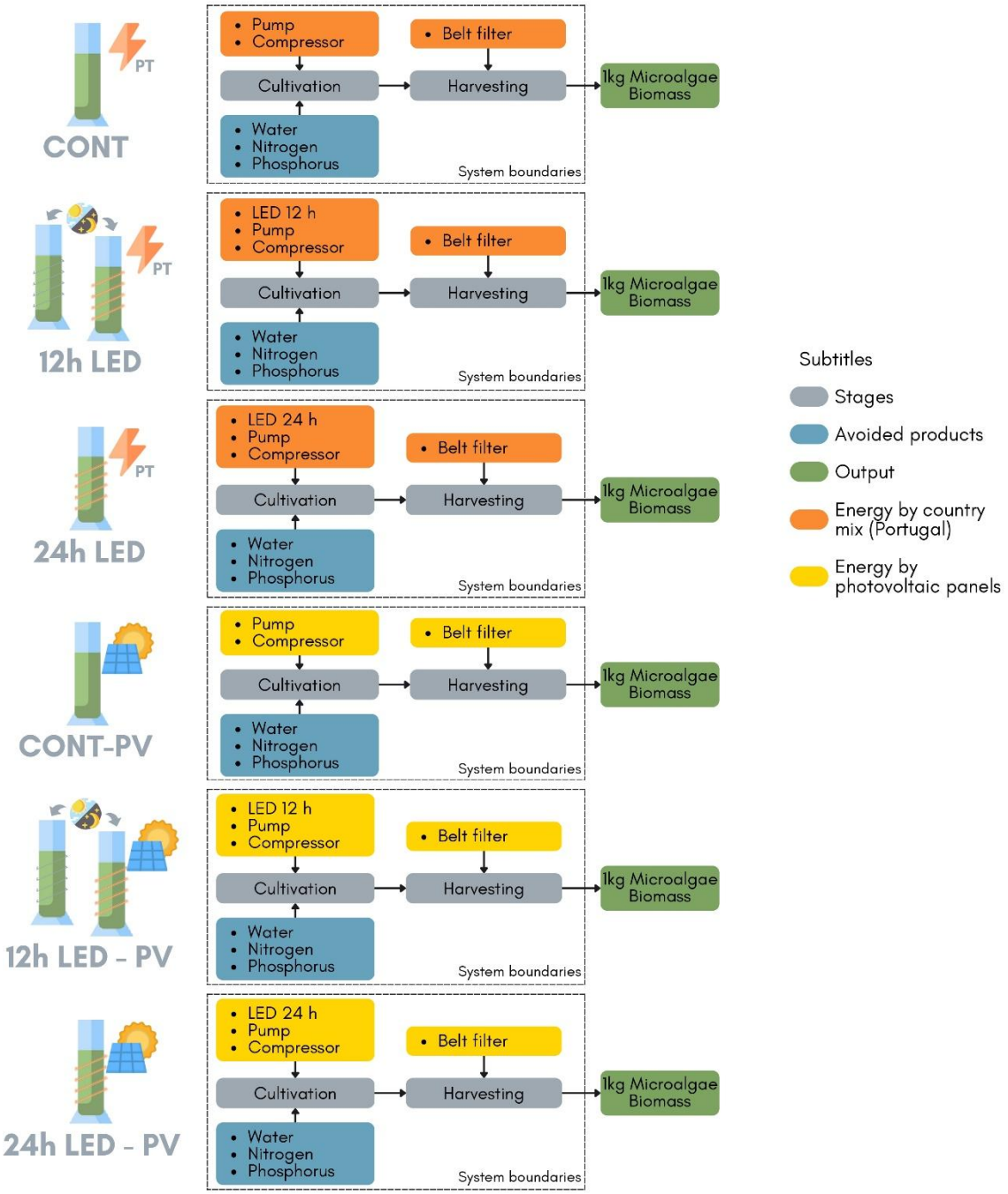

Fig. 9.1. System boundaries and cultivation scenarios for microalgae biomass production. *LED systems.

Six scenarios were proposed: the three experimental scenarios, considering the configurations of Control, 12h, and 24h lighting (CONT, 12H, 24H) using grid electricity, and three additional scenarios in which energy would be supplied by

photovoltaic panels (CONT-PV, 12H-PV, 24H-PV). The environmental benefit of using wastewater was evaluated based on modeling water, nitrogen, and phosphorus as avoided products, as previously done by Castro et al. (2023) and Ferreira et al. (2024). For this analysis, secondary data related to the demand for these inputs (water, nitrogen and phosphorus) in synthetic microalgae cultivation were used to estimate avoided products (see Table S3 in the Supplementary Files, Appendix D), based on the studies by Herrera et al. (2021). Energy demands were derived from modeling performed in Aspen Plus software. Construction data, as well as reactor and LED replacement, were excluded from the scope of the environmental analysis.

Life cycle modeling was carried out in SimaPro 9.1 using the academic PhD license (single-user, non-commercial), along with the ReCiPe impact assessment methodology, covering midpoint and endpoint categories (Goedkoop and Huijbregts, 2013). The midpoint categories analyzed were Global Warming (climate change), Stratospheric Ozone Depletion, Fine Particulate Matter Formation, Terrestrial Acidification, Marine Eutrophication, Freshwater Eutrophication, Terrestrial Ecotoxicity, Human Carcinogenic Toxicity, Mineral Resource Scarcity, and Fossil Resource Scarcity, given their relevance for wastewater-based microalgae systems (Arashiro et al., 2022). Endpoint analysis covered the ReCiPe damage categories—Human Health, Ecosystems, and Resources— (Schneider et al., 2018), within which climate-change-related effects are already included. The inventory was developed using primary data on biomass concentration and equipment energy consumption, with processes from the Ecoinvent 3.8 database integrated into the software. Table 9.1 summarizes the inventory for each system, including the background processes from the Ecoinvent database used to model the cultivation and harvesting steps.

Table 9.1. Inventory and background process from the Ecoinvent database for each scenario.

Background Process	Unit	Control	Control -PV	12h	12h-PV	24h	24h-PV
Control-Biomass (Functional Unit)	kg	1	1	1	1	1	1

Electricity, high voltage {PT}| production mix | APOS, S	GJ	0.04	0.00	0.72	0.00	1.33	0.00
Electricity, low voltage {PT}| electricity production, photovoltaic, 570kWp open ground installation, multi-Si | APOS, S	GJ	0.00	0.04	0.00	0.72	0.00	1.33
*Inorganic nitrogen fertiliser, as N {RoW}| nutrient supply from urea | APOS, S	g	-100.00	-100.00	-102.33	-102.33	-100.00	-100.00
*Inorganic phosphorus fertiliser, as P2O5 {RoW}| nutrient supply from diammonium phosphate | APOS, S	g	-16.00	-16.00	-16.16	-16.16	-16.24	-16.24
* Tap water {Europe without Switzerland}| tap water production, conventional treatment | APOS, S	ton	-2.33	-2.33	-2.39	-2.39	-2.33	-2.33

*Avoided Products.

9.2.4. Cost Assessment

The economic analysis used modeling outputs from Aspen Plus—encompassing biomass productivity, energy consumption, and life cycle inventory. However, for the cost assessment, the system was scaled to an annual production capacity, whereas the environmental assessment was conducted on a per-kilogram basis (i.e., 1 kg of biomass). A comparison of biomass selling prices was conducted across the six scenarios. In the photovoltaic energy scenarios, the costs of the panels were included in the acquisition price, while annual energy expenses were set to zero. The methodology followed the Percentage of Delivered-Equipment Cost approach, with specific parameters for solid and fluid processing plants described by Peters and Timmerhaus (2003). The minimum biomass selling price (MBSP) was calculated based on capital investment and processing costs, solving the cost for zero net present value (NPV).

Equipment costs were estimated using the Aspen database, supplemented by quotations from companies specializing in photovoltaic systems (Cunha, 2024).

The objective was to determine the MBSP for the evaluated scenarios. Table 9.2 summarizes the main parameters and assumptions used in the calculations.

Table 9.2. Summary of cost parameters for the economic analysis of the biomass production process.

Plant life	20 years	References
Internal rate of return	10%	(Masoumi and Dalai, 2021)
*Operating hours per year	7920	
*Lang factor	4.3 for Fixed Capital Investment (FCI)	
*Working capital cost	12% of FCI	
Operating labor	$24000/year per employee	
Supervisory and clerical labor	15% of labor cost	
Maintenance and repairs	6% of FCI	
Operating supplies	15% of maintenance and repairs	
Local taxes	1% of FCI	
Insurance	1% of FCI	
Overhead	60% of (operating labor, supervision, and maintenance)	
Capital charge	12% of FCI	
Depreciation	10% of FCI	
Administrative cost	25% of overhead	
Distribution and selling costs	5% of total expenses	(Peters and Timmerhaus, 2003)
Research and development	4% of total expenses	
Income tax rate	21%	
Annual-compounding discount rate	12%	(Assis et al., 2019)
LED Strip Cost	10 USD	(Ledagic, 2025)
LED Plate Cost	400 EUR +21%	(Cunha, 2024)
Utilities		
Electricity	$0,084/kWh	(Liu et al., 2025; Qi et al., 2023)

9.2.5. Sensitivity

To evaluate the influence of parameter uncertainty, we applied a one-factor-at-a-time sensitivity test to two headline results: the ReCiPe Single Score for environmental impacts and the minimum biomass-selling price (MBSP) for the cost model. Economic parameters examined were processing capacity, nutrient concentration in the wastewater, LED electricity demand, reactor purchase cost, photovoltaic-system cost, labour rate and the annual discount rate. For the LCA,

the analysis concentrated on biomass productivity and electricity demand, the two variables that are included in the inputs. Each parameter was adjusted independently by ±25 % while all others were kept constant. This ±25 % range mirrors the day-to-day variability reported by industrial operators and is widely employed in previous LCA and techno-economic studies of large-scale microalgae systems (Gurreri et al., 2024; Pechsiri et al., 2023).

9.3. Results and Discussion

9.3.1. Experimental Results

Experimental results revealed significantly higher biomass production in the LED systems relative to the Control, with the 12h (1.31 ± 0.12 g/L) and 24h (1.33 ± 0.08 g/L) systems showing no statistical difference but surpassing the control (1.14 ± 0.04 g/L). Wastewater treatment also proved effective: initial phosphorus (28.69 mg/L) and ammonia (100.98 mg/L) were substantially reduced, with complete ammonia removal in all systems. Phosphorus reductions reached 98.33% in the control, 99.76% in the 12h system, and 99.79% in the 24h system. These findings are consistent with other research demonstrating the potential of LEDs to increase microalgae production. For example, González-Camejo et al. (2019) reported a gain in volatile suspended solids from 333 ± 86 mg/L to 538 ± 101 mg/L when LED lamps were introduced to a mixed algae-bacteria culture in outdoor flat-panel reactors using urban wastewater. Building on these experimental outcomes, the following section details how the Aspen Plus model was used to simulate and evaluate the process at larger scales.

9.3.2. Simulation

For the three evaluated scenarios, the microalgae cultivation process, shown in Fig. 9.2, was divided into two main stages: microalgae cultivation and biomass harvesting. Following, Table 9.3 presents concentration data for NH_4^+ and PO_4^{3-} in the feed stream (Feed) and post-cultivation stream (S3), along with dry biomass yield for each scenario.

Initially, wastewater with a flow rate of 419 L/h (FEED) and compressed air (AIR) was injected into the cultivation system, represented by the growth reactor (R-

GROWRH), to achieve a 0.1 vvm. The operational conditions were maintained at 25 °C and 1.01 bar. The reactor was designed as an open vertical tank made of acrylic, with an estimated volume of 77,425.14 L for a 168-hour process cycle. To meet the total demand, 1,935 reactors, each with a 40 L capacity, would be required. In scenarios 2 and 3, each reactor has two LED strips. Excess gas inside the cultivation system is expelled through the reactor's top (GAS). Biomass collection is performed via belt filtration, with a concentration factor 5 and an 87.4% microalgae biomass recovery rate (Borowitzka and Moheimani, 2013; Ferreira et al., 2020).

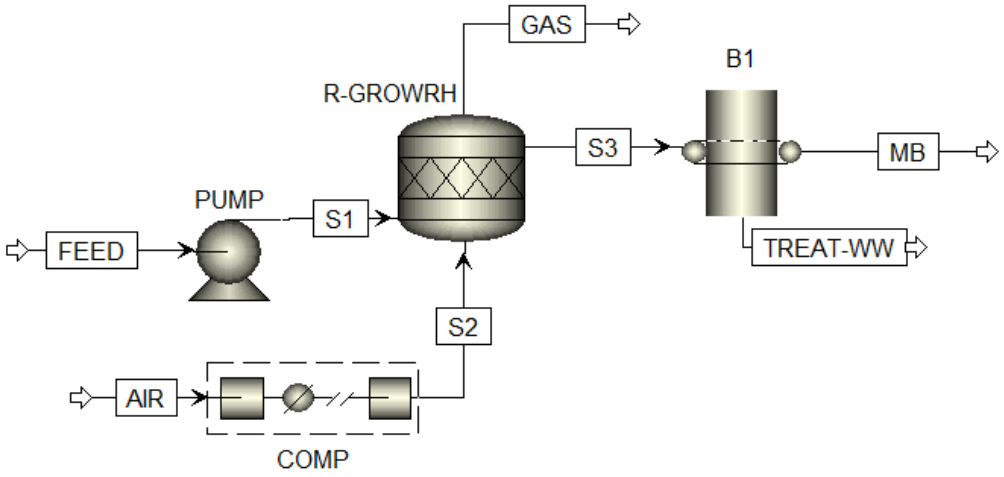

Fig. 9.2. Process Flow Diagram (PFD) of Microalgae Cultivation and Harvesting from Wastewater.

Table 9.3 presents concentration data for NH_4^+ and PO_4^{3-} in the feed stream (Feed) and post-cultivation stream (S3), along with dry biomass yield for each scenario.

Table 9.3. Ammonia and phosphorus concentration in the wastewater before and after microalgae cultivation and corresponding dry biomass yield.

Sample	Ammonia (mg/L)	Phosphate (mg/L)	Total Solids (kg/h)*
Wastewater	100.99	28.7	-
Control	7.8	0.0	0.42
12h	10.4	0.0	0.48
24h	22.1	0.0	0.49

**Dry basis*

The proposed scenarios estimated microalgae biomass production between 83.72 and 83.78 kg/h (wet basis), with approximately 99,44% moisture content. Based on experimental results, data validation confirmed the higher yields predicted in simulations for LED scenarios, with 12.5% and 14.3% increases, respectively, compared to the Control scenario. However, the ammonia concentration increases from 7.8 mg/L to 22.1 mg/L, as observed in the simulation results. This result differs from the experimental data, where NH_4^+ concentrations were reduced to zero after cultivation in all three scenarios. The observed ammonia accumulation trend in the evaluated scenarios suggests that up to 22% of ammonia nitrogen was lost during the cultivation experiments. These losses are likely due to NH_3 volatilization and the oxidation of ammonia nitrogen to nitrates by autotrophic bacteria (Eze et al., 2018). However, the model did not account for these potential losses, which explains the surplus nitrogen observed in the modeling results.

9.3.3. Life Cycle Assessment

Fig. 9.3 presents the environmental impacts of the scenarios relative to global average emissions, emphasizing normalized midpoint and endpoint impacts (dimensionless values). Detailed results from Characterization and Normalization are provided in Appendix D.

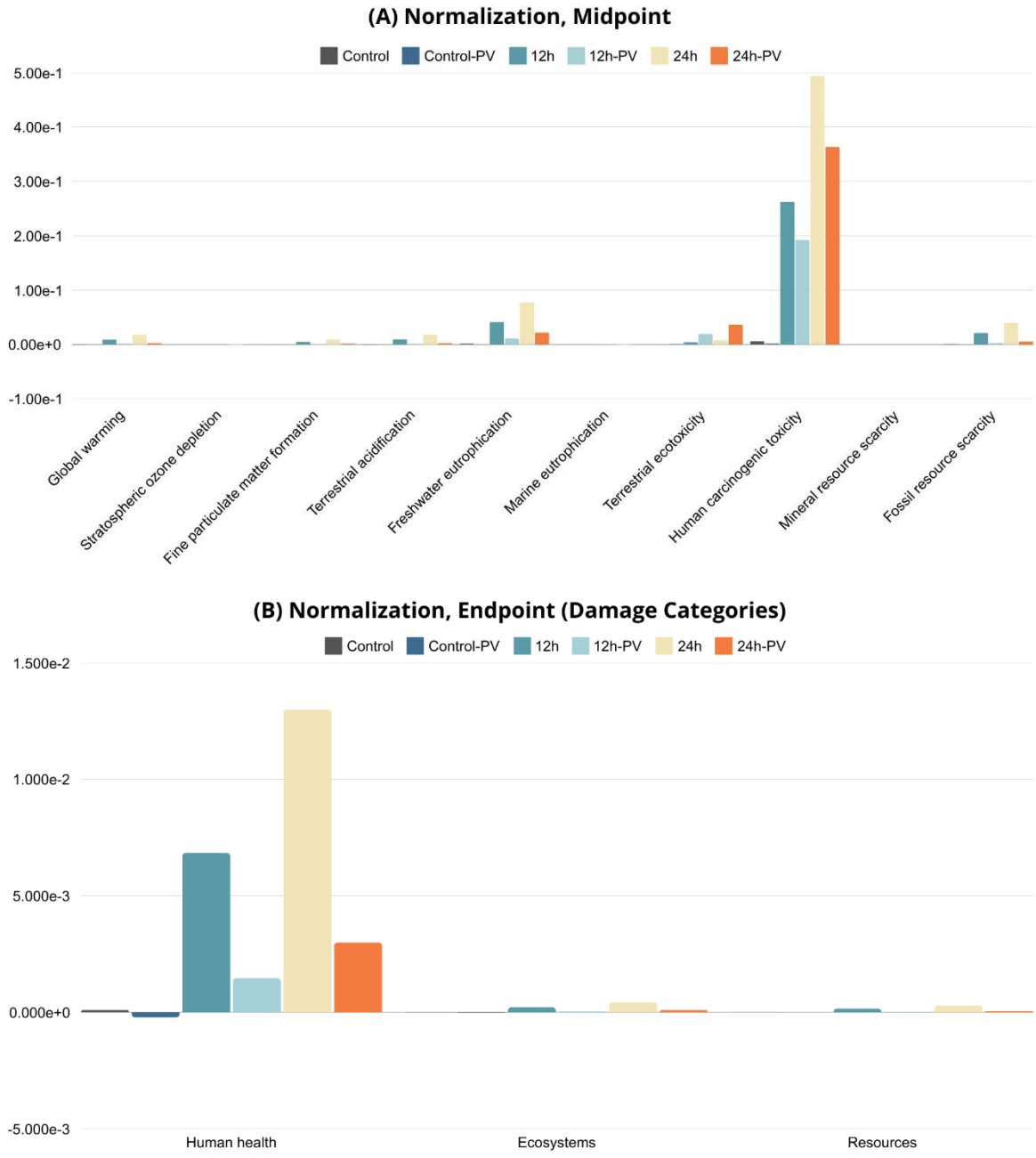

Fig. 9.3. Normalized midpoint (A) and endpoint (B) impacts.

