

IARA BARBOSA MAGALHÃES

**TECNOLOGIAS DE TRATAMENTO DE EFLUENTES E PRODUÇÃO DE
BIOMASSA: UMA ABORDAGEM DE CICLO DE VIDA**

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Engenharia Civil, para obtenção do título de *Magister Scientiae*.

Orientadora: Maria Lúcia Calijuri

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Assentimento:



Iara Barbosa Magalhães
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Maria Lúcia Calijuri
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“You want a revolution,

I want a revelation”

Hamilton, Act I - The Schuyler Sisters

BIOGRAFIA

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RESUMO

MAGALHÃES, Iara Barbosa, M.Sc., Universidade Federal de Viçosa, março de 2021. **Tecnologias de tratamento de efluentes e produção de biomassa: Uma abordagem de ciclo de vida.** Orientadora: Maria Lúcia Calijuri.

Neste estudo, a Avaliação de Ciclo de Vida foi utilizada como ferramenta para comparar a performance ambiental de diferentes tecnologias de cultivo de microalgas em águas residuárias. Uma primeira modelagem dos sistemas de cultivo foi realizada para as diferentes tecnologias de otimização da produtividade da biomassa algal em lagoas de alta taxa: (i) utilização da suplementação de carbono industrial e de emissões atmosféricas; (ii) utilização de sistemas híbridos, com crescimento aderido em reatores biofilme; e (iii) utilização de pré-desinfecção UV do efluente. Os impactos ambientais para a maioria das categorias foram reduzidos, em grande parte pelos benefícios do uso de águas residuárias como fonte de água e nutrientes, com os sistemas atingindo impactos negativos. A única exceção foram as categorias de eutrofização, com os maiores impactos normalizados junto com as categorias relacionadas à toxicidade. O melhor resultado de tecnologia de otimização da produtividade foi encontrado para o sistema que utiliza o fornecimento de CO₂ do gás de exaustão da combustão da gasolina, para 11 das 13 categorias avaliadas. O processo de maior impacto foi a suplementação de CO₂ industrial, seguido pelo uso de reator de biofilme e consumo de energia. Acoplar o fornecimento de CO₂ industrial e sistemas híbridos para aumentar a produtividade da biomassa não compensou em termos de impactos ambientais, principalmente em função do uso de pesticidas na cadeia produtiva do tecido do reator biofilme e produção industrial de carbono. A avaliação dos cenários foi realizada para aumento e piora do desempenho das taxas de fornecimento de CO₂ ($\pm 40\%$) e vida útil do reator de biofilme (± 20 dias). Oportunidades para melhores resultados deveriam considerar o uso de gás recuperado de diferentes indústrias e diferentes materiais de suporte para o crescimento de biomassa em sistemas híbridos. Uma segunda modelagem foi feita para comparação entre sistemas abertos (lagoas de alta taxa - LAT) e fechados (fotobiorreator de coluna de bolhas - FBR). A LAT teve impactos negativos para 7 das 13 categorias de impacto avaliadas, devido ao menor consumo energético (0,43 kWh por kg de biomassa). Embora o PBR ofereça maior produtividade de biomassa total, seu alto consumo de energia (177,4 kWh por kg de biomassa) causando ao menos 75% dos impactos ambientais. A LAT resultou em impactos ambiental menores que o PBR em 12 das 13 categorias analisadas. A única exceção foi a categoria de Eutrofização Marinha, principalmente devido à menor eficiência na recuperação de nitrogênio durante o cultivo em LAT. O processo mais impactante

desse sistema foi o consumo de CO₂ para suplementação (até 80% do impacto gerado). Ainda assim, a demanda de energia da FBR não foi compensada pelo uso de águas residuais como meio de cultivo. O balanço de energia mostra a LAT como um processo viável (NER = 10,68) e aponta a necessidade de uma redução de 97% na demanda de energia para PBR (NER = 0,03) para ser economicamente viável. Melhores resultados poderiam ser encontrados considerando o reuso de CO₂ do processo industrial e provendo energia de fontes renováveis.

Palavras-chave: Microalgas. Biomassa. Cultivo. Avaliação de Ciclo de Vida. Tratamento de Águas Residuárias.

ABSTRACT

MAGALHÃES, Iara Barbosa, M.Sc., Universidade Federal de Viçosa, March, 2021.
Wastewater treatment technologies and biomass production: a life-cycle approach.
Adviser: Maria Lúcia Calijuri.

In this study, Life Cycle Assessment was used to compare the environmental performance of different technologies for cultivating microalgae in wastewater. First modeling of the cultivation systems was carried out for the different technologies for optimizing the productivity of algal biomass in high-rate ponds: (i) use of industrial carbon supplementation and atmospheric emissions; (ii) use of hybrid systems, with growth adhered to biofilm reactors; and (iii) use of UV pre-disinfection of the effluent. The environmental impacts for most categories were reduced, mainly due to the benefits of using wastewater as a source of water and nutrients, with the systems reaching negative impacts. The only exception was the eutrophication categories, with the highest normalized impacts and the categories related to toxicity. The best result of productivity optimization technology was found for the system that uses CO₂ from the exhaust gas of gasoline combustion for 11 of the 13 categories evaluated. The process with the most significant impact was the supplementation of industrial CO₂, followed by biofilm reactor and energy consumption. Coupling the supply of industrial CO₂ and hybrid systems to increase biomass productivity has not paid off in terms of environmental impacts, mainly due to pesticides in the production chain of the biofilm reactor tissue and industrial carbon production. The assessment of the scenarios was carried out to increase and worsen the performance of the CO₂ supply rates ($\pm 40\%$) and the useful life of the biofilm reactor (± 20 days). Opportunities for better results should consider the use of gas recovered from different industries and different support materials for the growth of biomass in hybrid systems. Second modeling was done to compare open systems (high rate pond - HRP) and closed systems (bubble column photobioreactor - PBR). HRP had negative impacts for 7 of the 13 impact categories assessed due to the lower energy consumption (0.43 kWh per kg of biomass). Even though PBR offers higher total biomass productivity, high energy consumption (177.4 kWh per kg of biomass) causes at least 75% of the environmental impacts. HRP resulted in lower environmental impacts than PBR in 12 of the 13 categories analyzed. The only exception was the Marine Eutrophication category, mainly due to the lower efficiency in nitrogen recovery during HRP cultivation. The most impactful process of this system was the consumption of CO₂ for supplementation (up to 80% of the impact generated). Even so, PBR's energy demand has not been offset by using wastewater as a culture media. The energy balance

shows HRP as a viable process (NER = 10.68) and points out the need for a 97% reduction in energy demand for PBR (NER = 0.03) to be economically viable. Better results could be found considering the CO₂ reuse of the industrial process and providing energy from renewable sources.

Keywords: Microalgae. Biomass. Cultivation. Life Cycle Assessment. Wastewater Treatment.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

ACV	Avaliação de Ciclo de Vida
BR	Biofilm Reactor
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq	Conselho Nacional de Desenvolvimento Tecnológico e Científico
EEGC	Exhaust Gas from Gasoline Combustion
FBR	Fotobiorreator
HRP	High Rate Pond
ISO	International Organization for Standardization
LAT	Lagoa de Alta Taxa
LCA	Life Cycle Assessment
LESA	Laboratório de Engenharia Sanitária e Ambiental
NER	Net Energy Ratio
nPA	Núcleo de Pesquisas Ambientais Avançadas
PBR	Photobioreactor
pH	Potencial Hidrogenionico
UASB	Upflow Anaerobic Sludge Blanket
UFV	Universidade Federal de Viçosa
UV	Ultravioleta

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1 APRESENTAÇÃO

Esse trabalho foi parte dos estudos realizados no Núcleo de Pesquisas Ambientais Avançadas (nPA) do Departamento de Engenharia Civil da Universidade Federal de Viçosa (UFV) que englobam tratamento de efluentes e produção de biomassa algal, bem como a recuperação de recursos energéticos e materiais em saneamento.

O presente estudo visou a utilização da Avaliação do Ciclo de Vida (ACV) como ferramenta para análise da influência de diferentes processos de produção de biomassa algal em águas residuárias. Desse modo, pretendeu-se comparar os impactos ambientais dos diferentes sistemas produtivos, meios de cultivo e tecnologias propostas para aumento da produtividade de biomassa.

Essa pesquisa foi parte integrante do projeto “Recuperação de energia no saneamento ambiental: microalgas para biorremediação de efluentes e produção de bioenergia” aprovado junto ao CNPq, Processo 420429/2018-2.

Este documento foi dividido em dois capítulos principais, no formato de artigos científicos, além de introdução geral e conclusões gerais. No Capítulo I, avaliou-se a performance ambiental de diferentes tecnologias de aumento da produtividade de biomassa em lagoas de alta taxa por meio da análise de ciclo de vida. O mesmo já se encontra publicado no periódico *Algal Research* (ISSN: 2211-9264). No Capítulo II, avaliou-se uma comparação entre sistemas abertos e fechados para cultivo de microalgas em efluentes da indústria de processamento de carnes.

2 INTRODUÇÃO GERAL

Microalgas vem sendo estudadas como uma promissora fonte renovável para produção de biomassa, acoplando o cultivo à biorremediação de efluentes (JAVED et al., 2019; KETZER; SKARKA; RÖSCH, 2018; SHAHID et al., 2020). No cultivo em águas residuárias, um dos principais desafios é o balanço custo-energia-ambiente (SHAHID et al., 2020). Há uma menor produtividade de biomassa nesse meio de cultivo quando comparado ao uso de água limpa ou meio sintético, o que configura um dos principais obstáculos para se obter a viabilidade da tecnologia (JAVED et al., 2019). Dentre os principais fatores que influenciam o crescimento algal estão a temperatura, pH, luminosidade, concentração de nutrientes, concentração de CO₂, salinidade e presença de químicos tóxicos para as algas no meio de cultivo (OKORO et al., 2019). Alguns desses parâmetros podem ser influenciados pela utilização de efluentes de diferentes fontes, como industrial, esgoto doméstico ou agroindustrial (GOSWAMI et al., 2020). Outros fatores podem ser influenciados pelo sistema de cultivo escolhido, como lagoas de alta taxa (LAT) ou fotobiorreatores (FBR) fechados (DASAN et al., 2019).

Estratégias para otimizar o cultivo de microalgas e aumentar a produtividade da biomassa têm sido amplamente estudadas. Entre os avanços relatados na literatura, Assis et al. (2019) e Heubeck, Craggs e Shilton (2007) alcançaram maiores rendimentos de biomassa adicionando diferentes fontes de CO₂ para suprir a limitação de carbono do esgoto doméstico. Assemany et al. (2015) investigaram o uso esgoto doméstico pré-desinfetado por radiação ultravioleta (UV) em LATs. Os autores relataram resultados promissores dessa tecnologia para reduzir a comunidade bacteriana e promover uma biomassa de microalgas mais otimizada e segura, quando cultivada por meio de consórcios de microrganismos. Assis et al. (2017) operaram HRPs com recirculação de esgoto doméstico em reatores de biofilme (BR). A integração desses dois reatores formou um sistema híbrido para produção de biomassa e o uso do BR aumentou a produção de microalgas, quando comparada à uma LAT. Já em comparações entre os usos de diferentes reatores, os FBRs podem ter suas condições operacionais mais facilmente controladas, com menores chances de contaminação, menos perdas de CO₂ e maior produtividade que sistemas abertos como as LATs (GROBBELAAR, 2009; SUNDARRAJAN et al., 2019). Essas por sua vez apresentam menores custos operacionais, principalmente em relação ao consumo energético, sendo de mais fácil comercialização e reprodutibilidade em larga escala (DASAN et al., 2019; JORQUERA et al., 2010; XIAOGANG et al., 2020).

A viabilidade de cada tecnologia de cultivo pode ser avaliada em termos econômicos, produtivos e dos impactos ambientais associados. Apesar dos avanços no desempenho técnico dos sistemas de cultivo relatados na literatura, seu desempenho ambiental ainda requer maiores investigações. Estudos de Avaliação de Ciclo de Vida (ACV) permitem identificar processos críticos em um sistema, impulsionando a busca por inovações tecnológicas com redução dos impactos ambientais (YADAV; DUBEY; SEN, 2020). Essa ferramenta avalia as entradas e saídas de energia, processos e insumos desde a sua retirada do meio ambiente até sua disposição final e reciclagem, caracterizando seu ciclo de vida (ISO, 2006). Esses processos são, então, associados a indicadores de impacto ambiental, permitindo uma avaliação da sustentabilidade do processo de forma padronizada (HERRERA et al., 2020). Estudos recentes têm utilizado a ACV para avaliar diferentes tecnologias de cultivo de microalgas em águas residuárias (ARASHIRO et al., 2018; CASTRO et al., 2020; FERREIRA et al., 2020; HERRERA et al., 2020; SOUZA et al., 2019). Os resultados desses trabalhos apontam as vantagens da utilização de águas residuárias como meio de cultivo, mas a aplicação das tecnologias de cultivo ainda depende da otimização das etapas de cultivo, separação e tratamento do efluente.

Diante disso, o presente estudo visou utilizar a ACV como ferramenta para análise da performance ambiental de diferentes tecnologias de produção de biomassa algal em águas residuárias. Assim, compararam-se os impactos ambientais dos diferentes sistemas produtivos, meios de cultivo e tecnologias propostos para otimização da produtividade de biomassa. De forma a abranger diferentes arranjos de forma comparável, o trabalho foi dividido em dois capítulos:

- **Capítulo I:** Technologies for Improving Microalgae Biomass Production Coupled to Effluent Treatment: A Life Cycle Approach

- **Capítulo II:** Algal Biomass Production Coupled to Agro-industrial Wastewater Treatment: A Comparative Techno-Environmental Assessment of Open and Closed Systems

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3 HIPÓTESES

Os ganhos da adoção de tecnologias de aumento de produtividade no cultivo de microalgas em águas residuárias compensam os impactos ambientais gerados por essas tecnologias.

Do ponto de vista energético e ambiental, os sistemas abertos (LATs) são mais viáveis que sistemas fechados (FBR) para a produção de biomassa de microalgas em águas residuárias.

4 OBJETIVOS

4.1 Objetivo Geral

Avaliar os impactos ambientais das diferentes tecnologias otimizadas de produção de biomassa algal em águas residuárias por meio da Avaliação de Ciclo de Vida.

4.2 Objetivos Específicos

- Avaliar a performance (relação produtividade *versus* impactos ambientais) associados a diferentes tecnologias de produção de biomassa algal em águas residuárias, especificamente a influência:
 - Da utilização de sistemas híbridos (crescimento aderido em reator biofilme);
 - Da associação da suplementação de CO₂ de diferentes fontes;
 - Da pré-desinfecção UV do efluente.
 - De sistemas abertos e fechados, avaliando a performance de LATs e fotobiorreatores;
- Identificar os processos críticos e que mais impactam ambientalmente a produção;
- Propor soluções tecnológicas para os processos críticos.

5 ARTIGO 1. TECHNOLOGIES FOR IMPROVING MICROALGAE BIOMASS PRODUCTION COUPLED TO EFFLUENT TREATMENT: A LIFE CYCLE APPROACH¹

Abstract

Algal biomass production in wastewater is a promising value-adding alternative that should be coupled to the treatment system. The technical aspects of cultivation systems for improved biomass yield have been widely reported in the literature; yet, their environmental performance can still be further studied and compared. This study evaluated the environmental impacts associated with the production of 1 kg of biomass grown in high-rate ponds, with domestic effluent as culture media. Seven systems with three approaches to cultivation were modeled through the life cycle assessment method: using effluent pre-treated with ultraviolet disinfection; with supplementation from industrial CO₂ and with exhaust gas from gasoline combustion; coupled with a biofilm reactor for biomass attached growth. Environmental impacts were also compared to a base cultivation using the ReCiPe method, for 13 impact categories. The environmental impacts were reduced at least 30% by using wastewater as a water and nutrient source, with systems reaching negative impact values. The only exception was eutrophication categories, with the highest normalized impacts along with toxicity-related categories. The system using CO₂ supply from exhaust gas from gasoline combustion had the best performance in 11 categories, reducing impacts in at least 56% compared to a base cultivation scenario. The most impactful process was industrial CO₂ supplementation, followed by biofilm. Coupling industrial CO₂ supply and hybrid systems for increased biomass productivity did not compensate environmentally, increasing impacts up to 227%. Scenario evaluations were performed for increased and worsened performance of CO₂ supply rates ($\pm 40\%$) and biofilm reactor lifespan (± 20 days). Opportunities to improve lie in the use of recovered gas from different industries and different support materials for the attached growth of biomass in hybrid systems.

Keywords: Life cycle assessment; Algal biomass; High rate ponds; Biofilm Reactor; CO₂ supply; Ultraviolet disinfection.

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5.1 Introduction

The search for environmentally friendly technologies to supply the growing energy demand stimulates the study of renewable sources. Microalgae have been identified as a promising feedstock, being autotrophic organisms that use solar energy, carbon dioxide, and nutrients (such as nitrogen and phosphorus) to grow in aquatic media. They could require low-quality freshwater such as wastewater, brackish, or even highly saline water, also able to generate rich biomass in terms of carbohydrates, proteins, and lipids [1,2]. Today, there is a commercial application of many microalgae products considered being of high value (pigments, cosmetics, and pharmaceuticals). However, other products are not economically feasible yet, such as biofuels, biogas, biofertilizer and animal feed, given their low commercial value with investments higher than the final profit. Low productivity, high installation, and operating costs are barriers that still major drawbacks [3].

In this sense, the use of wastewater as culture media emerges as an economically attractive option, which integrates the potential of biomass production with effluent bioremediation [1,4,5]. Since wastewater treatment is a necessary step before discharge into water bodies, associating the production of a biomass rich in nutrients and organic matter (applicable to various recovery routes) to the process would be the ideal scenario [2]. For this reason, the cultivation of microalgae biomass has been evaluated in different wastewater, such as domestic sewage [6–9] and agro-industrial effluents [8,10,11].

Using wastewater as a culture medium, however, can result in lower algal biomass yield when compared to synthetic media, given productivity depends on factors inherent to the culture, such as pH, nutrient availability or presence of toxic compounds, and the cultivation system type [5]. Therefore, to make its large-scale application feasible, increasing productivity is one of the main optimization points. One of the most studied cultivation systems is high rate ponds (HRPs). The choice for the cultivation system depends on the investment cost, the desired product, the source of nutrients and the carbon fixation by the microalgae present in the media [12]. Ponds are usually shallow, implanted to receive sunlight and to promote photosynthesis. Culture media circulation is induced by paddles, which cause smooth horizontal movements around the HPR and turbulent vertical mixing [13]. Vertical mixing increases cell metabolism and the development of biomass, promoting light and dark cycles, increasing the diffusion of nutrients, and preventing sedimentation of biomass [13–17]. Strategies to improve HRPs cultivation performance and increase biomass productivity have been widely studied. Among

the advances reported in the literature, Assis et al. [7] and Heubeck et al. [18] achieved higher biomass yields by adding different sources of CO₂ to supply the carbon deficiency of domestic sewage. Assemany et al. [19] investigated the use of HRPs using domestic sewage pre-disinfected by ultraviolet radiation (UV). The authors reported that this technology was promising to reduce bacterial community and promote a cleaner and safer microalgae biomass when grown through microorganism consortia. Assis et al. [6] operated HRPs with domestic sewage recirculation in biofilm reactors (BR). Integrating these two reactors formed a hybrid system for biomass production and the use of the BR improved microalgae yield.

The feasibility of each technology can be assessed in technical, economic, social and environmental terms. Despite advances in the technical performance of cultivation systems reported in the literature, their environmental performance still requires further investigation. One of the primary tools used to assess the environmental performance of products and processes is the Life Cycle Assessment (LCA) [20]. The method allows environmental impacts quantification and enables spotting opportunities for improvement within the product life cycle, by analyzing the contribution of each input to the total environmental load [21]. In this way, it assists decision-making to reduce the negative environmental impacts caused by the process's entry into the cycle.

Cultivation is identified as a very impactful stage in the microalgae production chain [22]. The life cycle approach has shown that its environmental impact is associated with high energy consumption [10,11,23] and CO₂ supplementation [24]. On the other hand, Schneider et al. [25] reported that the use of effluent as a culture media contributes to the reduction of environmental impacts when compared to cultivation in synthetic media. This result is also corroborated by Bussa et al. [23], which verified a lower environmental impact in the production of biomass using different types of wastewater. Regarding the cultivation system, HRPs proved to be less economically impacting to treat wastewater in small communities when compared to other treatment units [26]. Goswami et al. [27] and Nagarajan et al. [3] recommend further studies on pilot-scale conditions, with studies aimed at the cultivation stage still incipient and to be better explored by techno-economic and life cycle analysis.

The present study evaluates the environmental performance of different algal biomass productivity technologies, using domestic sewage as a culture media. The investigated technologies were: (i) HRP preceded by UV disinfection; (ii) HRP with supplementation of industrial and reused CO₂ and (iii) the use of hybrid systems, with attached growth BR. The

critical points of each system were discussed, and the results were compared. The novelty of this work is the use of primary data, obtained through pilot-scale experiments and the comparison of different strategies to increase biomass productivity. In the literature reviewed, the authors did not find studies comparing these cultivation technologies through the LCA method.

5.2 Material and Methods

LCA is defined by ISO 14040 [28] as a compilation and evaluation of the inputs, outputs and potential environmental impacts of a product system throughout its life cycle. This method was initially used in the 60s and 70s, aiming to quantify environmental impacts systematically. That way it is possible to evaluate processes' resilience and overall sustainability of microalgae cultivation strategies [27], establishing a standardized comparison.

LCA was applied in this study to quantify the environmental impacts associated with 7 scenarios of microalgae cultivation in domestic effluent. Its application was carried out according to ISO 14040. The SimaPro © 9.0.0 software was used, with four main phases: goal and scope definition, life cycle inventory, environmental impact assessment and interpretation. Souza et al. [10] point out that one of the major difficulties of LCA studies is the use of primary data, which is scarce for this type of system in Brazil. Thus, the novelty of this work was using, mainly primary data reported from pilot-scale scientific experiments.

5.2.1. Data collection

For the present study, experimental data were obtained from the works by Assis et al. [7], Assis et al. [6] and Assemany et al. [19]. The studies were carried out in the experimental area of the Laboratory of Sanitary and Environmental Engineering at the Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil (20°45'14"S, 42°52'54"W).

Despite having different objectives, these studies achieved improvements in biomass yield, using microalgae cultivation in domestic sewage for biomass production in the experimental stage. Moreover, HRPs used in studies for the production of biomass had the same capacity. The pilot-scale HRPs used in the studies were operated in a continuous flow regime with dimensions: width 1.28 m, length 2.86 m, total depth of 0.5 m, useful depth of 0.3 m, surface area 3.3 m² and useful volume of 1 m³. They were built in fiberglass, with steel paddlewheels with six blades, powered by electric motors and wastewater continuously fed by

a water pump. The biomass produced in the experiments was a mix of bacteria and microalgae and measured using Total Volatile Solids (APHA-AWWA-WEF, 2012). *Chlorella vulgaris* was the predominant species in all cultivation systems. Productivity reported in the studies was given in $\text{g m}^{-2}\text{day}^{-1}$.

Assemany et al. [19] evaluated biomass yield for cultivation in two HRP: (C1) a base cultivation and (C2) one with UV pre-disinfection of the effluent. The base cultivation comprised an HRP receiving domestic effluent without disinfection, from an Up-flow Anaerobic Sludge Blanket (UASB) reactor. The second HRP received the same effluent, previously disinfected by UV radiation. The disinfection system used by the authors comprised 3 UV lamps, with a volume dosage of 5.64 Wh m^{-3} .

Assis et al. [7] studied two sources of CO_2 supplementation for microalgae cultivation in HRP: (C3) industrial CO_2 (99.9% concentration) and (C4) exhaust gas from gasoline combustion (EGGC). Two HRPs were operated continuously, fed with domestic effluent pre-treated in a septic tank. A carbonation column was used for the CO_2 addition. The carbonation columns for CO_2 supplementation were built according to Putt et al. [29], adding CO_2 in the lower parts of the HRPs.

Assis et al. [6] compared the biomass yield for HRP cultivation coupled to BR, characterizing the use of hybrid systems. Three HRPs were operated with domestic effluent pre-treated in a UASB reactor. One system (C6) consisted of an HRP adapted with a BR. The operation consisted of recirculating the effluent from the HRP in the BR during cultivation, forming attached biomass in the panels. Other HRP (C5) used both the BR and CO_2 supply (99.9% concentration) with a carbonation column, designed according to Putt et al. [29]. The biofilm panel for attached growth had an area of 1 m^2 , made with cotton fabric as a support material. The effluents from the HRP recirculated in the BR for 10 hours a day and useful volume was recirculated 10 times a day ($1 \text{ m}^3 \text{ h}^{-1}$). The last system (C7) consisted of a conventional HRP with industrial CO_2 supplementation. Total biomass yield consisted of both the suspended and attached growth.

More details about the experimental methodology of biomass production can be found in Assemany et al. [19], Assis et al. [6] and Assis et al. [7]. A summary of the key characteristics of the cultivation stage for the evaluated systems is shown in Table 5.1.

Table 5.1 Evaluated systems, with domestic sewage wastewater inlet in high rate ponds (HRP).