Negative normalized values indicate environmental benefits primarily due to avoided products from wastewater cultivation. Still, in most scenarios—excluding the Control—the reductions are insufficient to offset the overall burdens. Still, all positive normalized values remain below 1, indicating that the overall

environmental footprint of these scenarios is lower than the global average per person per year.

Integrating PV technology into microalgal wastewater treatment notably influences environmental outcomes. While microalgae-based treatment can effectively remove nutrients and valorize biomass, its energy requirements—especially in closed photobioreactors—elevate Human Carcinogenic Toxicity (HCT) when fossil fuel-based electricity is used. Portugal's reliance on natural gas and fuel oil for approximately 40% of its electricity mix results in higher NOx, SO2, and PM2.5 emissions, driving secondary pollutant formation and increased cancer risks (Dias et al., 2025; WHO, 2021). The incorporation of PV energy helps reduce operational emissions, as reflected in lower HCT values (0.192 in 12h-PV and 0.364 in 24h-PV). However, upstream impacts related to raw material extraction, manufacturing, and end-of-life management of PV equipment still contribute to this category (de Haes and Lucas, 2024). Even so, these impacts remain below the global reference, reinforcing the environmental feasibility of these configurations.

Under the 24-hour condition, for instance, HCT drops from 0.49 to 0.36, significantly lowering cancer-related risks. Similarly, the Global Warming Potential (GWP) decreases from 0.017 to 0.003 when PV replaces grid electricity, underscoring how renewable energy can alleviate direct fossil fuel emissions. Fig. 9.4 further illustrates these trade-offs with Single Score endpoint impacts (in points, or Pt), providing a holistic overview of each system's environmental performance.

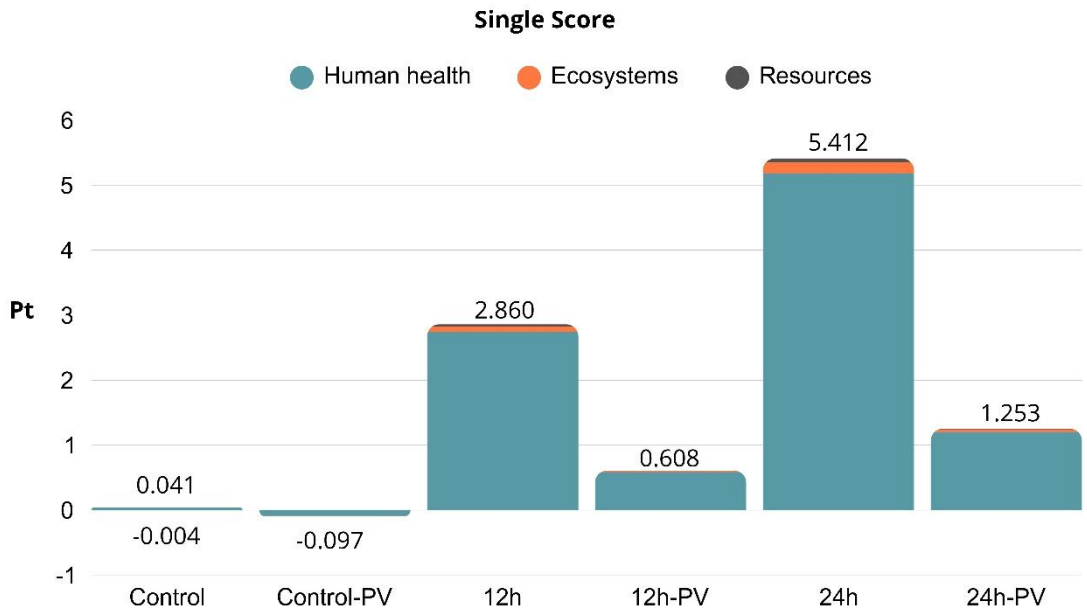

Fig. 9.4. Single score endpoint impacts for each system in points (Pt).

Despite improved biomass productivity in LED systems, increased energy demand contributed to substantially higher environmental impacts—approximately 78 times greater for the 12h system and 149 times greater for the 24h system than the Control. A more detailed examination showed that Fine Particulate Matter Formation and Global Warming (Human Health) accounted for about 87% and 86% of the total impacts for the 12h and 24h systems, respectively (Supplementary Materials, Appendix D). However, integrating PV systems mitigated these impacts, lowering them by 79% in the 12h system and 77% in the 24h system. Notably, the Control scenario shifted from 0.04 Pt to −0.10 Pt, indicating a net environmental benefit largely driven by reductions in Fine Particulate Matter Formation and Global Warming (Human Health).

Electricity consumption remains a central environmental hotspot for algal cultivation (Bhatt et al., 2024). Open systems, such as HRAPs, often show lower energy requirements than closed photobioreactors, making them more cost-effective (Magalhães et al., 2024). However, their reduced biomass productivity can limit commercial potential (Magalhães et al., 2022). Similar trade-offs occur in other wastewater-based cultivation strategies, including industrial CO_2 supplementation and hybrid systems, where significant environmental benefits

are achieved only when flue gas use offsets other emissions (Magalhães et al., 2021).

In a large-scale Italian study on LED-based systems for freshwater cultivation, nighttime LED use accounted for only 15% of total energy consumption, while aeration and thermoregulation were dominant contributors (Gurreri et al., 2024). Notably, infrastructure impacts were excluded, underscoring the importance of future research that fully integrates capital equipment footprints. In the present work, because the same processing capacities were adopted for all scenarios, LED use represented an incremental demand, thereby shifting the energy distribution. In the control system, the compressor was the main energy consumer (57%), whereas this share dropped to 0.8% under 12-hour LED operation and to 0.4% under 24-hour LED operation. These changes highlight how introducing LED illumination modifies overall energy dynamics in outdoor microalgal systems, emphasizing the need for comprehensive analyses that consider both operational and infrastructural factors to accurately evaluate economic and environmental performance.

9.3.4. Cost Assessment

The minimum selling price of the biomass produced was calculated for each of the six scenarios. Fig. 9.5 presents the equipment acquisition costs and minimum biomass selling price for each.

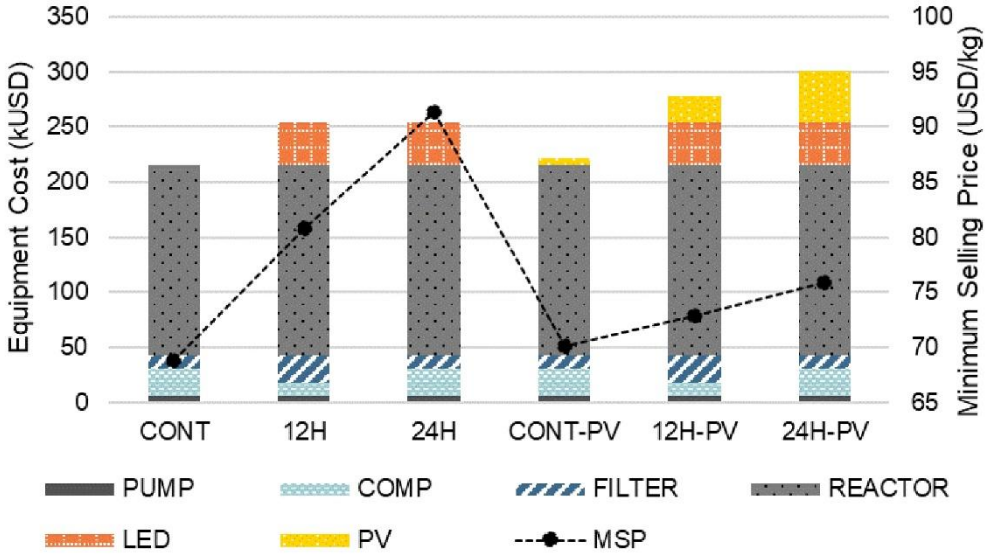

Fig. 9.5. Equipment Acquisition Cost and minimum biomass selling price (MBSP) for each scenario. PV = photovoltaic system.

The payback period across all evaluated scenarios was 5.23 years, largely influenced by the applied tax rate, with further details provided in the subsequent sensitivity analysis. Integrating PV systems cut operational costs for LED-based cultivation by 9.8 % under 12-hour lighting and 16.9% under 24-hour lighting; however, these gains did not surpass the control scenario. A 5.8% increase in productivity would be needed for the 12h-PV system to match the lowest Control cost (68.85 USD/kg). Both LED regimes resulted in higher total capital costs and selling prices than the Control, primarily due to the LED components, while PV integration further raised initial investments.

The medium scale proposed—producing under 4,000 kg/year for all scenarios—resulted in biomass costs ranging between $69 and $91/kg, reflecting limited economies of scale. The literature strongly indicates that scaling up microalgal production can substantially reduce costs (Clippinger and Davis, 2019; Schipper et al., 2021). At smaller scales (hundreds of kilograms per year), prices reportedly exceed 500 €/kg, whereas larger operations (thousands of kilograms per year) may achieve around 50 €/kg. Biomass produced in closed systems are unlikely to drop below 20 €/kg, yet open ponds using wastewater and flue gas could potentially reach under 2 €/kg, provided production volumes are

sufficiently high (approximately 10,000 tons of biomass per year) (Fernández et al., 2021).

Mallory et al. (2020) highlighted that although some literature cites sewage sludge prices around 50.70 USD/t, actual market values often do not exceed 5.07 USD/t — substantially lower than those found in this study. Nevertheless, incorporating microalgae as a final polishing step in wastewater treatment can help offset rising sludge disposal costs, which in Italy already range from €103/ton to €202/ton and have risen by 95% between 2016 and 2021 (Domini et al., 2022). Although cultivating microalgae involves higher upfront costs, it yields biomass suitable for applications such as animal feed, biofertilizers, or nutraceuticals and enhances nutrient recovery by capturing phosphorus and nitrogen (Calijuri et al., 2022). However, to remain competitive, microalgae cultivation must balance operational expenses against escalating sludge disposal costs. Further research on cradle-to-grave environmental footprints and life cycle costs—potentially integrating carbon credit trading or high-value by-product extraction (see Topic 3.5)—would help determine when microalgae-based approaches outperform traditional sludge disposal.

Similarly, a study by Nobre et al. (2024), which assumed a fixed biomass selling price of €0.40/kg, which is rather optimistic, reported a negative net present value (NPV) of €1.3 million and a 22-year payback period, even after accounting for nitrogen and phosphorus removal benefits. This result underscores the persisting economic challenges of microalgae-based systems, particularly when revenue from biomass sales is constrained or uncertain. It also highlights the importance of exploring additional revenue streams and cost reduction strategies to improve financial returns and shorten payback periods for microalgae-driven wastewater treatment, which will be further covered in topic 9.3.5.

The following section will address the sensitivity of these outcomes to key parameters, including tax rates, productivity, and equipment costs.

9.3.5. Sensitivity

Nine parameters were analyzed for cost sensitivity and two for the environmental assessment. Fig. 9.6 shows the results of the sensitivity analysis for the 12h-PV system, which was the closest price to the Control unit.

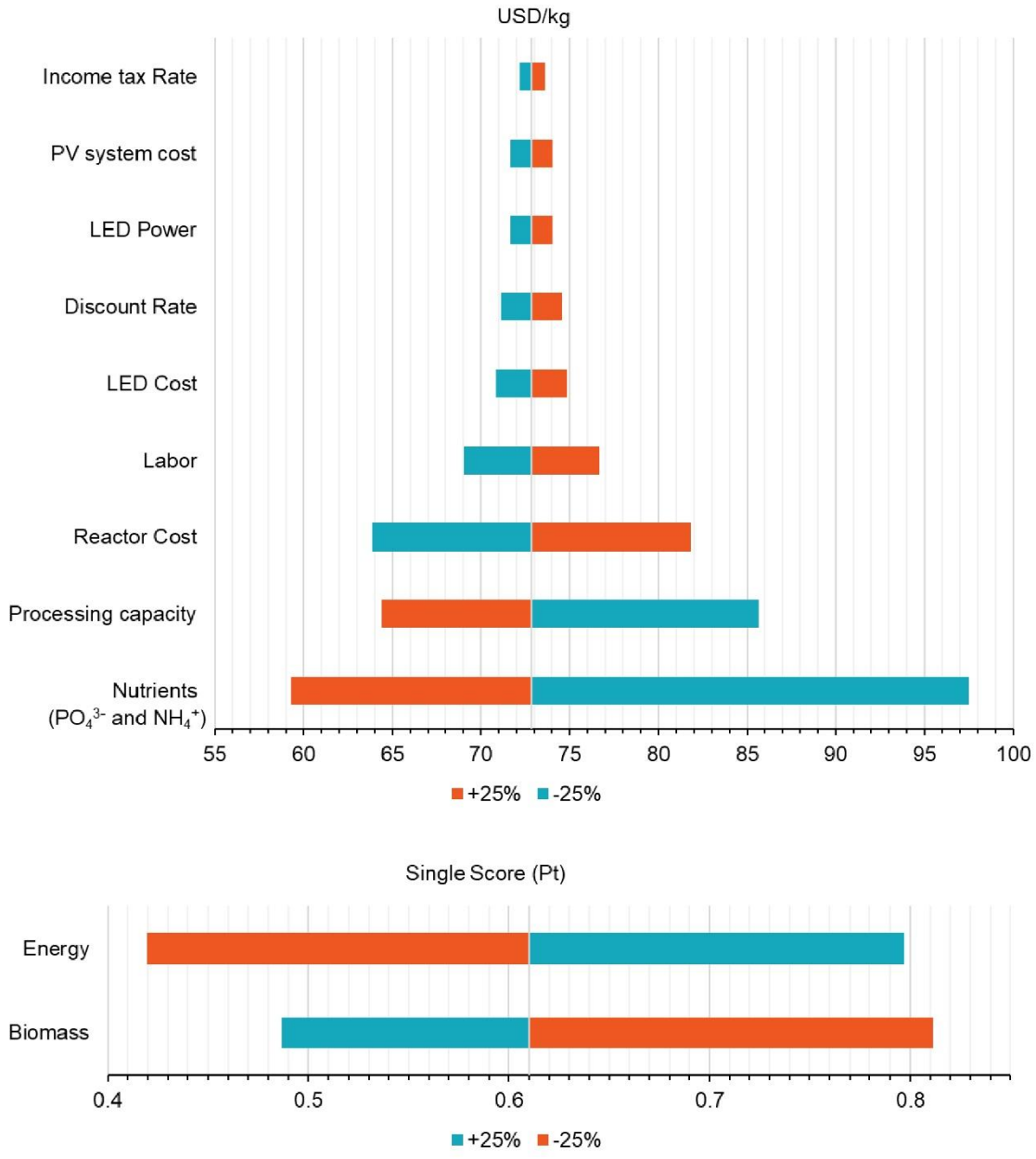

Fig. 9.6. Cost and environmental sensitivity analysis for the 12h-PV system.

Sensitivity analysis indicates that nutrient availability exerts the strongest effect on selling prices, with a 25% increase in nutrient levels reducing costs to 59.28 USD/kg and a 25% decrease raising them to 97.50 USD/kg. This finding

highlights the importance of maintaining sufficient nutrient concentrations, whether by blending different effluents (Magalhães et al., 2024) or leveraging nutrient-rich agro-industrial wastewater (Magalhães et al, 2022). However, additional treatment parameters particularly chemical oxygen demand (COD)—must be considered to confirm the overall feasibility of this approach.

Processing capacity emerged as the second most significant driver (85.62 USD/kg at −25%, 64.38 USD/kg at +25%), consistent with prior discussions emphasizing economies of scale as critical for reducing unit costs (see Topic 3.3). Reactor cost also significantly influenced overall feasibility, reducing to 63.89 USD/kg at a −25% variation but increasing to 81.80 USD/kg at +25%. Lower reactor costs can be achieved through innovative materials that fulfill requirements for transparency, corrosion resistance, chemical inertness, and ease of maintenance (Sathinathan et al., 2023). For instance, polymers such as PVC (0.46 EUR/kg) are more economical than acrylic (PMMA, ~0.66 EUR/kg) used in this study (Plastiker, 2025). Although plastic bags may offer further cost savings, their short lifespan, typically 3–5 months (Sathinathan et al., 2023), necessitates careful consideration of overall costs and environmental sustainability.

Notably, the annual discount rate was the only parameter affecting the payback period, following the model proposed by Peters and Timmerhaus (2003). In the reduced tax scenario, payback rose to 6.41 years, whereas a 25% tax increase dropped the payback to 4.42 years. However, if sanitation is viewed as a public policy concern rather than a market-driven endeavor (Mallory et al., 2020), governments and sanitation agencies may support longer payback periods than the ones proposed here, recognizing that these investments yield broader public health and environmental benefits and further dropping biomass prices.

Finally, labor, LED costs, discount rate, LED power, PV system cost, and income tax rate exhibited relatively smaller effects. Nonetheless, these factors remain essential to optimizing microalgae-based wastewater treatment, as reducing labor through automatization (Acién Fernández et al., 2019) and supportive policies (Mallory et al., 2020) contribute to lowering overall costs and enhancing economic sustainability.

Figure 6 also reveals that electricity use is the dominant driver of environmental performance: a 25 % cut in electricity demand lowers the impacts by 31 %, whereas a 25 % rise in biomass productivity reduces it by 20 % (in points, from the endpoint single score analysis). This result highlights the need for energy-optimized lighting. Our cultivation experiments (Section 3.1) showed no statistically significant gain when the photoperiod exceeded 12 h day^{-1}, indicating that future evaluation of a 0–12 h supplementation window could reduce power demand without sacrificing yield. Additional savings may be achieved through micro-controller-based dimming or on–off control (e.g., Arduino systems) and by adopting LEDs with higher photosynthetically active radiation efficiency (≥ 3.6 µmol photons s^{-1} W^{-1}) (Singh and Mishra, 2023). These measures could markedly lower the environmental footprint of LED-assisted wastewater cultivation, but each must be validated experimentally to confirm technical and economic viability.

9.3.6. Future Perspectives for Sustainable Production and Cost Reduction

Although the experiments in this study were conducted during spring, the impact of LED systems could be even more pronounced in winter months when natural light availability is lower. This study primarily addresses the influence of LED technology on biomass productivity, yet its benefits may extend beyond overall yield. LEDs can significantly boost high-value product accumulation, such as carotenoids (Zheng et al., 2022). Therefore, further research should explore pigment extraction and other product streams that could enhance the value proposition of LED-driven microalgal systems.

Considering seasonal operational strategies, such as extending the hydraulic retention time (HRT) during winter (Sutherland et al., 2020), integrating LED systems as removable external modules presents a promising approach to enhance economic performance. By reducing LED usage when sunlight is plentiful—typically in summer—and deploying it primarily in winter and autumn when productivity naturally declines, this strategy could help optimize costs.