Reference	Pre-Treatment	Reference	System	Description
	UASB	HRAP	C1	Base cultivation in HRP
Assemany et al., 2015	UASB and UV disinfection	uvRAP	C2	Effluent pre-disinfected by UV Reactor
Assis et al., 2019	Septic Tank	HPR1	C3	HRP with industrial CO ₂ supplementation (99.9% purity)
		HPR2	C4	HRP with CO ₂ supplementation (exhaust gas from gasoline combustion - EGGC)
Assis et al., 2017	UASB			Hybrid system, HRP + BR (attached growth in biofilm reactor)
		HS1	C5	HRP with industrial CO ₂ supplementation (99.9% purity)
		HS2	C6	Hybrid system, HRP + BR (attached growth in biofilm reactor)
		convHR P	C7	HRP with industrial CO ₂ supplementation (99.9% purity)

BR - Biofilm reactor; UV - Ultraviolet

It is noteworthy that, although all the scenarios used domestic wastewater as culture media, the anaerobic pre-treatment (septic tank and UASB reactor) resulted in culture media with different characteristics. For this reason, scenarios C3 (septic tank) and C7 (UASB reactor) used the same technology for increasing biomass production (Industrial CO₂ supply).

5.2.2 Goal, Scope Definition and System Boundaries

For this work, the LCA aimed to quantify the environmental impacts associated with biomass production. The approach adopted was from gate to gate, with the focus on the

cultivation stage, disregarding the life cycle of the treated effluent and/or the subsequent application of biomass for any by-products. System boundaries were defined to make the available data compatible and included the cultivation stages of each work, focusing on the different technologies that can be adopted in cultivation. The schemes were composed of the evaluated systems, energy additions, inputs, avoided products and emissions. All systems were composed of HRPs fed with domestic effluent. The existence of effluent pre-treatment was explicit, but it is not considered within the modeled frontiers.

The functional unit commonly adopted for effluent treatment processes is 1 m³ of treated effluent [20]. However, the present study aimed to assess whether the productivity gains compensate for the increases in inputs and energy necessary for the application of each technology here investigated. Thus, it was used 1 kg of biomass produced in each system as a functional unit.

The advantage of wastewater production is its ability to be easily coupled to the wastewater treatment [2], meeting the microalgae demand for nutrients in parallel to the mandatory bioremediation before its discharge in water bodies. Thus, to include this advantage in the modeled scenarios, nitrogen (N), soluble phosphorus (P) and water were considered as avoided products. This approach was also used in other studies that considered the production of wastewater biomass [10,11,30]. For this purpose, effluent characterization data reported in the previously mentioned studies were considered [6,7,19]. For system C4, to evaluate the recovery of the EGGC as a carbon source in the HRP, CO₂ was also modeled as an avoided product. As cutting criteria for data entry, structures with a useful life of over 10 years were not considered. This is the case for system units, such as ponds and treatment units, with a useful life of approximately 25 years [10,11].

Although some scenarios include the use of pure industrial CO₂, which is observed in other technical works that treat wastewater [23,31–33], its application to treat domestic sewage can be seen as somewhat strategic due to its cost. Currently, wastewater treatment plants (WWTP) are planned to comply with legal requirements. Plants based on circular economy and bioeconomy are still slowly progressing. WWTP are typically designed to offer operational simplicity and require low costs. Still, depending on the purpose of the WWTP, it is possible to adopt pure industrial CO₂ supplementation. In domestic sewage, carbon limits the growth of biomass, so the addition of CO₂ can contribute to increase the efficiency of nutrient removal [34]. Despite the possibility of using alternative sources of CO₂ in the cultivation of microalgae

[7,33–36], the WWTP may not have near industrial plants that generate CO₂ emissions, which makes supply unfeasible by increasing the cost of this approach. On the other hand, the composition of the flue gases or industrial activity varies according to the processes that generate it, so its use (with or without pre-treatment) needs to be investigated in each specific case. If the biomass-based product has a higher market price, there is greater flexibility for the use of gas sources with higher costs [7]. Thus, considering the points raised, the authors modeled scenarios with the supplementation of pure industrial CO₂.

Without disregarding the treatability aspect, each system reported different values of efficiency in nutrient removal. Seeking to simulate the systems in the best way, values of ammonia nitrogen (N-NH₃, used here as N) and soluble Phosphorus (P) after treatment were inserted as emission to water. These parameters are commonly considered among the effluent discharge standards. Their emission is decisive to trigger eutrophication of receiving water bodies, since P and N are essential elements for algae growth. Reported values of N, P and biomass productivity used for modeling the scenarios are summarized in Table 5.2 and boundaries for the seven systems in Figure 5.1.

Table 5.2 Reference parameters of N, Ps, biomass productivity and hydraulic retention time (HRT) from the systems.

<i>Parameters</i>	<i>Assemany et al., 2015</i>			<i>Assis et al., 2019</i>			<i>de Assis et. al, 2017</i>			
	Inlet	C1	C2	Inlet	C3	C4	Inlet	C5	C6	C7
N (mg L ⁻¹)	39.82	11.70	10.20	77.4	26.7	26.1	37.3	6.1	7.9	11.5
				0	0	0	0	0	0	0
P (mg L ⁻¹)	4.05	3.47	3.28	12.3	13.2	13.5	5.20	4.1	3.9	3.80
				0	0	0		0	0	
Productivity (g m ⁻² day ⁻¹)		11.43	9.30		6.00	6.12		6.7	6.2	6.27
								9	0	
HRT (day)		4.00			8.00			5.00		

HRT – Hydraulic Retention Time; N – Nitrogen; Ps – Phosphorus

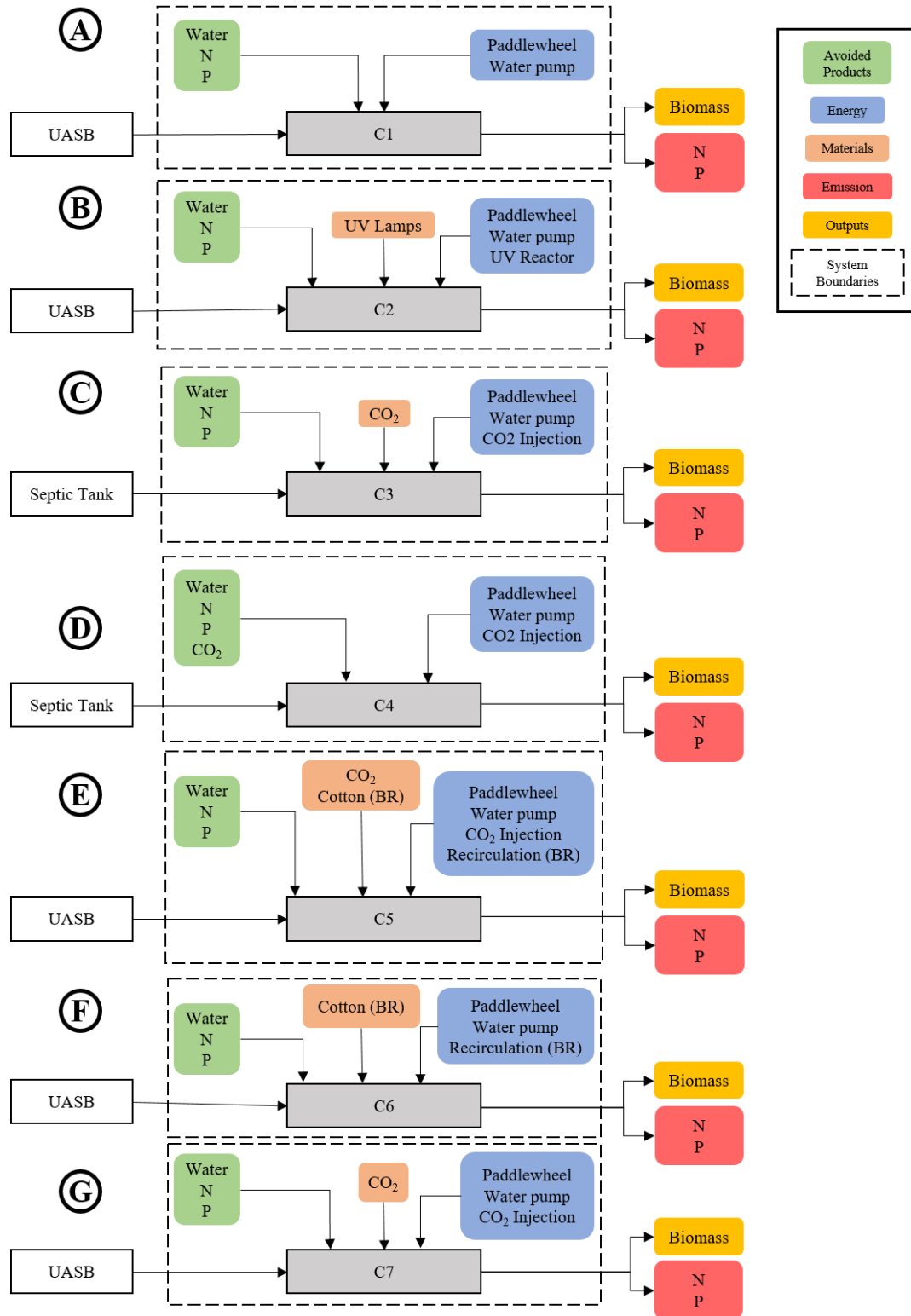


Figure 5.1- Systems boundaries: (A) Base cultivation – C1; (B) Effluent with UV pre-disinfection – C2; (C) Supplementation of industrial CO₂ – C3; (D) Exhaust Gas from Gasoline Combustion supplementation – C4; (E) Hybrid system with industrial CO₂ supplementation – C5; (F) hybrid system without CO₂ supplementation – C6; (G) With industrial CO₂ supplementation – C7. (N) Nitrogen; (P) Phosphorus; (BF) Biofilm Reactor.

5.2.3. Life cycle inventory

The system boundaries were defined from the arrival of the effluent to the system until the estimation of biomass production, seeking to perform a comparable analysis [37] which includes the cultivation phase.

Since the experiments were conducted on a pilot scale, the energy consumption of the units used differs significantly from that of a large-scale operation. Besides, they were carried out for purposes other than LCA, some data required for this approach were not reported (such as the specific consumption of motors for rotating the blades per experimental unit). In this way, the energy consumption data of this equipment was acquired through a bibliographic survey, for typical values from literature and equipment operation consumption data [38,39].

Also aiming for data standardization, CO₂ injection was based on the literature. Since data on the injected amount of gas for all works were not reported, the comparison between the systems becomes more coherent. A summary of some input calculation processes is shown in Table 5.3. The mathematical formulas used for such transformations, and areas and volumes required, are found in the Supplementary Material (Eq. 1 to Eq. 13 and Table S2, in Appendix A).

Table 5.3-Input calculation parameters.

		Value	Unit	References
Parameters	HRP area	3.3	m ²	
	BR area	1	m ²	
	Hybrid system area	4.3	m ²	
	HRP volume	1	m ³	
	HRP depth	0.3	m	
	CO ₂ injection	293	kg day ⁻¹ ha ⁻¹	Collet et al., 2011
Electricity consumption	Paddlewheel	2.7	kW ha ⁻¹	Albarelli et al., 2018
	CO ₂ injection	0.0222	kWh (kg CO ₂) ⁻¹	Collet et al., 2011
	Water pump	0.5	kW ha ⁻¹	Albarelli et al., 2018

	UV reactor	5.64 Wh m ⁻³	Assemany et al., 2015
BR Parameters	Textile Density (cotton)	105 g m ⁻²	
	Total Operation	40 Days	
	Recirculation	10 h day ⁻¹	
	BR Recirculation Flow	1 m ³ h ⁻¹	
	Power	13 W	

HRP – High rate algal pond; BR – Biofilm Reactor.

The processes used for scenario modeling in the software belong to the Ecoinvent 3.0 library database (see details in Supplementary Material, Table S1). When available, processes referring to global data (GLO) and Rest of the World (RoW) were used. For the energy mix, the German base (DE) was used. According to the 2017 International Energy Agency survey [40], the Brazilian mix is largely composed of renewable sources, with hydro power covering over 70% of its total generation. This is the base used for the Ecoinvent database, limiting its impacts and not representative of a global trend [11]. Thus, the choice for a more comprehensive and representative impact basis with larger study applicability is justified. Data reported in Table 5.2 were normalized for the production of 1 kg of biomass to compose the inventories for each scenario (Table 5.4).

Table 5.4 Inventories of modeled cultivation systems - Quantities necessary for the production of 1 kg of biomass.