In addition to improved product yields, integrating carbon credit trading also offers a promising avenue for enhancing the economic viability of wastewater-grown microalgae. However, at a minimum selling price (MSP) of around 60–70 USD/kg of biomass, the direct contribution from carbon credits remains relatively modest. Given that each kilogram of microalgal biomass can capture close to 2 kg of CO_2, the revenue from carbon credits would be roughly 1–2 USD/kg of algae—covering only a small fraction of overall production costs. Nonetheless, as carbon prices increase, carbon reduction strategies become more attractive (Dias et al., 2022), underscoring the role of sustainable technologies in long-term economic and environmental performance. For example, companies like Brilliant Planet (Chaudhary, 2024) cultivate algae in seawater for carbon capture, while other studies have shown that 75% of purified CO_2 used in microalgae cultivation can be effectively removed from the atmosphere at prices of 50–100 USD/ton (Manganaro et al., 2015). Similarly, López-Sánchez et al. (2023) proposed incorporating carbon credits into biorefineries producing distributed biogas and animal feed, estimating an approximately 38 million USD income for capturing 10 $MtCO_2$ eq.

These results suggest that LED technology could play an important role in enabling future innovations in wastewater-based microalgae cultivation. However, its widespread application will depend on advancements in cost reduction, energy efficiency, and policy support to ensure cleaner, more profitable, and resilient production processes.

9.4. Conclusion

LED-assisted microalgae cultivation in domestic wastewater boosted yields by up to 34% but increased capital and operating costs, leading to higher minimum biomass selling prices (80.76–91.37 USD/kg) compared with the control (68.85 USD/kg). Life cycle assessment revealed significantly elevated impacts—78 to 149 times greater for LED systems—although photovoltaic integration partially mitigated them. Sensitivity analysis underscored nutrient availability, scale, and reactor cost as key viability drivers. While LEDs offer

productivity gains, their added complexity and energy demand require careful balancing of economic and environmental outcomes.

Looking ahead, cost-effective reactor materials, automation, and a seasonal approach to LED deployment could help improve feasibility. Furthermore, tapping into multiple renewable energy pathways—such as solar energy for biomass drying (e.g., via solar dryers)—aligns well with the broader goal of leveraging sunlight at all stages, thereby reducing the overall environmental footprint. Co-product valorization, carbon credits, and supportive policy frameworks may also offset production costs. Ultimately, a holistic strategy that integrates technological efficiency, renewable energy, and regulatory support is vital for realizing cleaner, more economically viable microalgal wastewater treatment systems.

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10. Conclusões Gerais

A presente tese reforçou o potencial dos sistemas algais, especialmente das Lagoas de Alta Taxa (LATs) e de fotobiorreatores tubulares, como alternativas sustentáveis ao tratamento convencional de esgoto doméstico. A análise envolvendo LATs evidenciou tanto sua versatilidade quanto as limitações à aplicação em larga escala, como a remoção inconsistente de matéria orgânica. Ainda assim, pesquisas recentes em estratégias operacionais inovadoras, como técnicas de controle avançado baseadas em inteligência artificial e a mistura de diferentes efluentes, têm se mostrado promissoras para viabilizar sua difusão como tecnologia que promova a bioeconomia circular.

Ao investigar a operação em série de LATs, constatou-se um aumento significativo na remoção de patógenos (coliformes e *E. coli*), da ordem de 10%, em relação a sistemas operados individualmente. No entanto, parâmetros como o carbono orgânico total indicaram que a composição nutricional do meio também desempenha papel determinante na eficiência de remoção. Já o estudo das taxas de aplicação orgânica, situado entre 32,48 e 64,96 kg DBO ha^{-1} d^{-1}, não revelou diferenças estatisticamente significativas em termos de desempenho de tratamento ou produção de biomassa, sugerindo que operar em cargas mais elevadas, possivelmente por meio de mistura com outros efluentes, poderia trazer resultados mais expressivos.

Com relação à operação de reatores fechados ao ar livre, a complementação de luz LED demonstrou ganhos na produção de biomassa, que aumentou de 340 mg L^{-1} (controle) para 523 mg L^{-1} (12 h de LED) e 555 mg L^{-1} (24 h de LED). As análises de citometria de fluxo evidenciaram maior densidade celular e atividade enzimática no reator de 24 h, indicando o papel da luz na viabilidade celular. Em contrapartida, a análise econômica apontou um aumento no preço mínimo de venda da biomassa produzida com LEDs (≈68 $/kg no controle vs. 80–91 $/kg nos reatores com LED). A adoção de energia fotovoltaica reduziu os custos de operação do reator de 12 h para valores mais próximos ao controle, além de mitigar impactos ambientais em mais de 70%. A análise de sensibilidade indicou, ainda, grande influência do conteúdo nutricional do esgoto, da escala de processamento e do custo dos reatores no preço de venda final.

De maneira geral, os achados corroboram as hipóteses de que ajustes operacionais podem impulsionar a eficiência de sistemas algais. Também se evidenciou a importância de avaliações sistêmicas, como estudos de ciclo de vida e análises de viabilidade econômica, para embasar a adoção em larga escala. Conclui-se, portanto, que estratégias de operação otimizadas têm o potencial de viabilizar essas tecnologias, fortalecendo a economia circular e garantindo maior sustentabilidade no tratamento de esgoto doméstico.

11. Recomendações

Baseados nos resultados e discussões apresentados ao longo deste trabalho, surgem diversas oportunidades de pesquisa e ações práticas para aprimorar o projeto, a operação e a sustentabilidade de sistemas de tratamento esgoto doméstico via microalgas:

- *Projeto, Hidrodinâmica e Parâmetros Operacionais de LATs*

Modelagem CFD e Avaliações Hidrodinâmicas: Pesquisas futuras devem empregar CFD para otimizar velocidade de mistura, profundidade dos reatores e configurações geométricas, reduzindo zonas mortas e melhorando tanto a penetração de luz quanto a eficiência hidrodinâmica. O monitoramento contínuo de traçadores em condições abertas — organizadas em paralelo ou em série — pode refinar ainda mais diretrizes de projeto, sobretudo em operações de grande escala e longo prazo.

- *Ampliação de Cenários Operacionais e Fontes de Esgoto*

Taxas de Aplicação Orgânica e Mistura de Efluentes: Investigações em cargas orgânicas mais elevadas, incluindo a mistura de esgoto doméstico com efluentes industriais, podem esclarecer os limites máximos de desempenho das LATs. Essa abordagem ajuda a refinar coeficientes de remoção (por exemplo, para DBO, DQO, nitrogênio e fósforo) e a aprimorar estratégias de projeto e controle.

Colheita: Estudos aprofundados sobre métodos de colheita eficientes — via sedimentação, eletrocoagulação ou técnicas inovadoras baseadas em nanopartículas — podem reduzir custos e demanda energética.

- *Contaminantes Emergentes e Remoção de Patógenos*

Mecanismos de Detecção e Remoção: Como as normas para contaminantes emergentes ainda são pouco definidas, é fundamental investigar processos de fotodegradação, biodegradação e adsorção em LATs. Entender o papel de microplásticos e outros contaminantes em consórcios alga-bactéria também pode direcionar otimizações específicas de processo.

- *Estratégias Avançadas de Controle e Integração com IA*

Otimização Baseada em Dados: O uso de *Machine Learning* e IA pode auxiliar na previsão de produtividade de biomassa, remoção de nutrientes e estabilidade do processo. Pesquisas futuras devem focar no desenvolvimento de IA explicável, protocolos padronizados de modelagem e interfaces amigáveis, permitindo maior adoção e suporte à tomada de decisão em tempo real.

- *Cultivo Assistido por LEDs e Abordagens Sazonais*

Ajuste de Fotoperíodo e Espectro: Investigar diferentes intensidades de luz, fotoperíodos e composições espectrais contribui para compreender como esses fatores afetam a assimilação de nutrientes, a atividade metabólica e o acúmulo de compostos de alto valor;

Implantação Sazonal e Energia Renovável: O uso de módulos de LED removíveis ou ajustáveis em períodos mais frios e com menor incidência solar pode melhorar a viabilidade econômica, principalmente quando aliado a sistemas fotovoltaicos. A análise de receitas geradas por compostos de alto valor e créditos de carbono também pode fortalecer o retorno financeiro.

- *Sustentabilidade Econômica e Ambiental*

Avaliações de Ciclo de Vida e Análises Técnico-econômicas: A realização de ACVs detalhadas e análises de custos com inventários de dados padronizados facilitará comparações justas com outros métodos de tratamento de efluentes. A inclusão de impactos sociais, créditos de carbono e incentivos políticos tende a fortalecer a viabilidade das tecnologias de microalgas no longo prazo.

Biorrefinarias: Integrar LATs a outros processos ou adicionar sistemas de crescimento aderido pode abrir novas oportunidades de geração de coprodutos de maior valor, alinhando o tratamento de efluentes com a bioeconomia circular.

O estudo destes temas — abrangendo desde o projeto avançado de reatores e o uso diversificado de efluentes até técnicas de controle inovadoras e avaliações econômico-ambientais aprofundadas — pode acelerar a adoção e a

escalabilidade de tecnologias baseadas em microalgas para o tratamento sustentável de esgoto.

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11. Appendix

a. Appendix A

Supplementary Material for

Sustainable sanitary sewage treatment using high-rate algal ponds:

A comprehensive review of technology, challenges, and opportunities

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
S	After primary settling		12	0,6	3	275	550,000	0,15							El Hamouri et al. (1994)
S	After primary settling		24	0,5	3	275	458,333	0,15	69,00%		52,00%	88,00%		2,73	
S	After primary settling		4,7	0,3	3	275	275,000	0,15							
S	Primary settled	Winter	5	0,3	8			0,2	37,00%		32,00%				Sutherland et al. (2020)
S	Primary settled	Winter	330	0,3	8			0,2	45,00%		50,00%				
S	Primary settled	Winter	10000	0,3	8			0,2	32,00%		32,00%				
S	Primary settled	Spring	5	0,3	8			0,2	52,00%		39,00%				
S	Primary settled	Spring	330	0,3	8			0,2	61,00%		40,00%				
S	Primary settled	Spring	10000	0,3	8			0,2	41,00%		14,00%				
S	Primary settled	Summer	5	0,3	8			0,2	76,00%		42,00%				
S	Primary settled	Summer	330	0,3	8			0,2	90,00%		38,00%				
S	Primary settled	Summer	10000	0,3	8			0,2	69,00%		42,00%				
T	Facultative treated domestic wastewater			0,45	5	135			90,00%	59,65 %		90,37%			Banat et al. (1990)

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
T	Grease/sand trap and anaerobic pond treated domestic wastewater		3023	0,4	4,2	100	95,238	0,15	78,92%			32,00%			El Hamouri et al. (1995)
T	Grease/sand trap and anaerobic pond treated domestic wastewater		3023	0,4	4,2	113	107,619	0,15	21,89%			45,13%			
T	Septic tank treated domestic wastewater		192	0,32	4,5	203,99	135,280	0,2	69,80%			93,40%		1,76	Buchanan et al. (2018)
T	Septic tank treated domestic wastewater		208	0,43	6,4	203,99	137,056	0,2	73,50%			92,50%		2,19	
T	Septic tank treated domestic wastewater		226	0,55	9,1	203,99	123,291	0,2	61,10%			90,20%		1,86	
T	Facultative treated domestic wastewater		192	0,32	4,5	203,99	145,060	0,2	72,00%			72,00%		2,31	
T	Facultative treated domestic wastewater		208	0,43	6,4	203,99	137,056	0,2	83,00%			59,00%		2,12	
T	Facultative treated domestic wastewater		226	0,55	9,1	203,99	123,291	0,2	35,00%			51,00%		3,01	
T	Septic tank treated domestic wastewater		200	0,32	5	228	145,920	0,2				91,76%		2,13	Young et al. (2016)

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
T	anaerobic pond wastewater		10000	0,3	8			0,2	63,27%			76,00%			Sutherland et al. (2020b)
T	anaerobic pond wastewater		10000	0,3	8			0,2	62,47%			46,00%			
T	anaerobic pond wastewater	HRAP in series	10000	0,3	4			0,2	39,41%			51,79%			
Series	First-stage HRAP effluent	HRAP in series	10000	0,3	4			0,2	64,83%			31,25%			
S	Raw doemstic sewage		120	0,375	3,14	412	492,038	0,1		26,88%	11,11%	66,02%		2	Shelef et al. (1982)
T	Facultative treated domestic wastewater		48	0,45	8			0,15	85,00%		73,00%		81,00%		Picot et al. (1992)
S	Settling tank treated wastewater			0,3	8				80,40%	75,20%	47,50%				Chen et al. (2020)
S	Settling tank treated wastewater			0,3	4				93,60%	75,70%	40,70%				
T	Anaerobic digested domestic wastewater		31,8	0,3	8				74,29%	26,60%					Park et al. (2011)
S	Settling tank treated wastewater			0,4	–				73,76%						Wood et al. (1989)
T	Primary pond treated domestic wastewater		48	0,35	8			0,15	92,00%	30,54%	31,58%				Picot et al. (1992b)
T	Primary pond treated domestic wastewater		48	0,35	4			0,15	94,00%	47,81%	44,76%				
S	Primary treated	Autumn	12500	0,35	7			0,2	47,00%		37,00%				

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
	domestic wastewater														
S	Primary treated domestic wastewater	Winter	12500	0,35	9			0,2	53,00%		22,00%				Sutherland et al. (2013)
S	Primary treated domestic wastewater	Spring	12500	0,35	7			0,2	79,00%		49,00%				
S	Primary treated domestic wastewater	Summer	12500	0,35	5,5			0,2	77,00%		20,00%				
T	Step up-flow anaerobic reactor and gravel filter treated domestic wastewater		790	0,35	3	45	52,500		86,00%	86,00 %	66,00%	22,00%		1,23	El Halfiane and El Hamouri (2005)
S	Primary pond treated domestic wastewater		85	0,45	7,5			0,15	91,00%	51,95 %	15,32%			1,42	Craggs et al. (2003)
S	Primary pond treated domestic wastewater		128,1	0,3	7,5			0,15	85,00%	57,96 %	10,48%			1,49	
T	Facultative pond domestic wastewater			0,3	7,5									1	Davies-Colley et al. (2003)
T	Anaerobic digester treated domestic and laboratory wastewater				8									1	Davies-Colley et al. (2005)

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
T	Anaerobic digester treated domestic and laboratory wastewater				8									1	
S	Primary treated effluent (settler)		12500	0,35					64,29%		20,31%	50,94%			Carggs et al. (2003)
S	Primary treated effluent (settler)		12500	0,35					65,17%		23,44%	52,58%			
S	Primary treated effluent (settler)		12500	0,35					65,23%		21,63%	51,16%			
S	Primary treated effluent (settler)		12500	0,35					65,04%		17,83%	51,61%			
T	septic tank treated effluent		192	0,32	4,5	197	140,089		76,79%		16,67%	94,06%		1,60	Buchanan et al. (2018b)
T	septic tank treated effluent		208	0,43	6,4	197	132,359		78,04%		2,38%	91,42%		1,92	
T	septic tank treated effluent		226	0,53	9,1	197	114,736		52,79%		-2,38%	91,73%		1,72	
T	septic tank effluent further treated in a facultative pond		192	0,32	4,5	31,7	22,542		94,19%		10,84%	74,76%		2,65	
T	septic tank effluent further treated in a		208	0,43	6,4	31,7	21,298		75,00%		-31,33%	84,23%		2,81	

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
	facultative pond														
T	septic tank effluent further treated in a facultative pond		226	0,53	9,1	31,7	18,463		-2,91%		-15,66%	77,92%		2,63	
S	primary settled domestic wastewater	Air		0,3	8					66,00%	19,00%				Sutherland et al. (2016)
S	primary settled domestic wastewater	0.5% CO2		0,3	8					61,00%	22,00%				
S	primary settled domestic wastewater	2% CO2		0,3	8					61,00%	25,00%				
S	primary settled domestic wastewater	5% CO2		0,3	8					47,00%	19,00%				
S	primary settled domestic wastewater	10% CO2		0,3	8					35,00%	19,00%				
T	primary sedimentation chamber wastewater from a waste stabilization pond (WSP)		2,0826	0,25	2	266,49	333,113	0,20	93,32%	99,96%	72,33%	58,37%	54,48%		Alemu et al. (2018)
T	primary sedimentation chamber wastewater from a waste stabilization pond (WSP)		2,0826	0,25	4	266,49	166,556	0,20	96,84%	99,98%	67,91%	67,77%	63,22%		

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
T	primary sedimentation chamber wastewater from a waste stabilization pond (WSP)		2,0826	0,25	6	266,49	111,038	0,20	100,00%	85,42%	72,97%	74,86%	78,03%		
T	primary sedimentation chamber wastewater from a waste stabilization pond (WSP)		2,0826	0,25	8	266,49	83,278	0,20	100,00%	99,99%	99,94%	81,86%	75,59%		
T	primary sedimentation chamber wastewater from a waste stabilization pond (WSP)		2,0826	0,3	2	266,49	399,735	0,20	87,93%	99,96%	61,10%	54,96%	57,18%		
T	primary sedimentation chamber wastewater from a waste stabilization pond (WSP)		2,0826	0,3	4	266,49	199,868	0,20	96,87%	99,98%	70,92%	64,15%	64,19%		
T	primary sedimentation chamber wastewater from a waste stabilization pond (WSP)		2,0826	0,3	6	266,49	133,245	0,20	100,00%	99,28%	76,26%	70,11%	76,79%		
T	primary sedimentation chamber wastewater from a waste stabilization pond (WSP)		2,0826	0,3	8	266,49	99,934	0,20	100,00%	91,81%	100,00%	79,94%	74,92%		

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
S	Bar-screened domestic wastwater	Fall	120	0,4	3,4	409	481,176			75,20 %	95,70%				Shelef et al. (1992b)
S	Bar-screened domestic wastwater	Winter	120	0,5	4,25	398	468,235			62,80 %	93,60%				
S	Bar-screened domestic wastwater	Spring	120	0,35	2,9	412	497,241			72,70 %	95,20%				
S	Bar-screened domestic wastwater	Summer	120	0,25	2	428	535,000			83,30 %	97,20%				
T	Post UASB	Added Co2	3,30	0,2				0,15	76,80%		58,20%		43,10%	2	Couto et al. (2021)
T	Post UASB	Added Co2	3,30	0,3				0,15	40,90%		22,00%		40,60%	2	
T	Post UASB	Added Co2	3,30	0,4				0,15	39,40%		22,00%		42,20%	2	
T	Post UASB		3,30	0,2				0,15	84,40%		48,30%		42,20%	4	
T	Post UASB		3,30	0,3				0,15	47,70%		15,40%		42,10%	2	
T	Post UASB		3,30	0,4				0,15	62,50%		7,70%		40,40%	2	
T	Post UASB	UV pre disinfectio	3,30	0,4				0,15	56,60%		22,80%		47,50%	4	
T	Septic tank effluent		80,00	0,5	8			0,35	73,88%	20,83 %	16,18%		15,97%		Zhou et al. (2006)
Series	First-stage HRAP effluent	HRAPs in series	80,00	0,5	8			0,35	67,74%	10,81 %	6,90%		-0,39%		
T	Post septic tank		3,30	0,3	10			0,10	75,30%		13,40%		57,90%	1	Assis et al. (2020)
T	Post septic tank	hybdrid system (coupled biofilm reactor)	3,30	0,3	10			0,10	77,30%		16,20%		58,80%	1	
T	Post septic tank	CO2 at a concentration of 99.9%	3,30	0,3	8				65,10%	40,90 %	- 84,00%		31,70%	2	Assis et al. (2019)

App.	Effluent Source	Obs.	Area	D	HRT	BOD ef.	Organic load (kg BOD ha day)	v	Ammonia	TN	P	BOD (%)	COD (%)	Coliforms (log units)	Reference
T	Post septic tank	gas from the combustion of gasoline	3,30	0,3	8				65,70%	37,90 %	-11,30%		30,80%	2	
T	Post UASB	hybrid system without CO2 supplementation	3,30	0,3	5			0,15	84,00%		21,00%		33,00%	2	Assis et al. (2017)
T	Post UASB	hybrid system with CO2 supplementation	3,30	0,3	5			0,15	79,00%		25,00%		27,00%	2	
T	Post UASB		3,30	0,3	5			0,15	69,00%		27,00%		46,00%	1	
T	Post UASB		3,30	0,3	4			0,15	70,62%		14,32%				Assemany et al. (2015)
T	Post UASB	uv pre disinfection	3,30	0,3	4			0,15	74,38%		19%				
T	pre-treated by a full-scale upflow anaerobic sludge blanket (UASB) reactor and pre-disinfected by ultra-violet (UV) disinfection	HRAP with pre-ultraviolet disinfection treating anaerobic domestic sewage	3,30	0,3	8			0,15	41.15%	32.8%	-15,38%		52.18%	2,5	Assemany et al. (2017)
T	Post UASB								68,78%	43,50 %	-5,45%		-18,25%		Assemany et al. (2015b)
T	Post UASB	30% shading							58,94%	38,99 %	-6,06%		-8,47%		
T	Post UASB	80% shading							69,59%	33,13 %	1,21%		6,90%		
T	Post settling		1,50	0,3	4,5			0,002	94,00%		35,00%		68,00%		Arashiro et al. (2019)
S	Raw doemstic sewage		1,50	0,3	4,5			0,002	92,00%		26,00%		71,00%		
T	Post UASB		0.68		8				58,00%	55,00 %			37,00%		Vassalle et al. (20200

Parameter	Area	Depth	HRT	BOD ef.	Organic load (kg BOD ha day)	Mix. Speed	Ammonia	Total Nitrogen	Phosphorus	BOD (%)	COD (%)	Coliforms (log units)
Mean	2272,63	0,35	6,29	228,70	216,74	0,18	70,36%	61,71%	31,29%	67,74%	44,53%	2,00
Minimum	1,50	0,20	2,00	31,70	18,46	0,002	-2,91%	10,81%	-84,00%	22,00%	-18,25%	1,00
Maximum	12500,00	0,60	10,00	428,00	550,00	0,35	100,00%	99,99%	100,00%	94,06%	81,00%	4,00
Median	102,50	0,30	7,00	228,00	137,06	0,20	73,50%	61,00%	23,12%	70,11%	43,10%	2,00
n	80	90	81	33	32	62	79	35	74	35	31	36

Application	Area	Depth	HRT	BOD ef.	Organic load (kg BOD ha day)	Mix. Speed	Ammonia	Total Nitrogen	Phosphorus	BOD (%)	COD (%)	Coliforms (log units)
Seconday	4709,30	0,34	6,34	360,50	469,63	0,17	66,81%	60,84%	37,24%	60,05%		1,91
Tertiary	797,40	0,34	6,26	186,52	132,44	0,17	72,12%	64,86%	27,00%	70,69%	45,17%	2,01
Series	5040,00	0,40	6,00			0,28	66,28%	10,81%	6,90%	31,25%	-0,39%	

Parameter	Mean	Minimum	Maximum	Median	n
Area	2272,63	1,50	12500,00	102,50	80
Depth	0,35	0,20	0,60	0,30	90
HRT	6,29	2,00	10,00	7,00	81
BOD ef.	228,70	31,70	428,00	228,00	33
Organic load (kg BOD ha day)	216,74	18,46	550,00	137,06	32
Mix. Speed	0,18	0,00	0,35	0,20	62

TOTAL	96	%
Secondary	36	38%
Tertiary	58	60%
Series	2	2%

b. Appendix B

Effects of series operation of high-rate algal ponds treating sanitary sewage: remediation potential, biomass production and hydrodynamics evaluation

Supplementary Material

Environmental Conditions

Figure S1 presents monitoring data on photosynthetically active radiation and average daily temperature, based on data collected from INMET (2022) for the experiment period.

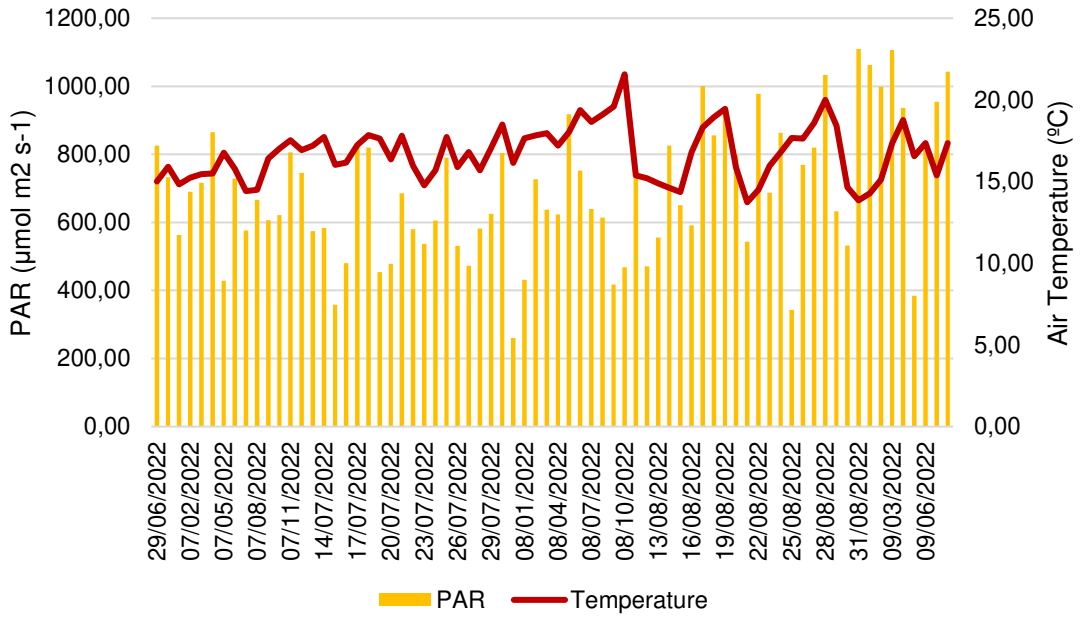


Figure S1. Climatic data for the operation period. Source: INMET.

Reynolds Number – Mixing Speed Data

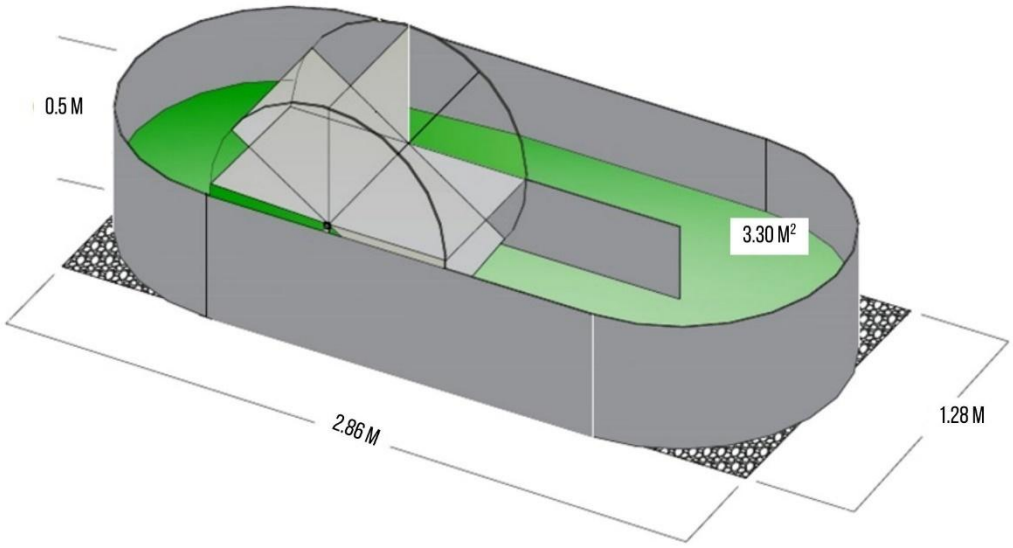


Figure S2. High-rate algal ponds design.

Table S1. Data collected for the mixing speed experiment.

Repetition	Time (s)	Δt (s)	Mixing Speed (m/s)
1	6.64	6.64	0.237951807
2	7.14	7.14	0.221288515
3	5.14	5.14	0.307392996
4	4.98	4.98	0.317269076
5	4.83	4.83	0.327122153
6	5.53	5.53	0.285714286
7	7.14	7.14	0.221288515
8	6.7	6.7	0.235820896
9	8.36	8.36	0.188995215
10	7.71	7.71	0.204928664
11	6.69	6.69	0.236173393
12	8.84	8.84	0.178733032
13	7.94	7.94	0.198992443
14	6.19	6.19	0.255250404
15	8.54	8.54	0.18501171
16	7.92	7.92	0.199494949
17	6.78	6.78	0.233038348
18	6.71	6.71	0.235469449
19	5.89	5.89	0.268251273
20	7.28	7.28	0.217032967

21	5.1	5.1	0.309803922
22	7.24	7.24	0.218232044
23	6.43	6.43	0.245723173
24	7.83	7.83	0.201787995
25	7.39	7.39	0.213802436
26	7.62	7.62	0.207349081
27	7.78	7.78	0.203084833
28	6.33	6.33	0.249605055
29	9.97	9.97	0.158475426
30	5.09	5.09	0.310412574

- Length: 1.58m
- Mean mixing speed: 0.2357 m/s

Considering the previous values:

$$R_h = \frac{A}{P} = \frac{d\ w_c}{w_c + 2d} = \frac{0.30\ \times 0.64}{0.64 + 2(0.30)} = 0.154$$

Finally, the Reynolds Number:

$$Re = \frac{\rho\ v\ R_h}{\mu} = \frac{1000\ \times 0.2357\ \times 0.154}{0.001003} = 36399.17$$

Bodenstein Number – Tracer Experiment Data

Total data points: 602

Peak concentration: 1711,111 (at 113 seconds)

Bo = 9,977292571

Minimized difference from Excel® Solver: 0,024133809

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
1	603,7462	0,0071029	0,220079716	-0,2129768	0,045359126
2	603,7361	0,0071028	0,298197956	-0,2910952	0,084736403
3	603,7261	0,0071027	0,349912888	-0,3428102	0,117518853
4	603,7692	0,0071032	0,387114068	-0,3800109	0,144408285
5	603,7664	0,0071031	0,414670947	-0,4075678	0,166111522
6	603,7636	0,0071031	0,435215024	-0,4281119	0,183279818
7	603,7609	0,0071031	0,450388115	-0,443285	0,196501631
8	603,7803	0,0071033	0,461309653	-0,4542064	0,206303413
9	603,7816	0,0071033	0,468790121	-0,4616868	0,213154709
10	603,7829	0,0071033	0,473442067	-0,4663387	0,217471819
11	603,7842	0,0071033	0,475743468	-0,4686401	0,219623566
12	603,8213	0,0071038	0,476076519	-0,4689727	0,21993543
13	603,8231	0,0071038	0,474752721	-0,4676489	0,218695512
14	603,8248	0,0071038	0,472029837	-0,464926	0,216156201
15	603,8266	0,0071038	0,468123788	-0,4610199	0,21253939
16	603,8407	0,007104	0,463217246	-0,4561132	0,208039286
17	603,8419	0,007104	0,457466016	-0,450362	0,202825925
18	603,8431	0,007104	0,451003878	-0,4438998	0,197047069
19	603,8444	0,0071041	0,443946332	-0,4368423	0,190831177
20	603,7973	0,0071035	0,436393543	-0,42929	0,184289943
21	603,7957	0,0071035	0,428432703	-0,4213292	0,177518315
22	603,7939	0,0071035	0,420139925	-0,4130365	0,170599124
23	603,7922	0,0071034	0,411581816	-0,4044784	0,163602759
24	603,8592	0,0071042	0,402816758	-0,3957125	0,156588408
25	603,8613	0,0071043	0,393895991	-0,3867917	0,14960785

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
26	603,8633	0,0071043	0,384864521	-0,3777602	0,142702804
27	603,8118	0,0071037	0,375761891	-0,3686582	0,135908885
28	603,8108	0,0071037	0,366622837	-0,3595192	0,129254041
29	603,8097	0,0071036	0,357477856	-0,3503742	0,122762089
30	603,8086	0,0071036	0,348353694	-0,3412501	0,116451606
31	603,8075	0,0071036	0,339273772	-0,3321702	0,110337012
32	603,9066	0,0071048	0,330258556	-0,3231538	0,104428361
33	603,9097	0,0071048	0,32132588	-0,3142211	0,098734875
34	603,9128	0,0071049	0,312491236	-0,3053864	0,093260841
35	603,7985	0,0071035	0,303768022	-0,2966645	0,088009831
36	603,7955	0,0071035	0,295167766	-0,2880643	0,082981035
37	603,7925	0,0071034	0,286700327	-0,2795969	0,078174418
38	603,7896	0,0071034	0,278374065	-0,2712707	0,07358777
39	603,8954	0,0071047	0,270196005	-0,2630914	0,06921706
40	603,8981	0,0071047	0,262171974	-0,2550673	0,065059323
41	603,9009	0,0071047	0,254306724	-0,247202	0,061108833
42	603,9036	0,0071047	0,246604052	-0,2394993	0,057359916
43	603,9893	0,0071058	0,239066894	-0,2319611	0,053805969
44	603,9948	0,0071058	0,231697419	-0,2245916	0,050441386
45	604,0004	0,0071059	0,224497114	-0,2173912	0,047258946
46	604,0059	0,007106	0,217466852	-0,2103609	0,044251709
47	603,9504	0,0071053	0,210606965	-0,2035017	0,041412928
48	603,9496	0,0071053	0,203917297	-0,196812	0,038734966
49	603,9489	0,0071053	0,197397267	-0,190292	0,03621104
50	603,9481	0,0071053	0,19104591	-0,1839406	0,033834158
51	604,0336	0,0071063	0,184861928	-0,1777557	0,031597071
52	604,0364	0,0071063	0,178843726	-0,1717374	0,02949374
53	604,0391	0,0071063	0,172989451	-0,1658831	0,027517206
54	604,0418	0,0071064	0,167297023	-0,1601906	0,025661044
55	603,9302	0,0071051	0,161764167	-0,1546591	0,023919439
56	603,9272	0,007105	0,156388438	-0,1492834	0,022285537
57	603,9242	0,007105	0,151167247	-0,1440623	0,020753934

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
58	603,9211	0,007105	0,146097882	-0,1389929	0,019319034
59	603,9288	0,007105	0,141177529	-0,1340725	0,017975431
60	603,9272	0,007105	0,136403289	-0,1292983	0,016718041
61	603,9257	0,007105	0,131772196	-0,1246672	0,015541908
62	603,9241	0,007105	0,127281228	-0,1201762	0,014442328
63	603,9298	0,0071051	0,122927326	-0,1158223	0,013414798
64	603,9301	0,0071051	0,1187074	-0,1116023	0,012455082
65	603,9305	0,0071051	0,114618345	-0,1075133	0,011559105
66	603,9308	0,0071051	0,110657045	-0,103552	0,010723012
67	603,9714	0,0071055	0,106820388	-0,0997148	0,00994305
68	603,9731	0,0071056	0,103105268	-0,0959997	0,009215943
69	603,9749	0,0071056	0,099508594	-0,092403	0,008538316
70	603,9766	0,0071056	0,096027299	-0,0889217	0,007907067
71	603,8874	0,0071046	0,09265834	-0,0855538	0,00731945
72	603,8843	0,0071045	0,089398707	-0,0822942	0,006772333
73	603,8813	0,0071045	0,086245425	-0,0791409	0,006263288
74	603,8783	0,0071045	0,083195559	-0,0760911	0,005789857
75	603,9301	0,0071051	0,080246216	-0,0731412	0,005349629
76	603,9306	0,0071051	0,077394549	-0,0702895	0,004940611
77	603,9311	0,0071051	0,074637758	-0,0675327	0,004560664
78	603,9316	0,0071051	0,071973094	-0,064868	0,00420786
79	604,0195	0,0071061	0,069397858	-0,0622917	0,003880262
80	604,024	0,0071062	0,066909404	-0,0598032	0,003576427
81	604,0284	0,0071062	0,06450514	-0,0573989	0,003294636
82	604,0016	0,0071059	0,06218253	-0,0550766	0,003033435
83	604,0021	0,0071059	0,059939091	-0,0528332	0,002791345
84	604,0025	0,0071059	0,057772399	-0,0506665	0,002567093
85	604,0029	0,0071059	0,055680082	-0,0485742	0,00235945
86	604,0031	0,0071059	0,053659828	-0,0465539	0,002167266
87	604,0027	0,0071059	0,051709379	-0,0446035	0,001989469
88	604,0023	0,0071059	0,049826535	-0,0427206	0,001825052
89	604,0019	0,0071059	0,048009148	-0,0409032	0,001673075

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
90	603,9986	0,0071059	0,046255129	-0,0391493	0,001532665
91	603,9985	0,0071059	0,044562442	-0,0374566	0,001402995
92	603,9984	0,0071059	0,042929107	-0,0358232	0,001283305
93	603,9982	0,0071059	0,041353194	-0,0342473	0,00117288
94	605,9873	0,0071293	0,03983283	-0,0327036	0,001069523
95	606,0717	0,0071303	0,038366191	-0,0312359	0,000975684
96	606,1561	0,0071312	0,036951506	-0,0298203	0,000889248
97	606,2405	0,0071322	0,035587054	-0,0284548	0,000809676
98	741,3499	0,0087218	0,034271164	-0,0255494	0,000652772
99	747,0307	0,0087886	0,033002213	-0,0242136	0,000586299
100	752,7115	0,0088554	0,031778626	-0,0229232	0,000525473
101	758,3923	0,0089223	0,030598874	-0,0216766	0,000469876
102	954,9877	0,0112351	0,029461476	-0,0182263	0,000332199
103	965,603	0,01136	0,028364992	-0,017005	0,000289169
104	976,2183	0,0114849	0,02730803	-0,0158231	0,000250371
105	986,8336	0,0116098	0,026289238	-0,0146794	0,000215486
106	1199,603	0,014113	0,025307307	-0,0111943	0,000125313
107	1211,818	0,0142567	0,024360968	-0,0101043	0,000102097
108	1224,033	0,0144004	0,023448992	-0,0090486	8,18772E-05
109	1236,248	0,0145441	0,022570189	-0,0080261	6,44182E-05
110	1648,999	0,0194	0,021723407	-0,0023234	5,39827E-06
111	1669,703	0,0196436	0,02090753	-0,001264	1,59761E-06
112	1690,407	0,0198871	0,020121479	-0,0002343	5,49142E-08
113	1711,111	0,0201307	0,019364209	0,00076651	5,87536E-07
114	1640,426	0,0192991	0,018634709	0,00066442	4,41455E-07
115	1644,713	0,0193496	0,017932001	0,00141756	2,00949E-06
116	1649	0,0194	0,017255139	0,00214486	4,60043E-06
117	1653,286	0,0194504	0,01660321	0,00284721	8,10663E-06
118	1140,308	0,0134154	0,015975328	-0,0025599	6,55329E-06
119	1117,945	0,0131523	0,015370638	-0,0022183	4,92105E-06
120	1095,581	0,0128892	0,014788315	-0,0018991	3,60668E-06
121	1073,218	0,0126261	0,014227559	-0,0016015	2,56469E-06