Parameters	Assemany et al., 2015		Assis et al., 2019		Assis et al., 2017		
	C1	C2	C3	C4	C5	C6	C7
Daily Productivity (g m ⁻² day ⁻¹)	11.43	9.30	6.00	6.12	6.79	6.10	6.27
HRT (day)	4.00		8.00		5.00		
Total Productivity (g m ⁻²)	45.72	37.20	48.00	48.96	33.95	30.50	31.35
Area (m ²)	21.87	26.88	20.83	20.42	22.61	25.16	31.90
Volume (m ³)	6.56	8.06	6.25	6.13	6.78	7.55	9.57

<i>Avoided Products</i>							
Water (kg)	6561.68	8064.52	6250.0 0	6127.4 5	6781.5 2	7548.6 1	9569.3 8
N (g)	261.29	321.13	483.75	474.26	252.95	281.56	356.94
P (g)	26.57	32.66	76.88	75.37	35.26	39.25	49.76
CO ₂ (g)				4.79			
<i>Input</i>							
<i>Material</i>							
Cotton (support material) (g)					89.91	100.08	
CO ₂ (kg)			4.88		3.31		4.67
UV Lamps (unit)		3.00					
<i>Electricity</i>							
Paddlewheel (kWh)	0.57	0.70	1.08	1.06	0.95	1.06	1.03
CO ₂ Injection (kWh)			1.08	1.06	0.74		1.04
Water Pump (kWh)	0.10	0.13	0.20	0.20	0.14	0.20	0.19
Pump for wastewater Recirculation (kWh)					0.65	0.65	
UV Reactor (kWh)		0.05					
Total (kWh)	0.67	0.87	2.36	2.32	2.48	1.91	2.26
<i>Output</i>							
<i>Emission to Water</i>							
N (g)	109.06	132.18	585.00	491.42	202.77	317.04	170.33
P (g)	22.77	26.45	82.50	82.72	27.80	29.44	36.36

HRT – Hydraulic Retention Time; N – Nitrogen; P – Phosphorus.

5.2.4 Assumptions and Analysis Limitations

In wastewater treatment, it is important to note that the comparison between the works can be affected by the variability of its physical-chemical characteristics. Domestic sewage presents a variable composition, both spatial and seasonal, and can differ in terms of volumes produced, temperature and compounds present [41]. Thus, comparing works carried out in different periods can cause limitations in direct comparison. Systems such as C3 and C7 used the same improvement technology (industrial CO₂ supply) but used different anaerobic pretreatments (septic tank and UASB reactor). However, the variability of domestic effluents is common within the context of wastewater treatment. To overcome this limitation and make the scenarios comparable, the efficiency of the treatment was considered, using the emissions of N and P to the water.

In the reference studies, HRPs were fed by gravity. In the scenario of sustainable WWTPs, ideally the system that precedes the HRP should be designed in order to avoid energy consumption when transporting the effluent to the biological system, such as the use of gravity. However, as predicted by Albarelli et al.[38] and used in Marsullo et al. [42], still, when adopting a WWT system with the use of HRPs as a secondary treatment, in most cases, it is necessary to use water pumps, considering that the plant was could not be designed in this configuration. Thus, the authors decided to take a more conservative approach and include the use of pumps in all scenarios.

Another operational point that differs for the reference studies is the HRT. Valigore et al. [43] point out that this parameter impacts biomass yield, with shorter times associated with higher productivity. It is important to note that operational design, such as raw effluent quality and discharge standards, also influence the ranges in which this parameter may vary. This can be seen in Table 2, in which the wastewater from the septic tank presents 2 and 3 times higher N and P concentration, respectively, compared to the wastewater from the UASB reactor. Therefore, they are quite different effluents, justifying the higher HRT adopted by Assis et al. [7]. Besides nutrients, the concentration of other sewage variables from both treatment technologies was different, as can be further seen in the original research by Assis et al. [7], Assis et al.[6] and Assemany et al. [19]. Thus, although HRT can influence productivity and nutrient outputs, data for all scenarios were normalized for the production of 1 kg of biomass and treatability aspects were used from primary data from the reference studies. Variations in this parameter were considered in the energy consumption calculations of each cultivation system and effluent quality reported in the studies. Thus, this parameter is indirectly impactful in the model.

The durability of the fabric used in the biofilm reactor was not reported by the study of Assis et al. [6]. Cotton consumption was estimated based on the time of use reported in the experiment. More appropriate values can influence factors for the impacts generated. To better investigate this input, it was subject to sensitivity analysis, covered in section 2.6.

Regarding modeling the amount of CO₂ (kg) consumed in each operation, this value was not reported on the reference studies. These studies performed carbon supply through pH control, and the amount of CO₂ used was not measured. Environmental and technical factors can change the pH during the cultivation, such as temperature, climate, the effluent organic matter load, light availability and carbon fixation rates. Park and Craggs [44] reported high

variability in CO₂ addition through pH control for two HRPs, attributing the results to variations in weather, algal concentration and degradation of organic matter. In the present study, authors used supply rates reported in Collet et. al [39], to model all scenarios, promoting standardization of parameters to compare results. Thus, a continuous supply of CO₂ throughout the operation was considered. To overcome this limitation of the model used, the authors performed scenario evaluation of CO₂ supply rates. The evaluation considered a worsened and improved scenario for the amount of CO₂ consumed in the operation, to model pH variations and how they could affect results. This analysis was covered in section 5.2.6.

Finally, for the lamp lifespan estimate in the system with UV disinfection, Assemany et al. [19] did not report their useful life. As an approximation, the production of all three units in the inventory was considered. More accurate estimates of energy consumption and lamp lifespan in operational scale systems can produce better environmental performance data.

5.2.5 Impact Assessment

To quantify the environmental impacts of each scenario, the ReCiPe midpoint (H) method was used. This choice was due to its focus on environmental issues, as pointed out and used by Souza et al. [10], Castro et al. [11] and Yadav et al. [22]. Besides, this method normalizes impact results using global emission standards, which made it possible to compare the systems and their ranking.

To rank system performance, a global assessment was made with aid of categories covered by ReCiPe. Considering inventory data, the focus was on the categories: Global Warming, Ozone Depletion, Terrestrial Acidification, Freshwater Eutrophication, Marine Eutrophication, Ozone Formation, Particulate Matter Formation, Metal Depletion, Fossil Depletion, Human Toxicity and Terrestrial Ecotoxicity. These were identified as the most relevant in effluent treatment and were used in other studies that addressed microalgae cultivation in wastewater [45].

Environmental impacts were assessed in terms of data characterization and normalization. Initially, the general analysis of the systems was through a score. In each category, the systems were assigned values from 1 to 7 based on their increasing impact for each category in the characterization results. For each category, systems with value 1 presented

the lowest impact and 7, the major. The sum of the ranks of each system in each category generated the final score. A lower score means lesser impacts and, therefore, a better system.

This analysis was complemented with the assessment of data normalization, based on European emission standards provided by the method used here. This normalization allows the assessment of the relative contribution in each category and helps to identify possible inconsistencies generated in comparative LCA [46].

5.2.6 Scenario Analysis

To identify the influence of the most impactful processes of the impact assessment, a scenario analysis was carried out for the lowest-ranked system. For these processes and considering the parameters reported from literature to calculate the inventory, two scenarios were evaluated for each: an improved scenario, using values that reduce the amount of key parameters; and a worsened one, imposing a maximum value to those same parameters. Both were compared with the base inventory previously reported for each impact category.

The influence of two processes was evaluated:

- (i) Durability of the fabric used in the BR: ± 20 days of the cotton textile, from the 40 days reported in Table 3. As discussed in topic 2.4, there was no evaluation of the time needed to change the BR fabric in the original work. Meaning, authors used the 40 days of operation as the base to calculate the amount of cotton (kg) needed.
- (ii) Influence of the CO₂ supply: $\pm 40\%$ carbon supplementation from the 293 kg day⁻¹ ha⁻¹ reported in Table 3. Since the reference studies did not report on the mass of CO₂ consumed during the operation, authors modeled the carbon utilization as continuous, and performed scenario evaluation for values $\pm 40\%$ from the 293 kg day⁻¹ ha⁻¹. This variation aims to simulate changes in the CO₂ consumption considering pH control for carbon supply. The variation in this rate changes the CO₂ amount (kg) and the CO₂ energy requirements (kWh).

The inventory for the scenarios is presented in Supplementary Materials (Table S3). The sensitivity for each category was evaluated in terms of the sensitivity coefficient [45,47], according to Eq. (1):

Sensistivity Coefficient (S)

$$= \frac{(\text{Output}_{\text{High}} - \text{Output}_{\text{Low}}) - \text{Output}_{\text{Default}}}{(\text{Input}_{\text{High}} - \text{Input}_{\text{Low}}) - \text{Input}_{\text{Default}}} \quad (1)$$

This way, it was possible to discuss the influence of each of the most impactful processes on the environmental load of each system.

5.3 Results and Discussion

5.3.1. Characterization of general impacts

The potential environmental impacts of cultivation systems, for each of the 13 categories considered, were compared in relative percentage. The ranking of the best systems, in terms of environmental impact from lower to highest, considering the characterization score, is shown in Table 5.5.

Table 5.5. Ranking of products evaluated according to the results of the impact assessment.

Impact category	Unit	C1	C2	C3	C4	C5	C6	C7
Global warming	kg CO ₂ eq	-4.30E+00	-2.35E+00	2.02E-01	-9.00E+00	2.06E+00	-1.74E+00	-8.13E-01
Stratospheric ozone depletion	kg CFC11 eq	-2.86E-05	-3.43E-05	-5.03E-05	-5.15E-05	-1.96E-05	-2.29E-05	-3.76E-05
Ozone formation, Human health	kg NO _x eq	-9.48E-03	-5.16E-03	-4.62E-03	-1.61E-02	1.68E-04	-4.80E-03	-7.08E-03
Fine particulate matter formation	kg PM2.5 eq	-9.14E-03	-3.04E-03	-4.40E-03	-1.56E-02	-3.94E-04	-5.08E-03	-7.24E-03
Ozone formation, Terrestrial ecosystems	kg NO _x eq	-9.59E-03	-5.18E-03	-4.66E-03	-1.63E-02	1.82E-04	-4.86E-03	-7.14E-03
Terrestrial acidification	kg SO ₂ eq	-1.63E-02	-9.41E-03	-8.41E-03	-2.85E-02	1.13E-03	-7.66E-03	-1.17E-02
Freshwater eutrophication	kg P eq	7.28E-03	8.55E-03	2.73E-02	2.68E-02	9.46E-03	9.75E-03	1.20E-02
Marine eutrophication	kg N eq	3.23E-02	3.92E-02	1.74E-01	1.46E-01	6.06E-02	9.45E-02	5.07E-02
Terrestrial ecotoxicity	kg 1,4-DCB	-1.01E+01	2.04E+02	1.43E+01	-4.38E+01	1.29E+01	-8.02E+00	1.41E+01
Human carcinogenic toxicity	kg 1,4-DCB	-4.29E-02	2.78E-02	-1.99E-02	-6.72E-02	1.02E-03	-1.88E-02	-3.61E-02
Human non-carcinogenic toxicity	kg 1,4-DCB	-2.84E-01	3.59E+00	-1.51E-01	-6.82E-01	1.73E-01	-2.06E-02	-1.39E-01
Mineral resource scarcity	kg Cu eq	-1.98E-02	-2.69E-03	-1.64E-02	-4.06E-02	-8.42E-03	-1.92E-02	-1.67E-02
Fossil resource scarcity	kg oil eq	-1.03E+00	-4.14E-01	-2.15E-01	-1.81E+00	2.72E-01	-4.31E-01	-4.87E-01
	Score	27	62	68	23	78	59	47
	Rank	2	5	6	1	7	4	3
Description	Base cultivation	UV reactor	Industrial CO ₂	CO ₂ supply	BR; Industrial CO ₂ supply	BR	Industrial CO ₂ supply	
Observation	Without any additions	Effluent pre-disinfected	Primary Treatment (Septic Tank)	EGGC	Hybrid System	Hybrid System	Secondary Treatment (UASB)	

EGGC - Exhaust Gas from Gasoline Combustion; UASB - Upflow Anaerobic Sludge Blanket.

System C4 presented itself as the least impacting in 11 of the 13 categories analyzed. Compared to the base system (C1), impacts were 1.57 times lower for Human Carcinogenic Toxicity (56% reduction) up to 4.54 times lower (352% reduction) for Marine Eutrophication. System C5 was the most impactful in 7 of the 13 categories, and the second most impactful in 3 more. Compared to C1, impacts increased up to 227% in the Terrestrial Ecotoxicity category, due to the use of industrial CO₂ and the BR. The major discrepancy for both systems was related to the Marine and Freshwater Eutrophication categories, in which C4 performed better. This result is mainly related to the values of N and P after treatment, inserted as emissions to water. In the analysis of contributions by processes, the subject is further addressed (Section 3.2).

The base cultivation system, C1, was second in the rank of the lowest negative environmental impacts. This may be associated with the productivity reported by Assemany et al. [19] being the highest among the systems presented. This system does not add any input (such as CO₂ and the biofilm reactor textile) or energy expenditure beyond the paddlewheel and pump.

Results can be further interpreted through normalization, shown in Figure 5.2. The category that had the greatest negative impact was Terrestrial Ecotoxicity, mainly for system C2. UV reactor use was the most impactful in 4 of the 13 categories, mainly in indicators related to toxicity. This result is attributed to the mercury present in its production (99.92% of the positive impact values). In a direct comparison between C1 and C2 [19], base cultivation proved to be favorable in all categories. Thus, despite the productivity gains reported with the use of UV lamps in the pre-treatment, the associated impacts discourage the application of this technology.