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
122	1001,279	0,0117798	0,013687599	-0,0019078	3,63988E-06
123	989,1401	0,0116369	0,013167689	-0,0015307	2,34319E-06
124	977,0007	0,0114941	0,012667109	-0,001173	1,37589E-06
125	1023,3	0,0120388	0,012185162	-0,0001463	2,1415E-08
126	1023,854	0,0120453	0,011721177	0,00032416	1,05082E-07
127	1024,409	0,0120519	0,011274504	0,00077737	6,04298E-07
128	1024,963	0,0120584	0,010844517	0,00121387	1,47348E-06
129	1100,966	0,0129525	0,010430611	0,00252193	6,36013E-06
130	1105,12	0,0130014	0,010032201	0,00296921	8,81621E-06
131	1109,275	0,0130503	0,009648723	0,00340157	1,15707E-05
132	1113,429	0,0130992	0,009279633	0,00381953	1,45888E-05
133	1189,843	0,0139982	0,008924406	0,00507375	2,57429E-05
134	1194,498	0,0140529	0,008582534	0,00547038	2,99251E-05
135	1199,153	0,0141077	0,008253529	0,00585415	3,42711E-05
136	1203,808	0,0141624	0,007936918	0,00622553	3,87572E-05
137	1328,462	0,015629	0,007632246	0,00799672	6,39475E-05
138	1335,147	0,0157076	0,007339074	0,00836854	7,00324E-05
139	1341,833	0,0157863	0,007056978	0,00872929	7,62005E-05
140	1348,518	0,0158649	0,00678555	0,00907937	8,24349E-05
141	1362,796	0,0160329	0,006524395	0,0095085	9,04116E-05
142	1365,693	0,016067	0,006273133	0,00979384	9,59194E-05
143	1368,591	0,0161011	0,006031398	0,01006967	0,000101398
144	1371,489	0,0161352	0,005798836	0,01033633	0,00010684
145	1332,804	0,01568	0,005575108	0,01010494	0,00010211
146	1331,62	0,0156661	0,005359883	0,01030623	0,000106218
147	1330,436	0,0156522	0,005152847	0,01049934	0,000110236
148	1329,253	0,0156383	0,004953693	0,01068458	0,00011416
149	1226,632	0,014431	0,004762127	0,00966884	9,34864E-05
150	1221,718	0,0143732	0,004577866	0,00979529	9,59476E-05
151	1216,804	0,0143153	0,004400638	0,0099147	9,83013E-05
152	1211,889	0,0142575	0,004230177	0,01002734	0,000100548
153	1186,861	0,0139631	0,004066232	0,00989684	9,79474E-05

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
154	1183,913	0,0139284	0,003908557	0,01001983	0,000100397
155	1180,964	0,0138937	0,003756917	0,01013678	0,000102754
156	1188,544	0,0139829	0,003611085	0,01037179	0,000107574
157	1188,348	0,0139806	0,003470842	0,01050972	0,000110454
158	1188,152	0,0139783	0,003335978	0,01064228	0,000113258
159	1187,956	0,013976	0,003206291	0,01076966	0,000115986
160	1210,055	0,0142359	0,003081584	0,01115436	0,00012442
161	1211,143	0,0142487	0,002961669	0,01128707	0,000127398
162	1212,232	0,0142616	0,002846366	0,01141519	0,000130306
163	1213,321	0,0142744	0,0027355	0,01153886	0,000133145
164	1251,323	0,0147214	0,002628902	0,01209254	0,00014623
165	1253,323	0,014745	0,002526412	0,01221856	0,000149293
166	1255,322	0,0147685	0,002427873	0,01234062	0,000152291
167	1257,322	0,014792	0,002333134	0,01245889	0,000155224
168	1258,484	0,0148057	0,002242053	0,01256364	0,000157845
169	1259,225	0,0148144	0,002154488	0,01265992	0,000160274
170	1259,966	0,0148231	0,002070308	0,01275282	0,000162634
171	1260,707	0,0148318	0,001989382	0,01284247	0,000164929
172	1268,602	0,0149247	0,001911587	0,01301314	0,000169342
173	1268,974	0,0149291	0,001836802	0,0130923	0,000171408
174	1269,347	0,0149335	0,001764914	0,01316858	0,000173411
175	1269,719	0,0149379	0,001695812	0,01324206	0,000175352
176	1255,423	0,0147697	0,001629389	0,01314029	0,000172667
177	1254,95	0,0147641	0,001565542	0,01319858	0,000174202
178	1254,478	0,0147586	0,001504172	0,01325439	0,000175679
179	1254,005	0,014753	0,001445186	0,01330781	0,000177098
180	1252,004	0,0147295	0,001388491	0,01334097	0,000177981
181	1251,653	0,0147253	0,001334	0,01339133	0,000179328
182	1251,302	0,0147212	0,001281628	0,01343957	0,000180622
183	1250,951	0,0147171	0,001231293	0,01348578	0,000181866
184	1232,015	0,0144943	0,001182917	0,01331138	0,000177193
185	1231,175	0,0144844	0,001136426	0,01334799	0,000178169

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
186	1230,335	0,0144745	0,001091745	0,01338278	0,000179099
187	1236,409	0,014546	0,001048806	0,01349718	0,000182174
188	1236,347	0,0145453	0,001007542	0,01353772	0,00018327
189	1236,285	0,0145445	0,000967887	0,01357664	0,000184325
190	1236,223	0,0145438	0,00092978	0,01361402	0,000185342
191	1240,365	0,0145925	0,000893161	0,01369937	0,000187673
192	1240,659	0,014596	0,000857972	0,01373802	0,000188733
193	1240,953	0,0145994	0,000824159	0,01377529	0,000189759
194	1241,247	0,0146029	0,000791668	0,01381124	0,00019075
195	1245,249	0,01465	0,000760447	0,01388954	0,000192919
196	1245,497	0,0146529	0,000730448	0,01392246	0,000193835
197	1245,745	0,0146558	0,000701623	0,0139542	0,00019472
198	1245,993	0,0146587	0,000673927	0,01398481	0,000195575
199	1253,861	0,0147513	0,000647317	0,01410399	0,000198923
200	1254,269	0,0147561	0,000621749	0,01413436	0,00019978
201	1254,678	0,0147609	0,000597183	0,01416373	0,000200611
202	1255,087	0,0147657	0,000573581	0,01419215	0,000201417
203	1251,671	0,0147255	0,000550905	0,01417464	0,00020092
204	1251,676	0,0147256	0,000529119	0,01419648	0,00020154
205	1251,68	0,0147256	0,000508189	0,01421746	0,000202136
206	1247,031	0,014671	0,00048808	0,01418287	0,000201154
207	1246,773	0,0146679	0,000468762	0,01419916	0,000201616
208	1246,516	0,0146649	0,000450203	0,01421469	0,000202057
209	1246,259	0,0146619	0,000432374	0,0142295	0,000202479
210	1246,002	0,0146588	0,000415247	0,0142436	0,00020288
211	1244,249	0,0146382	0,000398793	0,01423943	0,000202761
212	1244,086	0,0146363	0,000382987	0,01425332	0,000203157
213	1243,923	0,0146344	0,000367803	0,01426659	0,000203535
214	1243,758	0,0146324	0,000353217	0,01427923	0,000203896
215	1243,715	0,0146319	0,000339206	0,01429273	0,000204282
216	1243,673	0,0146314	0,000325748	0,0143057	0,000204653
217	1243,63	0,0146309	0,000312819	0,01431812	0,000205009

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
218	1243,588	0,0146304	0,000300401	0,01433005	0,00020535
219	1243,872	0,0146338	0,000288473	0,01434532	0,000205788
220	1243,878	0,0146339	0,000277016	0,01435684	0,000206119
221	1243,883	0,0146339	0,00026601	0,01436791	0,000206437
222	1245,254	0,01465	0,00025544	0,01439461	0,000207205
223	1245,313	0,0146507	0,000245287	0,01440545	0,000207517
224	1245,372	0,0146514	0,000235535	0,0144159	0,000207818
225	1245,431	0,0146521	0,000226169	0,01442596	0,000208108
226	1245,49	0,0146528	0,000217173	0,01443565	0,000208388
227	1246,871	0,0146691	0,000208532	0,01446054	0,000209107
228	1246,951	0,01467	0,000200234	0,01446978	0,000209374
229	1247,03	0,0146709	0,000192264	0,01447868	0,000209632
230	1247,11	0,0146719	0,000184609	0,01448727	0,000209881
231	1247,532	0,0146768	0,000177258	0,01449959	0,000210238
232	1247,574	0,0146773	0,000170197	0,01450714	0,000210457
233	1247,616	0,0146778	0,000163417	0,01451442	0,000210668
234	1247,14	0,0146722	0,000156905	0,01451533	0,000210695
235	1247,128	0,0146721	0,000150651	0,01452144	0,000210872
236	1247,116	0,014672	0,000144645	0,01452731	0,000211043
237	1247,104	0,0146718	0,000138877	0,01453293	0,000211206
238	1246,003	0,0146589	0,000133338	0,01452552	0,000210991
239	1245,948	0,0146582	0,000128019	0,01453019	0,000211126
240	1245,892	0,0146576	0,000122911	0,01453464	0,000211256
241	1245,837	0,0146569	0,000118006	0,0145389	0,00021138
242	1245,877	0,0146574	0,000113296	0,01454408	0,00021153
243	1245,859	0,0146572	0,000108772	0,01454839	0,000211656
244	1245,841	0,014657	0,000104429	0,01455252	0,000211776
245	1245,822	0,0146567	0,000100258	0,01455647	0,000211891
246	1246,018	0,014659	9,62523E-05	0,01456278	0,000212075
247	1246,026	0,0146591	9,24063E-05	0,01456672	0,000212189
248	1246,035	0,0146592	8,87133E-05	0,01457052	0,0002123
249	1246,043	0,0146593	8,51672E-05	0,01457416	0,000212406

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
250	1246,113	0,0146602	8,17621E-05	0,01457839	0,000212529
251	1246,119	0,0146602	7,84926E-05	0,01458173	0,000212627
252	1246,125	0,0146603	7,53532E-05	0,01458494	0,000212721
253	1246,131	0,0146604	7,23388E-05	0,01458803	0,00021281
254	1246,433	0,0146639	6,94445E-05	0,01459447	0,000212999
255	1246,447	0,0146641	6,66654E-05	0,01459742	0,000213085
256	1246,46	0,0146642	6,39971E-05	0,01460024	0,000213167
257	1246,474	0,0146644	6,14351E-05	0,01460296	0,000213247
258	1246,758	0,0146677	5,89752E-05	0,01460877	0,000213416
259	1246,775	0,0146679	5,66134E-05	0,01461133	0,000213491
260	1246,791	0,0146681	5,43457E-05	0,01461378	0,000213563
261	1246,808	0,0146683	5,21685E-05	0,01461616	0,000213632
262	1246,587	0,0146657	5,00782E-05	0,01461565	0,000213617
263	1246,583	0,0146657	4,80713E-05	0,01461761	0,000213675
264	1246,579	0,0146656	4,61444E-05	0,01461949	0,00021373
265	1246,574	0,0146656	4,42945E-05	0,01462128	0,000213782
266	1246,619	0,0146661	4,25184E-05	0,01462359	0,000213849
267	1246,617	0,0146661	4,08133E-05	0,01462527	0,000213898
268	1246,615	0,0146661	3,91763E-05	0,01462688	0,000213946
269	1246,613	0,014666	3,76047E-05	0,01462843	0,000213991
270	1246,505	0,0146648	3,60958E-05	0,01462867	0,000213998
271	1246,502	0,0146647	3,46473E-05	0,01463008	0,000214039
272	1246,498	0,0146647	3,32567E-05	0,01463143	0,000214079
273	1246,494	0,0146646	3,19217E-05	0,01463271	0,000214116
274	1246,284	0,0146622	3,064E-05	0,01463152	0,000214082
275	1246,274	0,014662	2,94096E-05	0,01463264	0,000214114
276	1246,264	0,0146619	2,82285E-05	0,0146337	0,000214145
277	1246,254	0,0146618	2,70946E-05	0,01463472	0,000214175
278	1246,457	0,0146642	2,60061E-05	0,01463819	0,000214277
279	1246,462	0,0146643	2,49611E-05	0,0146393	0,000214309
280	1246,467	0,0146643	2,3958E-05	0,01464036	0,00021434
281	1246,472	0,0146644	2,29951E-05	0,01464138	0,00021437

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
282	1246,412	0,0146637	2,20707E-05	0,0146416	0,000214376
283	1246,414	0,0146637	2,11833E-05	0,01464251	0,000214403
284	1246,415	0,0146637	2,03315E-05	0,01464337	0,000214428
285	1246,416	0,0146637	1,95138E-05	0,0146442	0,000214453
286	1246,408	0,0146636	1,87289E-05	0,01464489	0,000214473
287	1246,407	0,0146636	1,79755E-05	0,01464564	0,000214495
288	1246,406	0,0146636	1,72522E-05	0,01464635	0,000214516
289	1246,457	0,0146642	1,6558E-05	0,01464764	0,000214553
290	1246,459	0,0146642	1,58916E-05	0,01464833	0,000214574
291	1246,461	0,0146642	1,52519E-05	0,014649	0,000214593
292	1246,463	0,0146643	1,46379E-05	0,01464963	0,000214612
293	1246,524	0,014665	1,40485E-05	0,01465094	0,00021465
294	1246,527	0,014665	1,34828E-05	0,01465154	0,000214668
295	1246,531	0,0146651	1,29398E-05	0,01465213	0,000214685
296	1246,534	0,0146651	1,24186E-05	0,01465269	0,000214701
297	1246,572	0,0146656	1,19183E-05	0,01465363	0,000214729
298	1246,574	0,0146656	1,14381E-05	0,01465414	0,000214744
299	1246,577	0,0146656	1,09772E-05	0,01465463	0,000214758
300	1246,58	0,0146656	1,05348E-05	0,01465511	0,000214772
301	1246,547	0,0146653	1,01101E-05	0,01465515	0,000214773
302	1246,546	0,0146652	9,70258E-06	0,01465554	0,000214785
303	1246,545	0,0146652	9,3114E-06	0,01465592	0,000214796
304	1246,545	0,0146652	8,93593E-06	0,0146563	0,000214807
305	1246,489	0,0146646	8,57557E-06	0,014656	0,000214798
306	1246,486	0,0146645	8,22968E-06	0,01465631	0,000214807
307	1246,483	0,0146645	7,89771E-06	0,01465661	0,000214816
308	1246,48	0,0146645	7,57909E-06	0,01465689	0,000214824
309	1246,443	0,014664	7,27329E-06	0,01465676	0,000214821
310	1246,44	0,014664	6,97978E-06	0,01465702	0,000214828
311	1246,438	0,014664	6,69809E-06	0,01465728	0,000214836
312	1246,435	0,0146639	6,42773E-06	0,01465751	0,000214843
313	1246,478	0,0146644	6,16825E-06	0,01465828	0,000214865

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
314	1246,479	0,0146645	5,91922E-06	0,01465854	0,000214873
315	1246,48	0,0146645	5,68021E-06	0,01465879	0,00021488
316	1246,481	0,0146645	5,45083E-06	0,01465903	0,000214887
317	1246,683	0,0146669	5,23068E-06	0,01466163	0,000214963
318	1246,692	0,014667	5,0194E-06	0,01466195	0,000214973
319	1246,701	0,0146671	4,81663E-06	0,01466225	0,000214982
320	1246,71	0,0146672	4,62203E-06	0,01466255	0,000214991
321	1246,713	0,0146672	4,43527E-06	0,01466278	0,000214997
322	1246,717	0,0146673	4,25603E-06	0,014663	0,000215004
323	1246,721	0,0146673	4,08402E-06	0,01466322	0,00021501
324	1246,724	0,0146673	3,91894E-06	0,01466342	0,000215016
325	1246,717	0,0146673	3,76052E-06	0,0146635	0,000215018
326	1246,717	0,0146673	3,60848E-06	0,01466365	0,000215023
327	1246,717	0,0146673	3,46257E-06	0,0146638	0,000215027
328	1246,716	0,0146672	3,32255E-06	0,01466392	0,000215031
329	1246,591	0,0146658	3,18818E-06	0,01466259	0,000214991
330	1246,585	0,0146657	3,05922E-06	0,01466265	0,000214993
331	1246,58	0,0146656	2,93547E-06	0,01466271	0,000214995
332	1246,574	0,0146656	2,81671E-06	0,01466276	0,000214997
333	1246,568	0,0146655	2,70275E-06	0,0146628	0,000214998
334	1246,565	0,0146655	2,59338E-06	0,01466288	0,000215
335	1246,563	0,0146654	2,48843E-06	0,01466296	0,000215002
336	1246,56	0,0146654	2,38771E-06	0,01466302	0,000215004
337	1246,567	0,0146655	2,29106E-06	0,0146632	0,00021501
338	1246,567	0,0146655	2,19831E-06	0,0146633	0,000215012
339	1246,567	0,0146655	2,10931E-06	0,01466338	0,000215015
340	1246,567	0,0146655	2,0239E-06	0,01466347	0,000215017
341	1246,618	0,0146661	1,94195E-06	0,01466415	0,000215037
342	1246,62	0,0146661	1,8633E-06	0,01466425	0,00021504
343	1246,623	0,0146662	1,78783E-06	0,01466437	0,000215044
344	1246,625	0,0146662	1,71541E-06	0,01466446	0,000215046
345	1246,731	0,0146674	1,64592E-06	0,01466578	0,000215085

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
346	1246,737	0,0146675	1,57923E-06	0,01466591	0,000215089
347	1246,742	0,0146676	1,51524E-06	0,01466604	0,000215093
348	1246,747	0,0146676	1,45384E-06	0,01466616	0,000215096
349	1246,893	0,0146693	1,39492E-06	0,01466793	0,000215148
350	1246,901	0,0146694	1,33838E-06	0,01466809	0,000215153
351	1246,909	0,0146695	1,28413E-06	0,01466823	0,000215157
352	1247,013	0,0146707	1,23208E-06	0,01466951	0,000215194
353	1247,021	0,0146708	1,18212E-06	0,01466965	0,000215199
354	1247,027	0,0146709	1,13419E-06	0,01466977	0,000215202
355	1247,035	0,014671	1,0882E-06	0,01466991	0,000215206
356	1247,17	0,0146726	1,04407E-06	0,01467154	0,000215254
357	1247,177	0,0146727	1,00173E-06	0,01467167	0,000215258
358	1247,185	0,0146728	9,61094E-07	0,0146718	0,000215262
359	1247,193	0,0146729	9,22106E-07	0,01467194	0,000215266
360	1247,151	0,0146724	8,84697E-07	0,01467148	0,000215252
361	1247,151	0,0146724	8,48803E-07	0,01467152	0,000215253
362	1247,152	0,0146724	8,14361E-07	0,01467156	0,000215255
363	1247,153	0,0146724	7,81314E-07	0,01467161	0,000215256
364	1247,152	0,0146724	7,49605E-07	0,01467163	0,000215257
365	1247,151	0,0146724	7,19181E-07	0,01467165	0,000215257
366	1247,151	0,0146724	6,89989E-07	0,01467167	0,000215258
367	1247,15	0,0146724	6,61979E-07	0,01467169	0,000215259
368	1247,104	0,0146718	6,35104E-07	0,01467118	0,000215243
369	1247,102	0,0146718	6,09317E-07	0,01467118	0,000215243
370	1247,1	0,0146718	5,84576E-07	0,01467118	0,000215244
371	1247,099	0,0146718	5,60837E-07	0,01467119	0,000215244
372	1247,077	0,0146715	5,3806E-07	0,01467096	0,000215237
373	1247,075	0,0146715	5,16206E-07	0,01467095	0,000215237
374	1247,073	0,0146714	4,95238E-07	0,01467095	0,000215237
375	1247,071	0,0146714	4,75121E-07	0,01467095	0,000215237
376	1247,1	0,0146718	4,55818E-07	0,01467131	0,000215247
377	1247,1	0,0146718	4,37299E-07	0,01467133	0,000215248

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
378	1247,101	0,0146718	4,1953E-07	0,01467136	0,000215249
379	1247,102	0,0146718	4,02482E-07	0,01467139	0,00021525
380	1247,155	0,0146724	3,86125E-07	0,01467203	0,000215268
381	1247,158	0,0146724	3,70432E-07	0,01467208	0,00021527
382	1247,161	0,0146725	3,55375E-07	0,01467213	0,000215271
383	1247,163	0,0146725	3,4093E-07	0,01467216	0,000215272
384	1247,22	0,0146732	3,2707E-07	0,01467285	0,000215293
385	1247,223	0,0146732	3,13773E-07	0,0146729	0,000215294
386	1247,226	0,0146732	3,01015E-07	0,01467295	0,000215295
387	1247,229	0,0146733	2,88775E-07	0,01467299	0,000215297
388	1247,124	0,014672	2,77032E-07	0,01467177	0,000215261
389	1247,121	0,014672	2,65765E-07	0,01467175	0,00021526
390	1247,117	0,014672	2,54956E-07	0,01467171	0,000215259
391	1247,114	0,0146719	2,44586E-07	0,01467168	0,000215258
392	1247,198	0,0146729	2,34636E-07	0,01467268	0,000215288
393	1247,199	0,0146729	2,25091E-07	0,0146727	0,000215288
394	1247,201	0,014673	2,15933E-07	0,01467274	0,000215289
395	1247,202	0,014673	2,07147E-07	0,01467276	0,00021529
396	1247,141	0,0146722	1,98719E-07	0,01467205	0,000215269
397	1247,14	0,0146722	1,90632E-07	0,01467204	0,000215269
398	1247,139	0,0146722	1,82874E-07	0,01467204	0,000215269
399	1247,137	0,0146722	1,75431E-07	0,01467202	0,000215268
400	1247,183	0,0146727	1,6829E-07	0,01467257	0,000215284
401	1247,183	0,0146727	1,6144E-07	0,01467258	0,000215285
402	1247,184	0,0146728	1,54868E-07	0,0146726	0,000215285
403	1247,184	0,0146728	1,48563E-07	0,0146726	0,000215285
404	1247,272	0,0146738	1,42514E-07	0,01467365	0,000215316
405	1247,276	0,0146738	1,36712E-07	0,0146737	0,000215317
406	1247,28	0,0146739	1,31145E-07	0,01467375	0,000215319
407	1247,285	0,0146739	1,25804E-07	0,01467382	0,000215321
408	1247,258	0,0146736	1,2068E-07	0,0146735	0,000215312
409	1247,258	0,0146736	1,15765E-07	0,01467351	0,000215312

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
410	1247,258	0,0146736	1,1105E-07	0,01467351	0,000215312
411	1247,258	0,0146736	1,06526E-07	0,01467352	0,000215312
412	1247,307	0,0146742	1,02187E-07	0,0146741	0,000215329
413	1247,308	0,0146742	9,80234E-08	0,01467411	0,00021533
414	1247,31	0,0146742	9,40296E-08	0,01467414	0,00021533
415	1247,298	0,0146741	9,01983E-08	0,014674	0,000215326
416	1247,299	0,0146741	8,65228E-08	0,01467402	0,000215327
417	1247,299	0,0146741	8,29968E-08	0,01467402	0,000215327
418	1247,299	0,0146741	7,96143E-08	0,01467403	0,000215327
419	1247,407	0,0146754	7,63695E-08	0,0146753	0,000215364
420	1247,412	0,0146754	7,32566E-08	0,01467536	0,000215366
421	1247,416	0,0146755	7,02705E-08	0,01467541	0,000215368
422	1247,42	0,0146755	6,74059E-08	0,01467546	0,000215369
423	1247,335	0,0146745	6,46579E-08	0,01467446	0,00021534
424	1247,333	0,0146745	6,20217E-08	0,01467444	0,000215339
425	1247,332	0,0146745	5,94929E-08	0,01467443	0,000215339
426	1247,33	0,0146745	5,7067E-08	0,01467441	0,000215338
427	1247,427	0,0146756	5,47399E-08	0,01467556	0,000215372
428	1247,429	0,0146756	5,25075E-08	0,01467558	0,000215373
429	1247,432	0,0146757	5,0366E-08	0,01467562	0,000215374
430	1247,435	0,0146757	4,83118E-08	0,01467566	0,000215375
431	1247,384	0,0146751	4,63412E-08	0,01467506	0,000215357
432	1247,383	0,0146751	4,44508E-08	0,01467505	0,000215357
433	1247,383	0,0146751	4,26375E-08	0,01467505	0,000215357
434	1247,382	0,0146751	4,0898E-08	0,01467504	0,000215357
435	1247,446	0,0146758	3,92294E-08	0,0146758	0,000215379
436	1247,447	0,0146758	3,76288E-08	0,01467581	0,000215379
437	1247,449	0,0146759	3,60933E-08	0,01467583	0,00021538
438	1247,451	0,0146759	3,46205E-08	0,01467586	0,000215381
439	1247,416	0,0146755	3,32076E-08	0,01467545	0,000215369
440	1247,416	0,0146755	3,18524E-08	0,01467545	0,000215369
441	1247,415	0,0146755	3,05523E-08	0,01467544	0,000215369

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
442	1247,502	0,0146765	2,93053E-08	0,01467646	0,000215399
443	1247,505	0,0146765	2,8109E-08	0,0146765	0,0002154
444	1247,509	0,0146766	2,69616E-08	0,01467655	0,000215401
445	1247,512	0,0146766	2,58609E-08	0,01467659	0,000215402
446	1247,502	0,0146765	2,48051E-08	0,01467647	0,000215399
447	1247,503	0,0146765	2,37923E-08	0,01467648	0,000215399
448	1247,504	0,0146765	2,28208E-08	0,01467649	0,0002154
449	1247,505	0,0146765	2,1889E-08	0,01467651	0,0002154
450	1247,583	0,0146774	2,09951E-08	0,01467743	0,000215427
451	1247,586	0,0146775	2,01377E-08	0,01467746	0,000215428
452	1247,589	0,0146775	1,93152E-08	0,0146775	0,000215429
453	1247,592	0,0146776	1,85263E-08	0,01467753	0,00021543
454	1247,679	0,0146786	1,77696E-08	0,01467856	0,00021546
455	1247,684	0,0146786	1,70438E-08	0,01467862	0,000215462
456	1247,689	0,0146787	1,63475E-08	0,01467868	0,000215464
457	1247,695	0,0146788	1,56797E-08	0,01467875	0,000215466
458	1247,522	0,0146767	1,50391E-08	0,01467671	0,000215406
459	1247,516	0,0146767	1,44246E-08	0,01467664	0,000215404
460	1247,511	0,0146766	1,38353E-08	0,01467659	0,000215402
461	1247,505	0,0146765	1,32699E-08	0,01467652	0,0002154
462	1247,642	0,0146781	1,27277E-08	0,01467813	0,000215447
463	1247,645	0,0146782	1,22075E-08	0,01467816	0,000215449
464	1247,648	0,0146782	1,17086E-08	0,0146782	0,00021545
465	1247,65	0,0146782	1,12301E-08	0,01467822	0,00021545
466	1247,613	0,0146778	1,07711E-08	0,01467779	0,000215437
467	1247,614	0,0146778	1,03308E-08	0,0146778	0,000215438
468	1247,614	0,0146778	9,90851E-09	0,0146778	0,000215438
469	1247,615	0,0146778	9,50345E-09	0,01467781	0,000215438
470	1247,677	0,0146786	9,11494E-09	0,01467854	0,00021546
471	1247,679	0,0146786	8,74228E-09	0,01467857	0,00021546
472	1247,681	0,0146786	8,38484E-09	0,01467859	0,000215461
473	1247,683	0,0146786	8,042E-09	0,01467862	0,000215462

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
474	1247,64	0,0146781	7,71316E-09	0,01467811	0,000215447
475	1247,639	0,0146781	7,39775E-09	0,0146781	0,000215447
476	1247,638	0,0146781	7,09522E-09	0,01467809	0,000215446
477	1247,638	0,0146781	6,80505E-09	0,01467809	0,000215446
478	1247,637	0,0146781	6,52673E-09	0,01467808	0,000215446
479	1247,636	0,0146781	6,25978E-09	0,01467806	0,000215446
480	1247,636	0,0146781	6,00374E-09	0,01467806	0,000215446
481	1247,693	0,0146787	5,75815E-09	0,01467874	0,000215465
482	1247,696	0,0146788	5,5226E-09	0,01467877	0,000215466
483	1247,698	0,0146788	5,29668E-09	0,01467879	0,000215467
484	1247,701	0,0146788	5,07998E-09	0,01467883	0,000215468
485	1247,703	0,0146789	4,87215E-09	0,01467885	0,000215469
486	1247,678	0,0146786	4,6728E-09	0,01467856	0,00021546
487	1247,678	0,0146786	4,4816E-09	0,01467856	0,00021546
488	1247,678	0,0146786	4,29822E-09	0,01467856	0,00021546
489	1247,632	0,014678	4,12233E-09	0,01467802	0,000215444
490	1247,63	0,014678	3,95363E-09	0,014678	0,000215444
491	1247,628	0,014678	3,79182E-09	0,01467797	0,000215443
492	1247,625	0,0146779	3,63663E-09	0,01467794	0,000215442
493	1247,606	0,0146777	3,48779E-09	0,01467771	0,000215435
494	1247,605	0,0146777	3,34503E-09	0,0146777	0,000215435
495	1247,603	0,0146777	3,2081E-09	0,01467768	0,000215434
496	1247,602	0,0146777	3,07678E-09	0,01467767	0,000215434
497	1247,6	0,0146776	2,95082E-09	0,01467764	0,000215433
498	1247,733	0,0146792	2,83002E-09	0,01467921	0,000215479
499	1247,738	0,0146793	2,71416E-09	0,01467927	0,000215481
500	1247,743	0,0146793	2,60303E-09	0,01467933	0,000215483
501	1247,706	0,0146789	2,49645E-09	0,01467889	0,00021547
502	1247,706	0,0146789	2,39423E-09	0,01467889	0,00021547
503	1247,707	0,0146789	2,29619E-09	0,0146789	0,00021547
504	1247,708	0,0146789	2,20216E-09	0,01467892	0,000215471
505	1247,706	0,0146789	2,11197E-09	0,01467889	0,00021547

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
506	1247,705	0,0146789	2,02548E-09	0,01467888	0,00021547
507	1247,704	0,0146789	1,94252E-09	0,01467887	0,000215469
508	1247,704	0,0146789	1,86296E-09	0,01467887	0,000215469
509	1247,711	0,014679	1,78665E-09	0,01467895	0,000215472
510	1247,711	0,014679	1,71347E-09	0,01467895	0,000215472
511	1247,712	0,014679	1,64328E-09	0,01467896	0,000215472
512	1247,712	0,014679	1,57596E-09	0,01467896	0,000215472
513	1247,678	0,0146786	1,5114E-09	0,01467856	0,00021546
514	1247,677	0,0146786	1,44948E-09	0,01467855	0,00021546
515	1247,676	0,0146785	1,39009E-09	0,01467854	0,00021546
516	1247,674	0,0146785	1,33313E-09	0,01467852	0,000215459
517	1247,748	0,0146794	1,27851E-09	0,01467939	0,000215484
518	1247,75	0,0146794	1,22612E-09	0,01467941	0,000215485
519	1247,753	0,0146794	1,17587E-09	0,01467945	0,000215486
520	1247,755	0,0146795	1,12769E-09	0,01467947	0,000215487
521	1247,843	0,0146805	1,08147E-09	0,0146805	0,000215517
522	1247,848	0,0146806	1,03715E-09	0,01468056	0,000215519
523	1247,853	0,0146806	9,94642E-10	0,01468062	0,000215521
524	1247,858	0,0146807	9,53875E-10	0,01468068	0,000215522
525	1247,859	0,0146807	9,14776E-10	0,01468069	0,000215523
526	1247,86	0,0146807	8,77279E-10	0,01468071	0,000215523
527	1247,862	0,0146807	8,41317E-10	0,01468073	0,000215524
528	1247,843	0,0146805	8,06828E-10	0,01468051	0,000215517
529	1247,843	0,0146805	7,73752E-10	0,01468051	0,000215517
530	1247,842	0,0146805	7,4203E-10	0,01468049	0,000215517
531	1247,841	0,0146805	7,11607E-10	0,01468048	0,000215517
532	1247,789	0,0146799	6,82431E-10	0,01467987	0,000215499
533	1247,787	0,0146798	6,54449E-10	0,01467985	0,000215498
534	1247,784	0,0146798	6,27614E-10	0,01467981	0,000215497
535	1247,782	0,0146798	6,01878E-10	0,01467979	0,000215496
536	1247,921	0,0146814	5,77197E-10	0,01468142	0,000215544
537	1247,927	0,0146815	5,53526E-10	0,01468149	0,000215546

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
538	1247,932	0,0146816	5,30826E-10	0,01468155	0,000215548
539	1247,937	0,0146816	5,09055E-10	0,01468161	0,00021555
540	1247,913	0,0146813	4,88177E-10	0,01468133	0,000215541
541	1247,915	0,0146814	4,68154E-10	0,01468135	0,000215542
542	1247,916	0,0146814	4,48951E-10	0,01468136	0,000215542
543	1247,918	0,0146814	4,30535E-10	0,01468139	0,000215543
544	1247,83	0,0146804	4,12874E-10	0,01468035	0,000215513
545	1247,825	0,0146803	3,95937E-10	0,01468029	0,000215511
546	1247,821	0,0146802	3,79694E-10	0,01468025	0,00021551
547	1247,817	0,0146802	3,64117E-10	0,0146802	0,000215508
548	1247,917	0,0146814	3,49178E-10	0,01468138	0,000215543
549	1247,919	0,0146814	3,34852E-10	0,0146814	0,000215543
550	1247,922	0,0146814	3,21113E-10	0,01468143	0,000215545
551	1247,924	0,0146815	3,07937E-10	0,01468146	0,000215545
552	1248,061	0,0146831	2,95301E-10	0,01468307	0,000215593
553	1248,068	0,0146832	2,83184E-10	0,01468315	0,000215595
554	1248,075	0,0146832	2,71563E-10	0,01468324	0,000215597
555	1248,082	0,0146833	2,60418E-10	0,01468332	0,0002156
556	1247,975	0,0146821	2,49731E-10	0,01468206	0,000215563
557	1247,974	0,014682	2,39481E-10	0,01468205	0,000215562
558	1247,971	0,014682	2,29652E-10	0,01468201	0,000215561
559	1247,969	0,014682	2,20226E-10	0,01468199	0,000215561
560	1247,987	0,0146822	2,11187E-10	0,0146822	0,000215567
561	1247,986	0,0146822	2,02518E-10	0,01468219	0,000215567
562	1247,985	0,0146822	1,94205E-10	0,01468218	0,000215566
563	1247,984	0,0146822	1,86233E-10	0,01468216	0,000215566
564	1248,079	0,0146833	1,78588E-10	0,01468328	0,000215599
565	1248,083	0,0146833	1,71256E-10	0,01468333	0,0002156
566	1248,088	0,0146834	1,64225E-10	0,01468339	0,000215602
567	1248,092	0,0146834	1,57482E-10	0,01468344	0,000215603
568	1247,972	0,014682	1,51016E-10	0,01468202	0,000215562
569	1247,969	0,014682	1,44816E-10	0,01468199	0,000215561

Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
570	1247,965	0,0146819	1,38869E-10	0,01468194	0,000215559
571	1248,117	0,0146837	1,33167E-10	0,01468373	0,000215612
572	1248,121	0,0146838	1,27699E-10	0,01468378	0,000215613
573	1248,125	0,0146838	1,22455E-10	0,01468382	0,000215615
574	1248,13	0,0146839	1,17426E-10	0,01468388	0,000215616
575	1248,047	0,0146829	1,12603E-10	0,01468291	0,000215588
576	1248,046	0,0146829	1,07979E-10	0,01468289	0,000215587
577	1248,045	0,0146829	1,03544E-10	0,01468288	0,000215587
578	1248,043	0,0146829	9,92911E-11	0,01468286	0,000215586
579	1248,025	0,0146826	9,52128E-11	0,01468265	0,00021558
580	1248,023	0,0146826	9,13019E-11	0,01468262	0,000215579
581	1248,02	0,0146826	8,75515E-11	0,01468259	0,000215578
582	1248,018	0,0146826	8,3955E-11	0,01468256	0,000215578
583	1248,061	0,0146831	8,05062E-11	0,01468307	0,000215593
584	1248,062	0,0146831	7,71989E-11	0,01468308	0,000215593
585	1248,063	0,0146831	7,40273E-11	0,01468309	0,000215593
586	1248,065	0,0146831	7,0986E-11	0,01468312	0,000215594
587	1248,065	0,0146831	6,80695E-11	0,01468312	0,000215594
588	1248,066	0,0146831	6,52727E-11	0,01468313	0,000215594
589	1248,067	0,0146831	6,25908E-11	0,01468314	0,000215595
590	1248,068	0,0146832	6,00189E-11	0,01468315	0,000215595
591	1248,036	0,0146828	5,75527E-11	0,01468278	0,000215584
592	1248,035	0,0146828	5,51877E-11	0,01468276	0,000215584
593	1248,034	0,0146828	5,29199E-11	0,01468275	0,000215583
594	1248,033	0,0146827	5,07451E-11	0,01468274	0,000215583
595	1248,051	0,014683	4,86597E-11	0,01468295	0,000215589
596	1248,051	0,014683	4,66599E-11	0,01468295	0,000215589
597	1248,051	0,014683	4,47422E-11	0,01468295	0,000215589
598	1248,051	0,014683	4,29032E-11	0,01468295	0,000215589
599	1248,029	0,0146827	4,11398E-11	0,01468269	0,000215582
600	1248,028	0,0146827	3,94489E-11	0,01468268	0,000215581
601	1248,027	0,0146827	3,78273E-11	0,01468267	0,000215581