Next, the most impactful categories were Freshwater Eutrophication, Marine Eutrophication and Human Health, for most of the systems. This reaffirms the results of Arashiro et al. [45], which points to these as the most impactful found for other LCA studies applied to wastewater treatment.

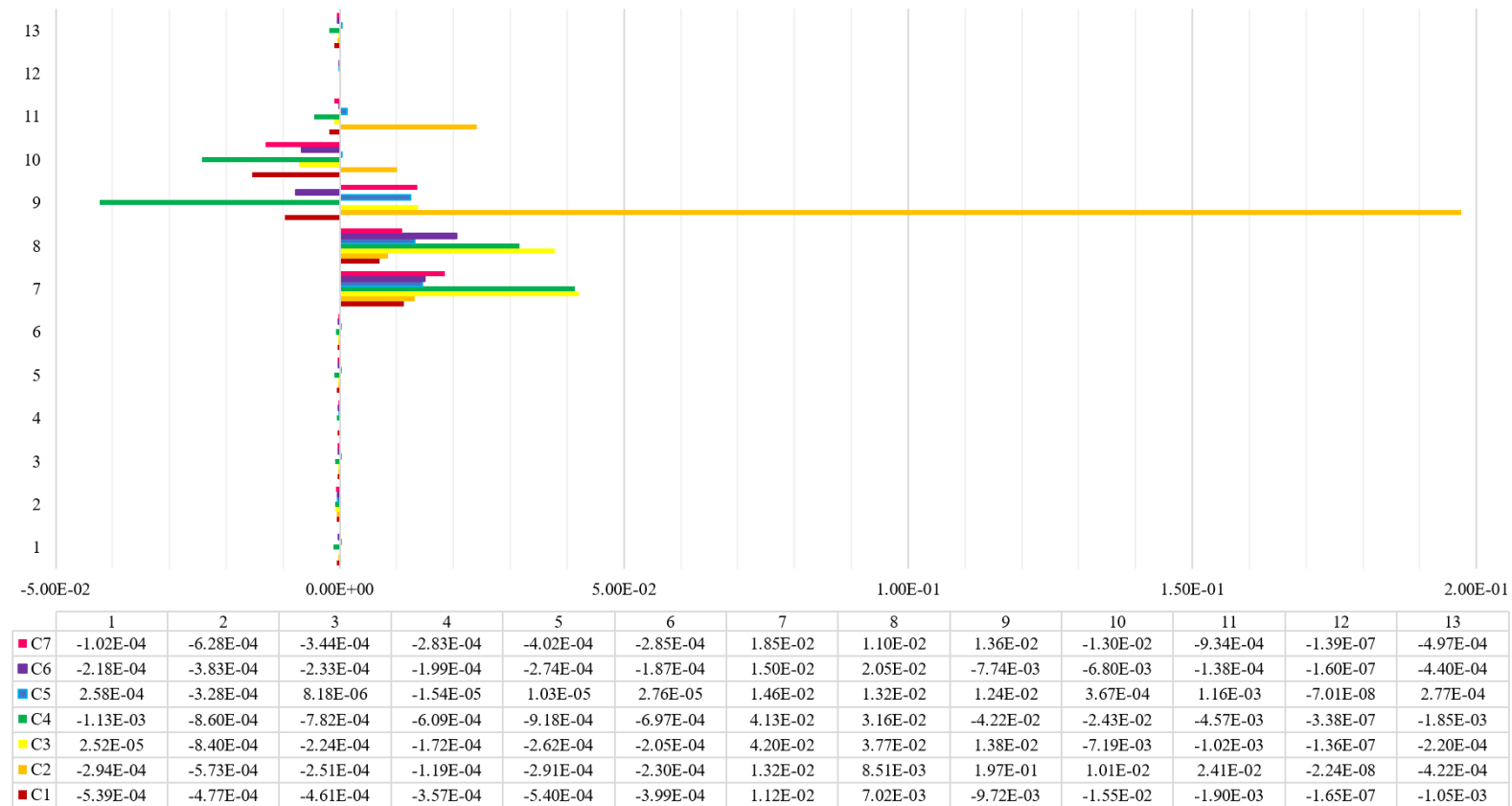
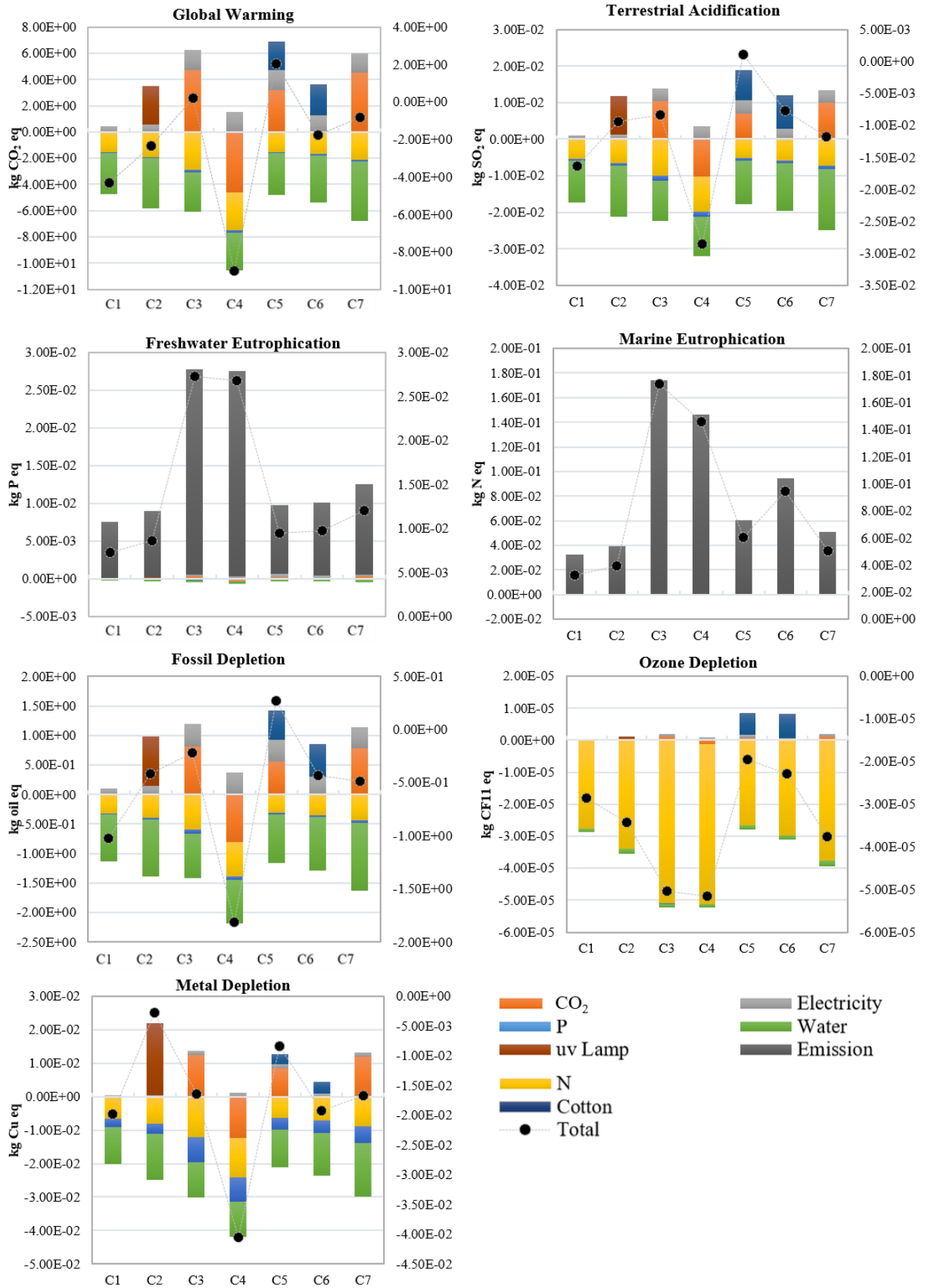


Figure 5.2- Results of the Normalization of the impact categories. (1) Global warming; (2) Stratospheric ozone depletion; (3) Ozone formation, Human health; (4) Fine particulate matter formation; (5) Ozone formation, terrestrial ecosystems; (6) Terrestrial acidification; (7) Freshwater eutrophication; (8) Marine eutrophication; (9) Terrestrial ecotoxicity; (10) Human carcinogenic toxicity; (11) Human non-carcinogenic toxicity; (12) Mineral resource scarcity; (13) Fossil resource scarcity.

5.3.2. Environmental impacts by category and input contribution

The environmental impacts of the modeled scenarios can be seen in Figure 5.3, for the 13 categories analyzed. These impacts were broken down by the contribution of inputs, avoided products and emission to water (column, main axis). Also, it is possible to observe the total impact value (line, secondary axis). The “Emissions” process detail contributions related to including N and P as emissions to water in the scenarios.



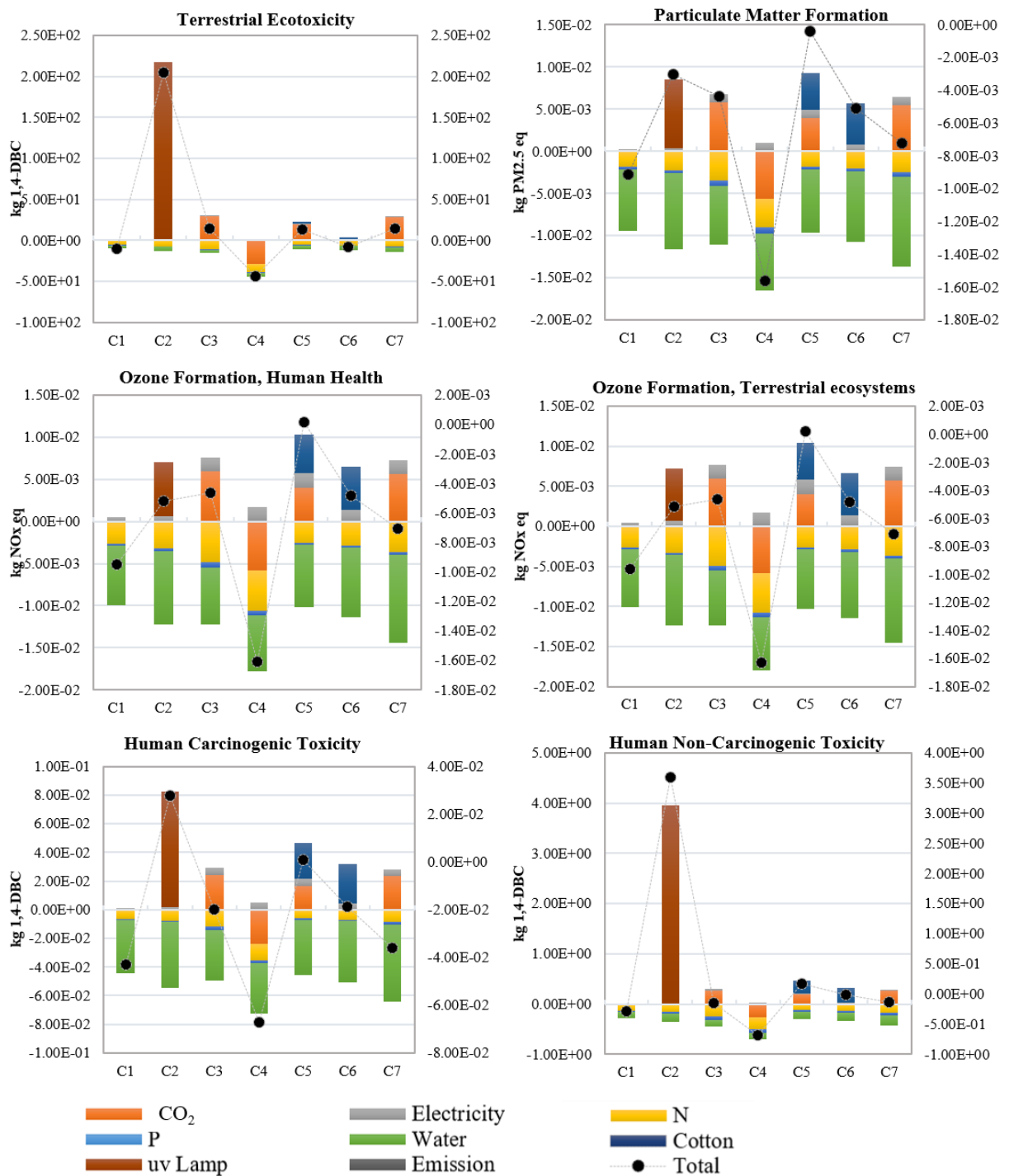


Figure 5.3 - Results by Impact Category and input contribution (columns, main axis) and total impact value (line, secondary axis).