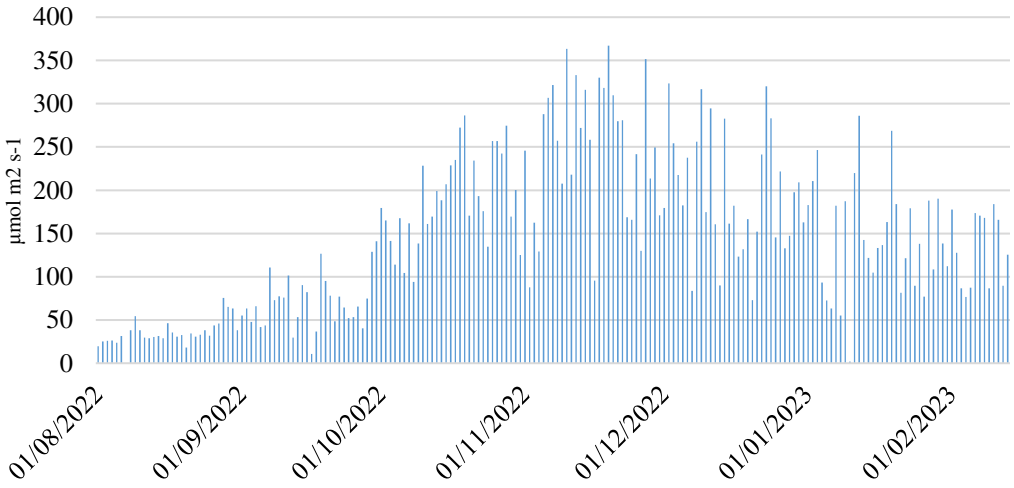
Time (s)	Conductivity	mg NaCl /L	Step 1	Difference	Residual
602	1248,027	0,0146827	3,62724E-11	0,01468267	0,000215581
603	1248,104	0,0146836	3,47814E-11	0,01468358	0,000215607

c. Appendix C

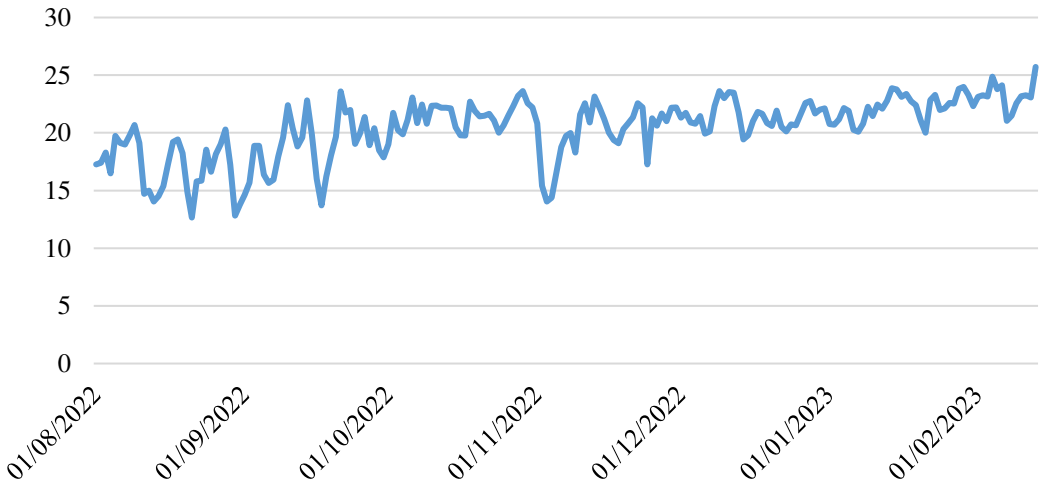
Condições Ambientais

A Figura S1 apresenta dados de monitoramento da radiação fotossinteticamente ativa, precipitação diária e da temperatura média diária, com base nos dados coletados do INMET (2022) para o período do experimento.

(A) Radiação Fotossinteticamente Ativa - Média Diária



(B) Temperatura Média Diária (ºC)



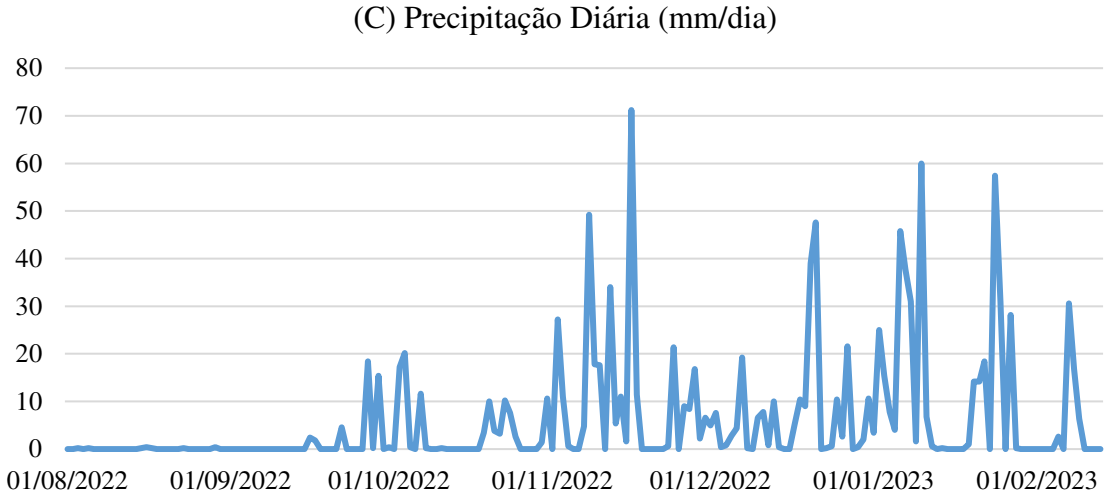


Figura S1. Dados climáticos para o período de operação: (A) RFA, (B) Temperatura Média Diária e (C) Precipitação Diária Total. Fonte: INMET.

d. Appendix D

Supplementary Files

for

Shining a light on outdoor algal systems for wastewater treatment: How artificial light enhancement impacts biomass costs and life cycle

Table S1. Chemical species considered in the simulations performed in Aspen Plus for microalgae cultivation.

Component ID	Chemical Species	Type
1	CO_2	Conventional
2	NH_4^+	Conventional
3	$H2PO_4^-$	Conventional
4	H_2O	Conventional
5	H_3O^+	Conventional
6	O_2	Conventional
7	N_2	Conventional
8	HPO_4^{--}	Conventional
9	PO_4^{---}	Solid
10	OH^-	Conventional
11	ALGAE	Conventional
12	NA_3PO_4	Conventional
13	NA^+	Conventional

Table S2. Chemical reactions considered in the simulations performed in Aspen Plus for microalgae cultivation.

Reaction ID	Fractional conversion	Fractional Conversion of Component	Reaction
1	1	NA_3PO_4	$Na_3PO_4 \rightarrow 3\ Na^+ + PO_4^{3-}$
2	0,5	PO_4^{---}	$PO_4^{3-} + H_2O \rightarrow HPO_4^{2-} + OH^-$
3	1	PO_4^{---}	$PO_4^{3-} + 2\ H_2O \rightarrow H_2PO_4^- + 2\ OH^-$
4(control)	1	$H_2PO_4^-$	$CO_2 + 0{,}123328\ NH_4^+ + 0{,}00358863\ H_2PO_4^- + 0{,}00358863\ HPO_4^{2-} + 0{,}953155\ H_2O \rightarrow ALGAE + 1{,}09059\ O_2 + 0{,}116151\ H_3O^+$
4 (12h)	1	$H_2PO_4^-$	$CO_2 + 0{,}111045\ NH_4^+ + 0{,}00334687\ H_2PO_4^- + 0{,}00334687\ HPO_4^{2-} + 1{,}01532\ H_2O \rightarrow ALGAE + 1{,}06759\ O_2 + 0{,}104351\ H_3O^+$
4(24h)	1	$H_2PO_4^-$	$CO_2 + 0{,}0919481\ NH_4^+ + 0{,}00318296\ H_2PO_4^- + 0{,}00318296\ HPO_4^{2-} + 1{,}02301\ H_2O \rightarrow ALGAE + 1{,}0965\ O_2 + 0{,}085582\ H_3O^+$

Table S3. Data for life cycle inventory calculations.

Stage	Process	Value	Unit	Source
Cultivation & Harvesting	Wastewater	10701.67	kg/day	Aspen Plus model output
	Electricity Pump	0.09	kWh	Aspen Plus model output
	Electricity Compressor	0.37	kWh	Aspen Plus model output
	Electricity LED	0.045	kWh	Livarno (2021)
	Energy (filter)	0.45	kWh	Borowitzka & Moheimani (2013)
	Biomass (Control)	10	kg/day	Aspen Plus model output + Experimental production
Avoided products from synthetic algae cultivation	Tap water	2.33	m^3 (kg algae)$^{-1}$	Herrera et al., 2021
	N	0.10	kg (kg algae)$^{-1}$	
	P	0.016	kg (kg algae)$^{-1}$	

Table S4. Characterization results (midpoint).

Category	Unit	Control	Control-PV	12h	12h-PV	24h	24h-PV
Global warming	kg CO2 eq	3.51E+00	-2.85E-01	7.49E+01	1.13E+01	1.39E+02	2.19E+01
Stratospheric ozone depletion	kg CFC11 eq	6.31E-07	-7.92E-08	1.70E-05	5.12E-06	3.18E-05	9.82E-06
Fine particulate matter formation	kg PM2.5 eq	6.00E-03	-9.64E-05	1.27E-01	2.51E-02	2.37E-01	4.78E-02
Terrestrial acidification	kg SO2 eq	1.94E-02	-1.31E-03	3.97E-01	5.07E-02	7.38E-01	9.79E-02
Freshwater eutrophication	kg P eq	9.22E-04	-2.41E-04	2.69E-02	7.39E-03	5.04E-02	1.43E-02
Marine eutrophication	kg N eq	4.46E-05	-4.56E-06	1.67E-03	8.41E-04	3.13E-03	1.61E-03
Terrestrial ecotoxicity	kg 1,4-DCB	-4.73E-01	1.37E+01	6.31E+01	3.01E+02	1.21E+02	5.61E+02
Human carcinogenic toxicity	kg 1,4-DCB	6.47E-02	2.16E-02	2.70E+00	1.98E+00	5.09E+00	3.75E+00
Mineral resource scarcity	kg Cu eq	-4.41E-03	2.17E-03	4.34E-02	1.54E-01	8.68E-02	2.91E-01
Fossil resource scarcity	kg oil eq	9.34E-01	-1.37E-01	2.08E+01	2.89E+00	3.88E+01	5.63E+00

Table S5. Normalization results (midpoint).

Impact category	Control	Control-PV	12h	12h-PV	24h	24h-PV
Global warming	4.38E-04	-3.56E-05	9.37E-03	1.42E-03	1.74E-02	2.73E-03
Stratospheric ozone depletion	1.05E-05	-1.32E-06	2.84E-04	8.54E-05	5.32E-04	1.64E-04
Fine particulate matter formation	2.35E-04	-3.77E-06	4.98E-03	9.80E-04	9.25E-03	1.87E-03
Terrestrial acidification	4.72E-04	-3.19E-05	9.69E-03	1.24E-03	1.80E-02	2.39E-03
Freshwater eutrophication	1.42E-03	-3.72E-04	4.14E-02	1.14E-02	7.75E-02	2.20E-02
Marine eutrophication	9.68E-06	-9.90E-07	3.62E-04	1.83E-04	6.79E-04	3.49E-04
Terrestrial ecotoxicity	-3.11E-05	9.03E-04	4.15E-03	1.98E-02	7.94E-03	3.69E-02
Human carcinogenic toxicity	6.28E-03	2.10E-03	2.62E-01	1.92E-01	4.94E-01	3.64E-01
Mineral resource scarcity	-3.68E-08	1.81E-08	3.61E-07	1.28E-06	7.23E-07	2.42E-06
Fossil resource scarcity	9.52E-04	-1.40E-04	2.13E-02	2.95E-03	3.96E-02	5.75E-03

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12. Attachments

a. Attachment 1

MAGALHÃES, Iara Barbosa; PEREIRA, Alexia Saleme Aona de Paula; SILVA, Thiago Abrantes; FERREIRA, Jéssica; BRAGA, Matheus Quintão; COUTO, Eduardo Aguiar; ASSEMANY, Paula Peixoto; CALIJURI, Maria Lúcia. Advancements in high-rate algal pond technology for enhanced wastewater treatment and biomass production: A review. Journal of Water Process Engineering. Elsevier BV, Sep. 2024. DOI 10.1016/j.jwpe.2024.105929

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Advancements in high-rate algal pond technology for enhanced wastewater treatment and biomass production: A review

Iara Barbosa Magalhães [a,*], Alexia Saleme Aona de Paula Pereira [a], Thiago Abrantes Silva [a], Jéssica Ferreira [a], Matheus Quintão Braga [a], Eduardo Aguiar Couto [b], Paula Peixoto Assemany [b], Maria Lúcia Calijuri [a]

[a] Federal University of Viçosa (Universidade Federal de Viçosa/UFV), Department of Civil Engineering, Advanced Environmental Research Group – nPA, Av. Peter Henry Rolfs, s/n, Campus Universitário, Viçosa, Minas Gerais 36570-900, Brazil
[b] Federal University of Lavras, Environmental Engineering Department, Campus Universitário, Lavras, Minas Gerais, Brazil

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Operation

ABSTRACT

This review examines recent advances in high-rate algal pond (HRAP) technology for wastewater treatment and the production of microalgae-rich biomass. It highlights the latest methods developed to enhance the efficiency and sustainability of these systems. Notably, these methods include two-stage cultivation, biomass recirculation, and strategies that collectively improve algal productivity and treatment efficacy, such as the implementation of hybrid systems. Hybrid systems combine HRAPs with other wastewater treatment technologies, such as biofilm reactors. By optimizing these configurations, HRAPs demonstrate effectiveness in nutrient removal, biomass production, and harvesting, enhancing their potential as a scalable and adaptable solution for effluent treatment and biomass production. Additionally, this study reviews HRAP performance in removing emerging contaminants, an important aspect in the context of current effluent treatment. This review establishes a significant advancement in HRAP technology, promoting its adoption as a sustainable and real-scale wastewater treatment option.

1. Introduction

The growing concerns over water pollution and the need for sustainable wastewater treatment technologies have driven research efforts towards innovative and environmentally friendly solutions [1]. High-rate algal ponds (HRAPs) emerged as a promising alternative for wastewater treatment due to their potential to remove pollutants while simultaneously producing valuable biomass [2,3]. HRAPs are usually shallow open ponds, with a continuous circulation of culture media enforced by a rotating paddlewheel [4]. This design favors the growth and accumulation of algal biomass, given that the stirring of the liquid subjects the cells to varying radiation intensities, effectively minimizing the photoinhibition effect [5].

The first application of HRAPs to wastewater treatment dates to the late 50s and early 60s, developed at the University of California [6–9]. Since then, researchers and industries have applied the technology to treat wastewater from domestic and industrial sources [10], also testing the biomass produced to several conversion routes [11,12]. Since their first application, researchers have proposed strategies to address technical challenges in HRAP technology applied to wastewater treatment, such as the low productivity of biomass and target biocompounds [1], overcoming seasonal variations [13] and reconciling these issues with economic and environmental sustainability [14,15].

Each year, 380 billion m^3 of municipal wastewater are generated globally [16]. In the context of increasing globalization, the challenge of municipal wastewater treatment becomes more pronounced as population growth and urbanization intensify the strain on water resources [17]. Its production is expected to increase by 24 % by 2030 and 51 % by 2050 [18]. Thus, this type of wastewater is a serious worldwide concern, especially in low-income countries that lack in wastewater treatment infrastructure. Municipal wastewater treatment covers 70 %, 38 %, and 28 % of wastewater generated in high-income, upper middle-income, and lower middle-income countries, respectively. In low-income countries, only 8 % of wastewater generated undergoes treatment [19]. Thus, the bioremediation and resource recovery potential of coupling HRAP technology to municipal wastewater treatment can contribute to

* Corresponding author.
E-mail address: iara.barbosa@ufv.br (I.B. Magalhães).

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increasing sanitation universalization and presents several advantages. Firstly, it provides a nutrient-rich and cost-effective source for algal growth, eliminating the need for expensive synthetic nutrients [20]. The wastewater's organic matter and dissolved nutrients serve as valuable resources that microalgae can assimilate, promoting rapid biomass production [1]. Secondly, using wastewater as a culture medium contributes to sustainable development by repurposing and recycling wastewater, transforming it into a valuable resource in the bioeconomy [21]. This arrangement helps efficient wastewater treatment and reduces the environmental burden of conventional wastewater disposal methods. Additionally, the HRAPs' ability to effectively remove nutrients and contaminants from the wastewater helps mitigate water pollution, protecting aquatic ecosystems and public health [22]. Still, there is a low uptake of the technology, with few real-scale units in operation [23].

One of the pointed bottlenecks is the HRAP design and operation standards, hindering the diffusion potential of the technology for sustainable wastewater treatment [24]. While several reviews and standards are consolidated for different pond treatment technologies [25,26], HRAPs still lack comprehensive and updated guides to practices and results of the technology, especially considering innovative and alternative operation strategies, besides design modifications recently proposed. Bridging this gap is vital to advancing HRAP technology dissemination, streamlining design processes, and optimizing system performance. This review aims to consolidate knowledge on HRAPs applied to sustainable municipal wastewater treatment, providing insights into design, operation, treatment efficiency, and challenges. For each topic, technical implications and critical assessment of future directions are also presented, aimed at consolidating current knowledge and potential and encouraging further cutting-edge developments.

2. Municipal wastewater treatment in high-rate algal ponds

2.1. Mapping

Research shows that HRAPs are promising for municipal wastewater treatment and resource recovery. However, this treatment approach is not widely spread, and there are few examples of large-scale operational systems [27]. Therefore, a review of functioning full-scale ponds was conducted in published literature, as summarized in Table 1.

The evaluation of constructed HRAPs for municipal wastewater treatment reveals a geographical distinction, primarily in developed countries like New Zealand and the United States. This trend correlates with Goswami et al.'s [14] study, which found intensive microalgae cultivation research in these countries for valuable products and sanitation. In real-scale studies, naturally growing wild algae were observed [28]. However, the main challenges in treatment lie in removal efficiency due to the operational control complexities [29], which may result in lower algal productivity and nutrient removal rates [30], coupled with heightened climate seasonality [31]. Moreover, being an outdoor open system, controlled and stable results at large scale and throughout continuous operation are not expected.

As reported by Sutherland et al. [30], the size of the HRAP can influence daily areal nutrient removal and biomass production. The study compared nutrient removal and microalgal productivity over three seasons for HRAPs of 5m^2, 330m^2, and 1 ha. The study highlights the implications of daily wastewater treatment efficiency and biomass productivity per land area for scale-up predictions, impacting biomass yield and costs. Thus, further research is recommended to optimize microalgal performance in full-scale HRAP.