Using CO₂ supplementation was primarily responsible for the environmental impacts in the categories of Global Warming, Terrestrial Acidification, Particulate Matter Formation, Fossil Depletion and Metal Depletion. Overall, CO₂ consumption was the input that most contributed to the generation of environmental impacts, for scenarios C3 and C5, in all categories, except for Freshwater and Marine Eutrophication. While the same is true for C7,

there is also the exception of the Terrestrial Acidification Category, in which Cotton stood out. This result is in line with that reported by Collotta et al. [24], who reported that when industrial CO₂ was used, it was the primary driver of environmental impacts. Despite this, considering the total impact, the scenarios with industrial CO₂ did not result in low environmental performance. Comparing scenarios C3 (domestic effluent after UASB with industrial CO₂), C7 (domestic effluent after septic tank) and C1 (base cultivation), scenarios that included industrial CO₂ had negative impacts in the same categories as C1, except for Global Warming for C3 and Terrestrial Ecotoxicity for C7 and C3. Thus, of the 13 categories studied, scenarios C3 and C7 generated an impact in four and three categories, respectively, and of those, two categories generated an impact in all scenarios. This was because the impacts of CO₂ consumption are offset by the benefit of using wastewater. Thus, the use of industrial CO₂ in the context investigated was not, in environmental terms, unfeasible. In economic terms, future studies can further investigate the feasibility of this approach. In particular, on a real scale, it would be interesting to understand to what extent the use of pure CO₂ would be practical, considering the treatment efficiency and desired biomass productivity, edaphoclimatic conditions, volume and type of effluent treated, among other possible important aspects for wastewater treatment.

For C2, UV lamps were the input that most contributed to environmental impact in all categories evaluated. Using the lamps had an impact mainly on Terrestrial Ecotoxicity and Human Toxicity. It also contributed to Terrestrial Acidification, Particulate Matter Formation, Fossil Depletion and Metal Depletion.

Regarding hybrid systems (C5 and C6), the impact associated with the fabric used as a support material in BR was also highlighted. Using cotton fabric had a greater influence on the categories of Global Warming, Fossil Depletion, Ozone Depletion, Particulate Matter Formation, Ozone Formation, Human Toxicity, Terrestrial Acidification and, to a lesser extent, Metal Depletion. The main substances responsible for these impacts are those associated with insecticides in the cotton production chain, as will be further discussed in the following section.

Energy consumption is identified as one of the major setbacks in cultivation [22], being the second most impactful after CO₂ consumption [23,24,48]. Souza et al. [10] e Castro et al. [11] investigated the environmental impacts related to microalgae biofilm application in soil and the production of a microalgae-biomass-based biofertilizer to recover P, respectively. The studied systems included the production of biomass in HPR, with CO₂ supply, fed with effluent from a meat processing industry. The authors related the energy demand during cultivation was

impactful, mainly to rotate the HPR paddlewheels. This process was the input that most contributed to generating environmental impacts in all the scenarios investigated by Castro et al. [11]. For that study, the energy for the cultivation system was the second most impactful input. However, it is highlighted that the articles mentioned used primary data of pilot scale units and that for the present study authors modeled secondary data from literature survey. This way, the energy input from those works could be overestimated. It is known that the energy consumption by production (kWh / kg of produced biomass) is lower when in large-scale operations [36]. Thus, the approach used in this study collaborated to better model the results that would be found upon scaling-up.

Still, the low production associated with high energy consumption was a major drawback. That way, it is recommended that efforts should be directed towards reducing that relation and reducing energy-related to energy consumption. Choi et al. [49] related the importance of reducing the energy requirements of biomass production and treatment, given it is the main responsible for the CO₂ emission in the life cycle of microalgae biorefineries.

Authors believe the use of a renewable energy source could be a strategic option. Using photovoltaic panels contributes to the reduction of environmental impacts in Global Warming (major), Particulate Matter Formation, Terrestrial Acidification [10,11], Terrestrial Ecotoxicity and Fossil Depletion [10]. There was an increase in the impacts of shwater Eutrophication due to the presence of various carcinogenic heavy metals involved in the construction of photovoltaic panels [10].

Among the most impactful categories after normalization, Freshwater and Marine Eutrophication stand out, whose results are associated with nutrient removal efficiencies in each scenario. In the Freshwater Eutrophication case, the phosphorus amount (kg P eq) considered as emission to water after pond cultivation was the primary agent causing impacts. In this sense, system C4 proved to be the most impactful, mainly due to its amount of P. Assis et al. [7] observed that although the percentage of N removal was high (C3 = 65.5%; C4 = 66.1%), there was an increase in phosphorus in the final effluent in HRP. The increase in phosphorus, both soluble and particulate, can be attributed to the excretion and mortality of aquatic organisms in the culture medium. [7,50,51]. Considering the European discharge standards [52], the values presented are not satisfactory ($P > 2\text{mg L}^{-1}$). As for the Marine Eutrophication category, the values obtained are mainly related to the release of ammonia nitrogen. For the values reported in Table 2, the nitrogen amount of systems C3 and C4 did not achieve the European standards

for effluent discharge ($N > 15 \text{ mg L}^{-1}$) [52], with these scenarios presenting as the most impactful in the Marine Eutrophication category.

It is important to highlight that the culture media of scenarios C3 and C4 came from the anaerobic pretreatment in a septic tank, whereas in the other scenarios, the pretreatment of the culture medium occurred in a UASB reactor. Besides the higher concentration of N (77.40 mg L^{-1}) and P (12.30 mg L^{-1}) in wastewater from the septic tank compared to the UASB reactor (N between 37 and 40 mg L^{-1} ; P between 4 and 5.2 mg L^{-1}), systems C3 and C4 showed lower N removals (about 65%) than the other systems ($> 70\%$) and an increase in P (7 to 9% increase) rather than removal, as previously discussed. Thus, the characterization of the culture media is reflected in the treatment efficiency and in the quality of the effluent to be discharged. It is observed that in scenarios C3 and C7, in which the same technology for improving biomass production was adopted, the environmental impacts related to these scenarios were different due to the quality of the final effluent. For Freshwater (kg P eq) and Marine Eutrophication (kg N eq), the impacts of system C3 were 2.28 and 3.43 times higher than that of C7, respectively. The study of Couto et al. [53] highlights that nutrient removal through microalgae assimilation is a better alternative, when compared to removal by nutrient loss (volatilization and chemical precipitation). With domestic wastewater as culture media, the technology for improving biomass production through CO_2 supply positively contributes to the recovery of nutrients by biomass. With higher nutrient recovery efficiency, the broader the prospects of reusing biomass as a raw material for several bioproducts, especially as biofertilizer, showing a context of a circular and green economy. The valorization of nutrients from wastewater and its application in agriculture has been researched as alternative routes to conventional and promising fertilizers for soil and plant quality improvements [10,11,54].

Considering the total environmental load, most of the impacts in all scenarios/categories were negative (mainly due to the use of wastewater and EGGC). With the exception of C2 and the Eutrophication Categories for all the systems, impacts were reduced at least 30% by the use of water, N and P as avoided products. The scenarios that showed the best performance were C1 and C4, which only cause positive environmental impacts in the Freshwater and Marine Eutrophication categories, followed by C7, which only cause positive environmental impacts in the Freshwater and Marine Eutrophication and Terrestrial Ecotoxicity categories.

Water is the avoided product that most contributes to reducing the impacts of all scenarios in 9 categories. The major exception is the Ozone Depletion category, in which the

consumption of N fertilizer was decisive ($-5.10E-05$ up to $-2.67E-05$ kg CF11 eq). The impacts generated by the use of BR in this category, for C5 and C6, are mainly associated with the use of pesticides in the cotton production chain. Cotton production processes cause heterogeneous and complex impacts, with the use of water and pesticides causing the most significant environmental problems, specifically in the cultivation stage [55]. Besides, it is estimated that only cultivation is responsible for the consumption of about 11% of pesticides compared to world consumption [55,56]. According to Souza et al. [10], the use of any nitrogen-based fertilizer increases the nitrogen content in the soil and favors the emission of nitrogen oxides (NO_x), a potential gas for oxygen depletion. In this context, due to its production chain, the use of cotton in BR for the algal production of hybrid systems (systems C5 and C6) was not environmentally positive, contributing significantly to the impacts associated with Ozone Depletion.

5.3.3. Scenario analysis and Interpretation

Scenario analysis was carried out for processes identified as most impactful, namely: CO₂ supplementation rate and BR cotton. Both these processes were present in system C5, which presented itself as the most impactful from the previous topic. To complement this analysis, the authors also evaluated normalization results for the changes proposed. Table 5.6 presents the results for each impact category.

Table 5.6 – Scenario evaluation results.

Impact category	Characterization						Sensitivity Coefficient		Normalization				
	Unit	C5	Cotton (-)	Cotton (+)	CO ₂ (-)	CO ₂ (+)	Cotton	CO ₂	C5	Cotton (-)	Cotton (+)	CO ₂ (-)	CO ₂ (+)
Global warming	kg CO ₂ eq	2.06E+00	1.35E+00	4.18E+00	6.60E-01	3.60E+00	-1.38E+00	1.79E+00	2.58E-04	1.69E-04	5.24E-04	8.26E-05	4.51E-04
Stratospheric ozone depletion	kg CFC11 eq	-1.96E-05	-2.19E-05	-1.29E-05	-2.00E-05	-1.92E-05	4.58E-01	-5.23E-02	-3.28E-04	-3.65E-04	-2.15E-04	-3.34E-04	-3.20E-04
Ozone formation, Human health	kg NO _x eq	1.68E-04	-1.37E-03	4.79E-03	-1.57E-03	2.07E-03	-3.67E+01	2.70E+01	8.18E-06	-6.68E-05	2.33E-04	-7.62E-05	1.01E-04
Fine particulate matter formation	kg PM _{2.5} eq	-3.94E-04	-1.87E-03	4.03E-03	-2.03E-03	1.34E-03	1.50E+01	-1.07E+01	-1.54E-05	-7.30E-05	1.57E-04	-7.93E-05	5.22E-05
Ozone formation, Terrestrial ecosystems	kg NO _x eq	1.82E-04	-1.38E-03	4.86E-03	-1.58E-03	2.11E-03	-3.42E+01	2.53E+01	1.03E-05	-7.75E-05	2.73E-04	-8.90E-05	1.19E-04
Terrestrial acidification	kg SO ₂ eq	1.13E-03	-1.63E-03	9.41E-03	-1.96E-03	4.56E-03	-9.74E+00	7.19E+00	2.76E-05	-3.97E-05	2.30E-04	-4.78E-05	1.11E-04
Freshwater eutrophication	kg P eq	9.46E-03	9.40E-03	9.63E-03	9.36E-03	9.58E-03	-2.46E-02	2.81E-02	1.46E-02	1.45E-02	1.48E-02	1.44E-02	1.47E-02
Marine eutrophication	kg N eq	6.06E-02	6.05E-02	6.09E-02	6.06E-02	6.07E-02	-6.84E-03	2.14E-03	1.32E-02	1.31E-02	1.32E-02	1.31E-02	1.32E-02
Terrestrial ecotoxicity	kg 1,4-DCB	1.29E+01	1.20E+01	1.57E+01	4.89E+00	2.10E+01	-2.86E-01	1.56E+00	1.24E-02	1.16E-02	1.51E-02	4.72E-03	2.03E-02
Human carcinogenic toxicity	kg 1,4-DCB	1.02E-03	-7.30E-03	2.60E-02	-5.98E-03	8.52E-03	-3.27E+01	1.78E+01	3.67E-04	-2.64E-03	9.37E-03	-2.16E-03	3.08E-03
Human non-carcinogenic toxicity	kg 1,4-DCB	1.73E-01	8.56E-02	4.37E-01	9.76E-02	2.52E-01	-2.03E+00	1.11E+00	1.16E-03	5.74E-04	2.93E-03	6.55E-04	1.69E-03
Mineral resource scarcity	kg Cu eq	-8.42E-03	-9.43E-03	-5.37E-03	-1.19E-02	-4.80E-03	4.83E-01	-1.05E+00	-7.01E-08	-7.86E-08	-4.47E-08	-9.91E-08	-4.00E-08
Fossil resource scarcity	kg oil eq	2.72E-01	1.07E-01	7.67E-01	2.02E-02	5.59E-01	-2.43E+00	2.48E+00	2.77E-04	1.09E-04	7.82E-04	2.06E-05	5.70E-04

Overall, C5 was more sensitive to the changes in BR than CO₂. In terms of the sensitivity coefficient and normalization results, Ozone Formation categories were the most sensitive to both processes. Nitrogen oxides were the substance that most contributed to the impacts for worsened scenarios in the Human Health related category (97.9% for BR, 96.6% for CO₂ and 86.4% for the original C5). Upon analyzing the normalized impacts, however, these categories were not the most impactful.