However, to elucidate HRAP technology, the current review will address the abovementioned studies along with pilot and bench-scale findings. Still, where pertinent, the aim is to consolidate potential considerations for scale-up in large-scale applications. This approach addresses the scarcity of data in large-scale implementations. The overview of the 32 reviewed studies, comprising 96 experimental results, operational parameters, and treatment efficiencies (see Supplementary File).

2.2. Application

HRAPs are stabilization ponds that strongly depend on climatic conditions, particularly well-suited for use in warm and tropical climate regions. Their relatively high area requirement makes them an alternative for application in small communities, such as rural areas and remote locations, where decentralized sanitation units are needed. Notably, HRAPs offer the advantage of biomass recovery, which can transform conventional wastewater treatment plants into sustainable systems [32].

In Australia, HRAPs (Table 1) are primarily situated in rural areas, with limited access to experienced operators and low energy availability [33]. An interesting case is the HRAP located in Kingston on Murray, South Australia, which outperformed a waste stabilization pond in Lyndoch, resulting in reduced treatment time, construction costs, and increased water availability for reuse, particularly crucial in water-

Table 1
Location, size, and performance of large-scale high-rate algal ponds for sanitary wastewater treatment.

Location	First-year operational report	Surface area (m^2)	Operational flow (m^3 day^{-1})	Removal efficiency	Reference
Lawrence - United States	1956	–	97.7	N-NH_4 – 61.4 % P – 90.6 %	[14]
Rabat – Morocco	1991	3023	259.2	N-NH_4 – 23-78 % P – 40- 72 %	[175]
St. Helena – United States	1970	20,000	–	–	[29,176]
Hollister – United States		50,000	–	–	[176]
Christchurch - New Zealand	2009	14,000	175[a]	BOD – 47-52 % N-NH_4 – 64-67 % P – 14-24 %	[31]
Chiclana de la Frontera - Spain	2014	10,000	4000	–	[27,29]
Kingston on Murray - Australia		192-226	12	BOD – 91-94 %[b] N-NH_4 – 52-78 % P – (-2)-16 %	[40]
	2008			BOD – 78-86 %[c] N-NH_4 – (-2)-94 % P – (-31) - 10 %	
Cambridge- New Zealand	–	10,000	362.5	N-NH_4 – 32 - 46 % P – 32 - 42 %	[30]
Peterborough - Australia	2019	2 ponds of 5000m^2 each	475	–	[62]

[a] 500 m^2/d and 0.35 depth reported.
[b] Septic tank treated domestic wastewater.
[c] Facultative pond-treated domestic wastewater.

b. Attachment 2

MAGALHÃES, Iara Barbosa; REIS, Miriam Costa Fateixa; PEREIRA, Alexia Saleme Aona de Paula; BRAGA, Matheus Quintão; ASSEMANY, Paula Peixoto; CALIJURI, Maria Lúcia. Effects of series operation of high-rate algal ponds treating sanitary sewage: hydrodynamics, remediation potential, and biomass production. Algal Research, [s. l.], vol. 89, p. 104091, jul. 2025. DOI 10.1016/j.algal.2025.104091. Disponível em: http://dx.doi.org/10.1016/j.algal.2025.104091.

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Effects of series operation of high-rate algal ponds treating sanitary sewage: hydrodynamics, remediation potential, and biomass production

Iara Barbosa Magalhães [a,*], Miriam Costa Fateixa Reis [a], Alexia Saleme Aona de Paula Pereira [a], Matheus Quintão Braga [a], Paula Peixoto Assemany [b], Maria Lúcia Calijuri [a]

[a] Federal University of Viçosa (Universidade Federal de Viçosa/UFV), Department of Civil Engineering, Advanced Environmental Research Group – nPA, Av. Peter Henry Rolfs, s/n, Campus Universitário, Viçosa, Minas Gerais 36570-900, Brazil
[b] Federal University of Lavras, Environmental Engineering Department, Campus Universitário, Lavras, Minas Gerais, Brazil

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ABSTRACT

Operational modifications are a promising, cost-effective strategy to increase the efficiency of algal systems applied to sewage treatment. In this context, this study investigates the effectiveness of operating High-rate Algal Ponds (HRAPs) in series for sanitary sewage treatment, focusing on improving technical performance by assessing pollutant removal efficiencies and biomass production. A hydrodynamic analysis determined Bodenstein's value of 9.7 for HRAPs, indicating a complete mixing regime and suggesting potential benefits to series operation. Subsequently, two systems were operated, each comprising two HRAPs: one operated in series (with a hydraulic retention time (HRT) of 5 days in each HRAP) and one operated in parallel (with an HRT of 10 days in each HRAP). The series operation achieved higher coliforms and *E. coli* removal (97.79 % and 98.33 % compared to 84.09 % and 89.56 % in parallel), while no statistically significant differences were observed in nutrient removal or biomass production. However, the parallel system exhibited 14 % higher TOC removal and biomass with greater lipid (19 %), protein (76 %), and carbohydrate (44 %) content, while the series had higher ash content (65 % higher). Discussions approach the roles of hydrodynamics, nutrient balance, and senescence to explain the performance of each system. Future research should focus on reaching a plug flow regime by optimizing mixing, besides testing more than two ponds in series.

1. Introduction

High-Rate Algal Ponds (HRAPs) have garnered increasing attention as a cost-effective and sustainable technology for wastewater treatment, owing to their relatively low construction costs [1], scalability [2], and capacity to integrate conventional wastewater treatment plants [3]. By removing nutrients and organic matter from wastewater, HRAPs also facilitate the production of algal biomass, offering additional resource recovery potential [4]. Despite these benefits, achieving economically viable full-scale HRAPs still necessitates further optimization of treatment processes and resource recovery strategies [5,6], leading researchers to explore operational modifications as a promising approach for low-cost improvements [7].

Several operational enhancements—including the implementation of hybrid systems [8–10], the addition of carbonation columns [11], and modifications to operational depth [12] —have been proposed to boost HRAP performance. One especially notable strategy is the arrangement of ponds in series, a concept established in the 1960s for improving the performance of maturation ponds through enhanced hydraulic regimes [13]. This configuration has also demonstrated potential for improving nutrient removal and promoting biomass production in HRAPs [7,14]. For example, Sutherland et al. [7] reported significantly higher total chlorophyll-a productivity when HRAPs were operated in series (2.9 ± 0.7 kg/day) compared to parallel operations (1.2 ± 0.1 kg/day), attributing these gains to shorter hydraulic retention times (HRT) that supported higher daily biomass productivity and nutrient removal efficiency. Notably, however, these series-configured HRAPs employed settling tanks between the ponds, introducing additional infrastructure and masking the effects of a purely direct, uninterrupted flow path on pathogen removal.

Recent modeling work suggests that an HRAP series arrangement could optimize pathogen removal—particularly for *Escherichia coli* (*E. coli*)—when plug-flow conditions are approximated [15]. Operations that approach plug flow, widely recognized for favoring processes

* Corresponding author.
E-mail address: iara.barbosa@ufv.br (I.B. Magalhães).

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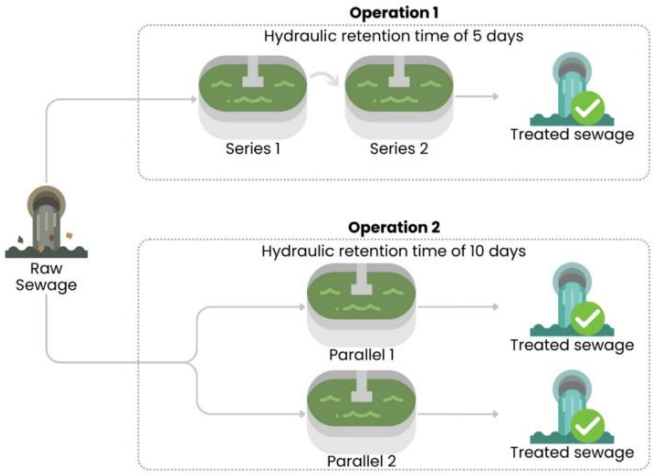

Fig. 1. Schematic operation.

governed by first-order decay kinetics, can be cultivated by lengthening the flow path or employing baffles and multiple pond stages [16,17]. To quantify axial dispersion in such systems, researchers frequently use the Bodenstein number (Bo). Low Bo values (≤20) signify complete mixing, whereas higher values (≥100) align with near-plug-flow conditions [18,19]. While HRAPs often exhibit lower Bo values at impeller blades, pond outlets, and bends (<7), the elongated straight sections—comprising most of the reactor volume—can achieve Bo values between 200 and 540 [20].

Building on this foundation, the present study introduces a direct series configuration for HRAPs—one that bypasses intermediate settling stages. This uninterrupted flow path is hypothesized to accentuate plug-flow behavior, and thereby enhance the removal of contaminants governed by first-order decay kinetics. Notably, while prior research has recognized improved nutrient and organic matter removal under series operation, the full implications for pathogen removal under practical operational conditions remain underexplored.

Thus, we compare HRAPs operated in parallel to those directly linked in series, examining their respective performances in pathogen removal (coliforms and *E. coli*), nutrient and organic matter removal, biomass productivity and characterization. We also integrate hydrodynamic insights via the Bodenstein and Reynolds numbers to contextualize observed performance within flow regimes. Ultimately, this study aims to highlight how targeted operational configurations, specifically direct series operation, can bolster cost-effective wastewater treatment and resource recovery—achieving these gains without the added complexity of auxiliary settling infrastructure.

2. Material and methods

The research was conducted at the Sanitary and Environmental Engineering Laboratory (LESA), Department of Civil Engineering, Federal University of Viçosa (UFV). The experiments were carried out from June to September 2022, covering autumn and winter. Sanitary sewage was used as a culture medium, collected weekly after treatment in a septic tank at the Sewage Treatment Station located in the Romão dos Reis neighborhood in Viçosa - Minas Gerais, operated by the Autonomous Water and Sewage Service (SAAE - Viçosa).

The experiments were conducted in pilot-scale HRAPs (1000 L each), with the following characteristics: width: 1.28 m; length: 2.86 m; total depth: 0.5 m; useful depth: 0.3 m; and surface area: 3.3 m^2. These HRAPs are made of fiberglass with steel blades and six blades. During operation, the blades are driven by a 1 hp. electric motor. The rotation was reduced by a reducer coupled to the motor and is controlled by a frequency inverter (WEG, CFW-08 series).

2.1. Hydrodynamic assessment: bodenstein number (Bo)

An evaluation of the Bo of the HRAPs was carried out. The methodology was adapted from NaCl tracer experiments in a stimulus-response approach [18,20,21]. The Aqua TROLL 500 Multiparameter probe was fixed in the outlet of HRAP, coupled with a Temperature-Conductivity Sensor. The continuous reading was set to record NaCl concentration data each 1 s. The HRAPs were filled with tap water in the same configurations previously described. A final 0.293 mg NaCl /L solution for the 1000 L HRAPs was used as a reference, following previous studies [18]. Thus, a concentrated 60 mg NaCl/L solution was prepared, and 5 L was added to the HRAP.

The mixing time was stipulated as the time necessary for variations in conductivity to reach <5 % of the final stable value [20]. The Bo is calculated as a function of the decrease in the output signal in relation to the input signal, with both signals, in this case, being experimentally determined by the conductivity values. To investigate the mixing characteristics inside the reactor, the HRAP was operated in a closed condition, with no inlet or outlet flow. The data from the conductivity probe measurements was processed according to the method proposed by [20]. Eq. 1, following the Solver tool from Excel®, was used to calculate the final Bo value.

$$\frac{C}{C_\infty} = \sqrt{\frac{Bo}{4\pi\theta}} \sum_{j=1}^{\infty} exp\left[-\frac{Bo}{4\theta} (j-\theta)^2 \right] \quad (1)$$

Where $\frac{C}{C_\infty}$ is the dimensionless concentration and θ is the dimensionless time $\frac{t}{t_o}$.

A complementary mixing speed experiment was also conducted with a Polystyrene floater to evaluate the Reynolds Number of the pond [22] later. The methodology included conducting the test 30 times ($n = 30$) and noting the time it took for the floater to traverse the straight channel

c. Attachment 3

Magalhães, I.B., Assis, M.L., Pereira, A.S.A.P., Assemany, P.P., Calijuri, M.L. Effects of organic loading rate in high-rate algal ponds: bioremediation potential, implications to design and operation. In 17th IWA Conference on Small Water and Wastewater Systems (SWWS) and 9th IWA Conference on Resource Oriented Sanitation (ROS), 2024, Curitiba. Available at: https://abes-dn.org.br/anaiseletronicos/47_swwsros2024/194_theme_v.pdf

Effects of organic loading rate in high-rate algal ponds: bioremediation potential, implications to design and operation

Magalhães, I.B.*, Assis, M.L.*, Pereira, A.S.A.P.*, Assemany, P.P.**, and Calijuri, M.L.*

* Federal University of Viçosa, Department of Civil Engineering, Advanced Environmental Research Group – nPA, Av. Peter Henry Rolfs, s/n, Campus Universitário, Viçosa, Minas Gerais, 36570-900, Brazil.

**Federal University of Lavras, Environmental Engineering Department, Campus Universitário, Lavras, Minas Gerais, Brazil

Highlights:

- Three ponds were operated with increasing sewage flows to evaluate the effects of organic loading rates (kg BOD ha^{-1} day^{-1}).
- Hydraulic retention times of 5, 6.7, or 10 days did not differ significantly in treatment efficiency or biomass production.
- Further studies should investigate higher organic loads besides prioritizing HRAPs as tertiary treatment units.

Keywords: microalgae biomass; wastewater treatment; raceways; operational strategies.

INTRODUCTION

The Organic Loading Rate (expressed in kg BOD ha^{-1} day^{-1}) is one of the parameters used in the design of facultative ponds, with established removal coefficients for different operational ranges (Ho & Goethals, 2020). However, for High-Rate Algal Ponds (HRAPs), design methods lack standardization. Although studies have reported operational organic loading rates ranging between 100 and 150 kg BOD ha^{-1} day^{-1} (Craggs et al., 2014), the values for HRAP applications are not well defined in the literature. This type of system has been applied both as a secondary and tertiary treatment unit for domestic wastewater (DW) treatment with varying results. Despite the increasing adoption of HRAPs as an attractive alternative for decentralized treatment in small communities (Fallowfield et al., 2018), the establishment of reliable removal coefficients adjusted to variations in loading rates remains a substantial gap. Obtaining concrete data for removal coefficients under different operational scenarios is essential for formulating models and guidelines that provide accurate sizing and optimal performance. Thus, the aim of this study was to evaluate the operation, regarding treatment efficiency and biomass production, of HRAPs subjected to different organic loading rates.

METHODOLOGY

The experiment was conducted at the Laboratory of Sanitary and Environmental Engineering of the Federal University of Viçosa from August 2022 to February 2023. DW, collected after a septic tank, was used as the cultivation medium. Three HRAPs of $1m^3$ each were operated with different organic loading rates (measured in kg BOD ha^{-1} day^{-1}): 200 L/day (with a Hydraulic Retention Time - HRT = 5 days) for HRAP 1, 150 L/day (HRT = 6.7 days) for HRAP 2, and 100 L/day (HRT = 10 days) for HRAP

3. The DW had a mean biochemical oxygen demand (BOD) of 107.18 ± 50.45 mg/L, which resulted in organic loading rates of 64.96, 48.72, and 32.48 kg BOD ha^{-1} day^{-1} for HRAPs 1, 2, and 3, respectively. Environmental conditions and physical and chemical parameters of the DW and HRAPs were monitored weekly following APHA (2012) standards. The monitored variables included BOD, ammoniacal nitrogen ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), soluble phosphorus (Ps), soluble chemical oxygen demand (CODs), soluble total organic carbon (TOC), and volatile suspended solids (VSS). Chlorophyll-a (Chl-a) was extracted with 80% ethanol and measured by spectrophotometry; concentrations were determined using equations provided in the Dutch standard. Dissolved oxygen, pH, and temperature were measured with a Hach HQ40d probe. The phytoplankton community was analyzed for the inoculum and at the end of the operation for each HRAP, including organism identification, and density. The organic matter removal coefficients (k_{BOD}) were calculated for each treatment using von Sperling (2015) models based on BOD data for the complete mixing model (Bodenstein number of the HRAPs = 9.7). The results underwent normality tests followed by the Kruskal-Wallis test with a 5% probability, using PAST 4.03 software.

RESULTS AND CONCLUSIONS

The results for removal efficiencies and biomass production for each system are presented in Table 1.

Variable	Unit	DW	HRAP 1	Remov.	HRAP 2	Remov.	HRAP 3	Remov.	p*
Load	kg BOD/day /ha	-	64.96	-	48.72	-	32.48	-	X
BOD	mg/L	107.18 (50.45)	68.6 (21.13)	35.99%	80.24 (25.9)	25.13%	103.67 (34.69)	3.28%	0.1492
CODs	mg/L	164.75 (81.44)	129.5 (91.88)	21.40%	119.43 (31.44)	27.51%	98.92 (31.36)	12.72%	0.2195
pH	-	7.63 (0.22)	6.13 (1.19)	X	6.72 (1.24)	X	6.48 (1.09)	X	0.5738
DO	mg/L	0.79 (0.36)	10.66 (2.49)	X	10.61 (2.35)	X	10.61 (2.14)	X	0.9475
Temp.	°C	23.65 (1.77)	23.08 (2.11)	X	23.38 (2.64)	X	22.78 (2.26)	X	X
$N\text{-}NH_4^+$	mg/L	96.12 (57.96)	23.23 (12.21)	75.83%	12.4 (11.02)	87.10%	19.03 (8.07)	80.20%	0.1339
$N\text{-}NO_3^-$	mg/L	11.38 (9.55)	43.56 (24.63)	-282.81%	60.91 (58.53)	-435.33%	58.29 (53.71)	-412.29%	0.9341
Ps	mg/L	10.51 (4.22)	8.88 (2.88)	15.46%	8.13 (3.01)	22.68%	9.62 (3.18)	8.47%	0.357
TOC	mg/L	34.89 (13.47)	36.73 (12.34)	39.77%	23.3 (8.6)	22.66%	20.49 (3.94)	36.45%	0.735
Chl-a	mg/L	-	1.35 (0.73)	X	1.75 (0.87)	X	1.61 (0.94)	X	0.6352
VSS	mg/L	-	100.75 (50.3)	X	157.54 (112.48)	X	119.57 (69.57)	X	0.7254

*p: p-value for the Kruskall-Wallis test.

Table 1. Treatment efficiencies and biomass production for each pond (average values and standard deviation between parenthesis).

In our preliminary analysis, no significant difference in treatment performance across different flow rates was found, indicating a lack of distinctive treatments. The average BOD removal efficiencies were 35.99%, 25.13%, and 3.28% for HRAPs 1, 2, and 3, respectively. Comparable results were observed by El Hafiane and Hamouri (2005) operating HRAPs with effluent from UASB with and without gravel filters. They applied a rate of 52 kg BOD/day/ha, achieving 22% BOD removal. Inlet BOD concentration was low at 45 mg/L after the gravel filter. Biomass production ranged from 131-185 mg VSS/L, with chl-a productivity averaging 0.05-0.08 g/m^2/day, statistically equal among treatments.