Regarding Human Carcinogenic Toxicity, the use of Chromium IV in the cotton production chain was the most impacted substance. For the BR worsened scenario, this substance was responsible for the highest emissions in kg 1.4-DBC (47.7% water, 37.6% soil, and 5.78% air). The main responsible for this range was associated with the change in the insecticide compounds present in the cotton production process [55,56].

For CO₂ scenarios, Terrestrial Ecotoxicity was the most relevant category. Although the sensitivity coefficient did not present high variability (1.56E+00), normalized impacts varied from 1.24E-02 in the original C5 scenario to 4.72E-03 (-62.05%) for the improved scenario and 2.03E-02 (+38.59%) for the worsened one.

The variation in the useful life of cotton as a support material for BR was modeled because, in these reactors, algal biomass aggregates cumulatively [6]. Therefore, it was expected that by increasing the useful life, less cotton mass would be required and lower pesticide contents would be accounted for in the cotton production process. However, the environmental impacts related to the lower demand for this support material have not been amortized. Still, it is possible to note that even for the improved scenario, C5 still presented itself with the highest impacts. Also, it is possible to conclude that better results could come from advances in BR technology than in carbon uptake, based on the sensitivity coefficients. A technological investigation to optimize BRs would, for example, replace the use of cotton with other support materials already successfully explored for the production of algal biomass, such as nylon [57], velvet [58], polyester [59–61] or ceramic foam filters [62]. Besides the environmental disadvantages presented in its production chain, cotton has been identified in the literature as a material with less durability when compared to synthetic composition materials [59]. Given its natural origin, cotton fibers can be used as an organic nutrient for some microorganisms present in wastewater [60,63]. Therefore, due to the greater degradability of cotton compared to other synthetic materials and the innovative character of BR to produce and

harvest algal biomass, it is recommended that new LCAs be carried out with the use of other support materials in BR for algal production.

These results follow several studies suggesting further research on microalgae: sustainability could lie on CO₂ supply advances, aiming at higher biomass yield or the use of recovered gas from different sources [24,31,47]. Although many authors recently studied BR technology [6,30,61,64], evaluation through LCA is still sparse and results point this to this process as a way towards environmentally friendly perspectives to cultivation.

5.3.4 Selection of the best alternative

It is important to note that the use of avoided products, although consistent from the point of view of modeling and literature [10,11] was decisive when the LCA is focused only on cultivation. The positive effects of the use of wastewater modeled in this way meant that the systems had less environmental impacts compared to cultivations in synthetic media, a conclusion also reported by [47].

Using EGGC presented itself as the best alternative in terms of environmental performance. This was due to the fact that this system provided interesting biomass productivity, from a technical point of view, even using a non-pure CO₂ source. This result is in line with that proposed by Collotta et al. [24], Bussa et al. [23] and Porcelli et al. [31] which attest that the use of CO₂ from other processes is the main way forward in the search for less impacting cultivation. Since productivity values were close, in the present study, the use of EGGC proved to be more favorable in environmental terms. However, as mentioned earlier in Section 2.2, each source of CO₂ emissions needs to be assessed, particularly in technical, environmental and economic terms. There are concerns about the application of CO₂ emission gases from industrial activities without pre-treatment, as some toxic compounds can inhibit the growth of microalgae [65].

There is still sparse literature showing whether alternative sources of CO₂ are an economical option, evaluating the feasibility of the project including initial investment, operating costs and mainly the market price of the produced biomass [7]. Assis et al. [7] carried out an investment analysis to identify the economic viability considering the use of biomass (produced in HRP with the addition of pure industrial CO₂ and EGGC, fed with domestic effluent) for energy purposes and as a source of protein for animal feed. The authors found that,

within the experimental context on which the analysis was based, none of the CO₂ sources had a viable investment in the 20-year horizon and that, for the energy use of biomass, the Net Present Value (NER) is higher for the EGGC, while for its application in animal feed the NER is higher for industrial CO₂. In addition, different sources, quantities and quality of CO₂ affect the biochemical composition of microalgae [65]. Thus, future work to assess the technical, environmental and economic feasibility that includes the final use of biomass should be encouraged.

Most scenarios had a large part of the negative impacts. The most unfavorable scenarios were C5, which generated an environmental impact in 10 of the 13 categories, and C2, which had a very high impact in the Terrestrial Ecotoxicity category when compared to the others. Therefore, all other strategies to increase productivity (CO₂ supplementation and hybrid system) proved to be more favorable and can be considered for the cultivation of microalgae. This result suggests that future studies should focus on these strategies to make them even more interesting and viable.

It should be noted that hybrid systems have the advantage of optimizing the harvesting stage through attached growth, increasing the efficiency of biomass collection and dispensing high energy consumption (compared to centrifugation) and chemical products (such as the case of coagulation/flocculation) [10,11,30,66,67]. A future LCA may investigate the recovery of value-added products to verify the sustainability of the process. Recovery routes that do not require complete drying as a way of preparing biomass should be prioritized to avoid the impacts associated with excessive energy consumption. These routes include anaerobic digestion and hydrothermal processes, such as carbonization, liquefaction and hydrothermal gasification. This is because the biomass separation and drying processes are responsible for a large part of the energy demand of the entire process, which is one of the major challenges [66]. In this sense, processes that do not require drying of biomass tend to have a more favorable energy balance [53].

5.4 Conclusions

For the systems assessed through LCA, coupling technologies for increasing biomass productivity did not compensate in terms of environmental impacts. The most impactful process was related to the use of industrial carbon supplementation in the systems. Using UV pre-

disinfection of the effluent generated greater impacts in toxicity categories, mainly due to the production of the lamps. As for hybrid systems, the use of cotton as a support material caused greater environmental impacts, mainly due to insecticides in the production chain, and new assessments with different fabrics are recommended.

The lowest environmental impacts found, for most categories, were in the scenario that includes the use of EGGC for CO₂ supplementation (system C4). With technical studies and based on the LCA result for the same production, the use of gases from other processes is the best cultivation proposal.

Finally, this study highlights the potential of LCA as a tool to help optimize microalgae production coupled to wastewater treatment. The results presented contribute to the discussion of improvements in the biomass cultivation stage, seeking more sustainable chains and expanding its application on a larger scale.

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6 ARTIGO 2. ALGAL BIOMASS PRODUCTION COUPLED TO AGRO-INDUSTRIAL WASTEWATER TREATMENT: A COMPARATIVE TECHNO-ENVIRONMENTAL ASSESSMENT OF OPEN AND CLOSED SYSTEMS

Abstract

Microalgae-based wastewater treatment can be applied to the bioremediation of agro-industrial wastewater, aiming at a circular economy approach. The present work compared the technical-environmental feasibility of operating a bubble column photobioreactor (PBR) and a high rate pond (HRP) for the production of microalgae biomass and wastewater treatment from a meat processing unit. The environmental assessment was conducted through life cycle assessment and energy balance, using 1kg of biomass as the functional unit. Environmental impacts were assessed through the ReCiPe methodology for 13 impact categories. Results revealed that energy consumption was the major contributor to the generation of PBR impacts (over 75%). The HPR had negative impacts for 7 of the 13 impact categories assessed, mainly due to lower energy consumption. Still, HPR presented higher impacts than PBR in eutrophication, due to the lower nitrogen recovery during cultivation. The most impactful process of HPR cultivation was CO₂ consumption (up to 80% of the total impacts). Energy balance through the Net Energy Ratio also resulted in the HPR advantage over the PBR (NER = 10.98 and 0.03, respectively). Thus, the results found here and in the literature encourage the use of HRP and reveal new trends to optimize PBR, such as the use of hybrid systems.

Keywords: Life-cycle assessment; Algal biomass; photobioreactors; High rate ponds; bubble column reactor; wastewater treatment.

6.1 INTRODUCTION

Microalgae-based wastewater treatments are one of the clean technologies applied to effluent bioremediation. Seeking a circular economy approach, the resource recovery capacity of this renewable source makes its application attractive in the context of agro-industrial effluents. This sector requires high water demand throughout the production process, consuming between 1.6-9 m³ of water per ton produced and generating 2-20 L of wastewater per head of cattle or pig (World Bank Group, 2007). The effluent generated can be rich in organic matter, nutrients, pathogens and contain antibiotics and heavy metals (Aziz et al., 2019). Algae can assimilate the nutrients present in the wastewater for its growth (Nagarajan et al., 2020). Biomass, in turn, can be converted into by-products such as biofuels, bio-fertilizers, and animal feed (Shahid et al., 2020).

Among the limitations to the application of this technology, the lower biomass productivities are one of the major drawbacks, when compared to freshwater cultivation. The challenges in cultivation conditions arise, among others, from the variation in light availability, temperature, pH, CO₂ fixation rate, and available area (Couto et al., 2020; SundarRajan et al., 2019; Xiaogang et al., 2020). The feasibility of each cultivation system may vary depending on the investment cost, the desired final product, nutrient source, and the carbon fixation (Klinthong et al., 2015; Okoro et al., 2019). Closed systems such as photobioreactors (PBR) have more controlled operating conditions, with lower CO₂ losses and higher productivity than open systems, such as High Rate Ponds (HRP) (Grobelaar, 2009; SundarRajan et al., 2019). Open systems, in turn, have lower operating costs, especially considering energy consumption, with easier reproducibility on a commercial scale (Dasan et al., 2019; Jorquera et al., 2010; Xiaogang et al., 2020).

Beyond technical criteria, the comparison of the cultivation systems can be carried out in environmental terms. One of the tools used to assess the environmental sustainability of production processes is the life cycle assessment (LCA). The method makes it possible to quantify the environmental impacts in the products' life cycle, thus highlighting the bottlenecks in the production chain. The LCA approach has been applied to microalgae cultivation, their resource recovery routes, and different culture media (Arashiro et al., 2018; Castro et al., 2020; Ferreira et al., 2020; Herrera et al., 2020; Hulatt and Thomas, 2011; Souza et al., 2019). In studies that compared cultivation systems, Dasan et al. (2019) studied, using LCA and Net Energy Ratio (NER), biofuel production in HRP, bubble column PBR and horizontal tubular

PBR, using freshwater as the culture media and adding chicken compost as a nutrient source. The results showed that energy consumption was the major responsible for the impacts generated and that no system offered a favorable energy balance ($NER > 1$). Jorquera et al. (2010) also studied the performance of different reactors for freshwater cultivation, namely raceway ponds, tubular and flat-plate PBR. For biomass production, the pond and the flat-plate reactor got better results in the energy balance ($NER > 1$). Considering the cultivation in wastewater, Abu-Ghosh et al. (2015) studied a comparison between PBR and HRP for the production of oil-rich biomass. The work focused on proposing a productive arrangement with lower energy consumption, integrating both systems of series cultivation to optimize growth. However, there is no study of the environmental impacts and emissions associated with the application of this system.

The present work aims to carry out a technical-environmental comparison of open and closed systems for the production of biomass and effluent treatment from a meat processing unit. The comparison was made for a HRP and a PBR bubble column in terms of life cycle assessment and energy balance.

6.2 MATERIALS AND METHODS

6.2.1 Data Collection

The present study used the results of experiments conducted by Costa (2016) and Tango (2015) that operated on HRP and PBR, respectively. Experiments were carried out in the external area of the Laboratory of Sanitary and Environmental Engineering (LESA) on the campus of the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais ($20^{\circ} 45'14''S$, $42^{\circ} 52'54'' W$). The local climate is warm and temperate, with an average annual temperature of $20.6^{\circ}C$, and the systems were operated in the spring.

The culture media used in HRP was collected from the wastewater treatment plant installed in a meat processing facility, after primary treatment (flotation). A 4% (v/v) of the inoculum of this same effluent was added to the media, but, additionally to the primary treatment, it underwent secondary treatment (activated sludge) and tertiary treatment (HRP). The culture medium used in PBR was the same used in HRP. A 10% (v/v) inoculum collected

from a high rate pond was added to the culture media, preceded by a UASB reactor and applied to the treatment of domestic effluent.

The HRP used by Costa (2016) was 1.28 m wide, 2.86 m long, 0.5 m deep and had 3.3 m² of surface area, 0.3 m of useful depth and 1 m³ of useful volume. It was built with fiberglass, with six stainless steel blades. The paddles were powered by an electric motor of 1 hp to maintain the flow in the units. The speed was reduced by a reducer coupled to the motor and controlled by a frequency inverter (WEG brand CFW-10 series), which provided a speed of approximately 0.10 to 0.15 ms⁻¹ for the necessary mixing of the liquid media. Figure 6.1A shows the HRP scheme that was used in this study.

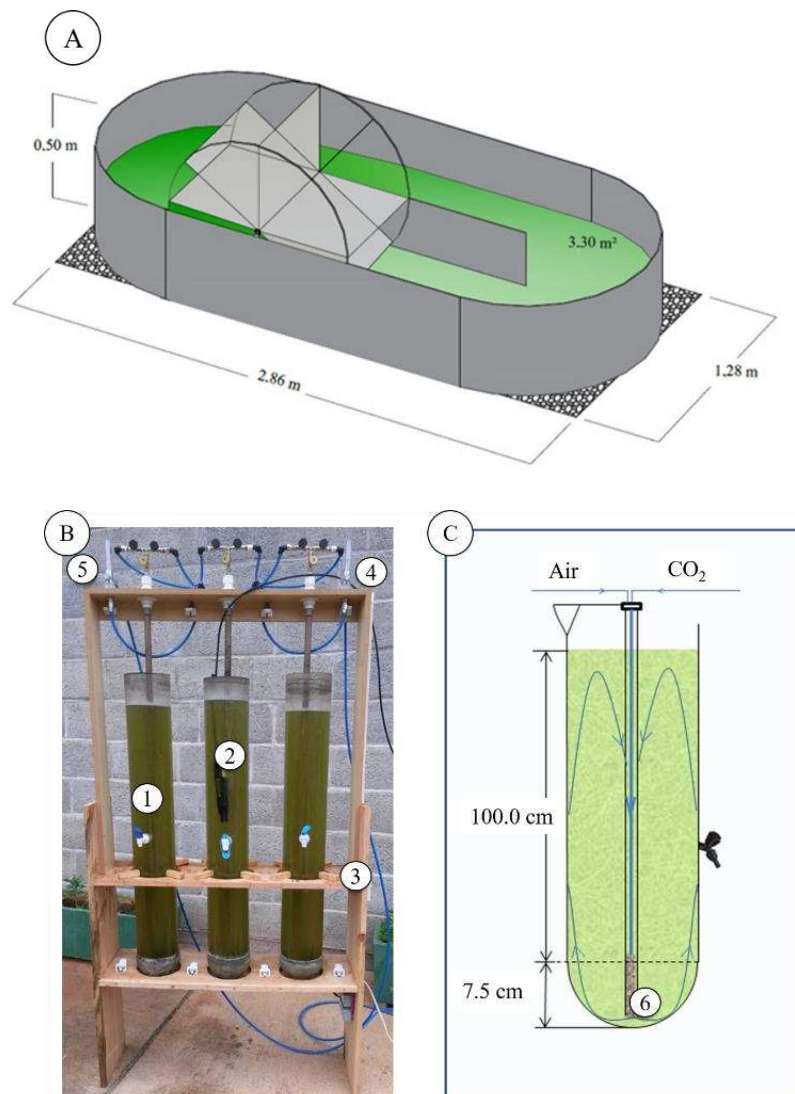


Figure 6.1 - (A) HRP and (B and C) PBR schemes used for biomass production.

During the operation, the CO₂ supply was controlled from the pH variation in the unit. Synthetic CO₂ was added to a 99% pure gas cylinder per carbonation column coupled to the

HRP. The column used was made of PVC and built according to Putt et al. (2011), presenting a 0.10 m diameter and 2.20 m height. The gas flow was $1 \text{ L}\cdot\text{min}^{-1}$, controlled by flowmeters with a capacity of 0 to $15 \text{ L}\cdot\text{min}^{-1}$. An aquarium pump (Atman, AT304) was used to promote the recirculation of the effluent through the column of carbonation, the recirculation flow being $4 \text{ L}\cdot\text{min}^{-1}$.

The bubble column PBR operated by Tango (2015) was composed of three independent acrylic tubes, with half-sphere-shaped bottom, with the same dimensions, and a 15 cm diameter, 14.4 cm internal diameter, 3 mm wall thickness, and 15 L of useful volume in each tube. The tubes were arranged in parallel on a wooden support in a vertical position, to better avail the solar lighting throughout the day.

Culture media mixing was carried out full-time, by bubbling air ($10 \text{ L}\cdot\text{min}^{-1}$) enriched with CO_2 . The air for mixing was supplied by a diaphragmatic air compressor, 0.25 kW power, and conducted to each acrylic tube through a pneumatic hose followed by a PVC tube connected to a cylindrical porous stone disperser (As-001 22 mm long and 12 mm diameter). For flow control, precision valves and flowmeters 0 to $15 \text{ L}\cdot\text{min}^{-1}$ were installed. Figure 6.1B shows the key components of the system, and Figure 6.1C shows the dimensions of the acrylic tubes used.

The CO_2 supply was controlled based on the pH variation in the unit which was kept between 6 and 8. For such an automated system, a probe was used to measure pH and temperature in real-time (H200 sc200 controller and analog sensor differential pH for effluents), with an electrical signal emission system compatible with a solenoid valve (Jefferson, 2016BV221) to control the addition of CO_2 .

More details about the experimental methods of biomass production can be found in Costa (2016) and Tango (2015). Table 6.1 shows a summary of the key parameters of the cultivation stage for the modeled systems.

Table 6.1- Parameters of the experiments in HRP and PBR. Source: Adapted from Costa (2016) and Tango (2015).

System Parameter	PBR			HRP		
	Inlet	Post-Treatment	Efficiency (%)	Inlet	Post-Treatment	Efficiency (%)
N-NH ₄ ⁺ (mg.L ⁻¹)	23.10	0.00	100.00	20.50	8.30	59.50
N-NO ₃ ⁻ (mg.L ⁻¹)	2.40	2.60	-8.30	1.40	2.20	-57.14
NTK (mg.L ⁻¹)	96.60	-	-	40.00	-	-
Ps (mg.L ⁻¹)	1.60	0.00	100.00	4.60	0.00	100.00

Biomass Concentration (mg.L ⁻¹)	966.66	855.00
HRT (day)	3.00	10.00

6.2.2 Life-Cycle Assessment

The life cycle assessment was performed according to NBR ISO 14040. Two cultivation systems were modeled: (i) the HRP with CO₂ supply, and (ii) the bubble column PBR. Inventory was carried out based on the results of the experiments. The goal of the LCA was to quantify the environmental impacts of cultivation systems, considering the treatment of effluent and the biomass production. The functional unit adopted for the comparison and calculation of the inventory was 1 kg of biomass produced. Figure 6.2 shows system boundaries and the main inputs. The systems were defined in a “gate to gate” approach, considering only the cultivation stage of each experiment. Material and energy inputs for each system were calculated based on the results of biomass concentration (mg L⁻¹) for each experiment.

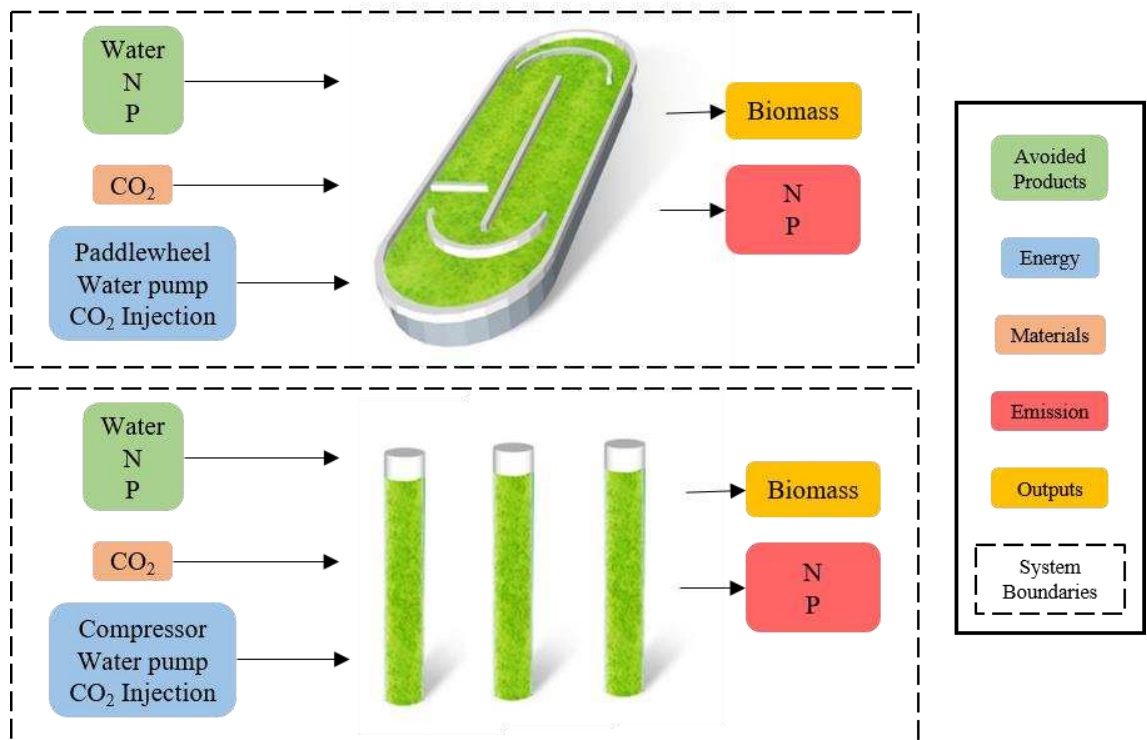


Figure 6.2 - System Boundaries and Inputs.

To include the use of wastewater as the culture media, water, nitrogen and phosphorus were considered as avoided products. Inputs with over 10 years of useful life were not included in the analysis, which excludes experimental units and general equipment (Castro et al., 2020;

Ferreira et al., 2020; Souza et al., 2019). The treatability of the systems was modeled by considering the results of phosphorus and nitrogen from the effluent after passing through the systems as emissions to water. The calculations of CO₂ and energy consumption for the systems were performed using secondary data, according to Table 6.2.

Table 6.2 - Literature data for energy demand.

HRP Parameters	Value	Unit	Reference
CO ₂ Injection	293.00	kg day ⁻¹ ha ⁻¹	(Collet et al., 2011)
Paddlewheel	2.70	kW ha ⁻¹	(Albarelli et al., 2018)
Energy for CO ₂ Injection	22.20	Wh (kg CO ₂) ⁻¹	(Collet et al., 2011)
Water Pump	0.50	kw ha ⁻¹	(Albarelli et al., 2018)
PBR Parameters			
Energy	177.40	kWh kg ⁻¹ biomass	(Gouveia et al., 2016)
CO ₂ Injection	1.96	g L ⁻¹	(Zhang et al., 2015)

The environmental impacts were quantified using the SimaPro software (PRÉ Sustainability BV, Netherlands, version © 9.0.0) and the Ecoinvent database (Goedkoop et al., 2016). When available, global data (GLO) and Rest of the World (RoW) were used. For energy consumption, it was decided not to use the Brazilian mix in system modeling, as it is mostly composed of renewable sources (Castro et al., 2020). Thus, aiming for more representative values of energy impacts, the German mix (DE) was used, which had a higher percentage of fossil fuel use.

The ReCiPe midpoint (H) method was used. It has already been used in other research aimed at environmental sustainability in the cultivation of microalgae (Arashiro et al., 2018; Castro et al., 2020; Ferreira et al., 2020; Souza et al., 2019). According to Arashiro et al. (2018), the most relevant categories for assessing wastewater treatment are Climate Change, Ozone Depletion, Terrestrial Acidification, Freshwater Eutrophication, Marine Eutrophication, Ozone Formation, Particulate Matter Formation, Metal Depletion, Fossil Depletion, Human Toxicity, and Terrestrial Ecotoxicity. Thus, these categories were considered in the present study. The

impacts were assessed according to the characterization and normalization, and an analysis of the contribution of each process to the total impact.

6.2.3 Energy Efficiency Analysis

One of the major differences between cultivation systems is their energy consumption (Jorquera et al., 2010). This parameter is identified as crucial in the environmental performance of microalgae cultivation (Bussa et al., 2020). In this way, an analysis of the energy efficiency of the experiments was conducted by assessing the Net Energy Ratio (NER). The parameter considers the ratio between the energy produced and consumed by the systems, and can be calculated according to Equation (1):

$$\begin{aligned} \text{NER} &= \text{Net energy ratio} & (1) \\ &= \frac{\sum \text{Energy Produced (biomass)}}{\sum \text{Energy Requirements}} \end{aligned}$$

The energy produced was calculated using the biomass productivity and energy expenses were based on system consumption, both according to Jorquera et al. (2010). For NER results higher than 1, cultivation systems can be considered economically viable for large-scale production (Suparmaniam et al., 2019).

6.3 RESULTS AND DISCUSSIONS

6.3.1 Life-cycle inventories

From the biomass concentration values (mg L^{-1}), data were normalized according to the functional unit of 1 kg of biomass. Table 6.3 presents the inventory and quantitative of each process used as data entry in the software. The energy consumption for the HRP was calculated by equipment, considering the supply by the pump, paddle rotation and CO_2 supply by the carbonation column. For the PBR, the value was based on the total for the production of 1 kg of algae estimated by Gouveia et al. (2016). Emissions to water came from the removal values found in Table 6.1.

Of the inventory values, the biggest highlight is the different energy consumptions of the reactors. The value for the PBR was much higher than the HRP, with a difference of 176.97 kWh for the production of 1 kg of biomass. As a result, the treatment of 1 m³ of effluent requires 171 kWh in the PBR against 0.37 kWh in the HRP. For CO₂, consumption in the PBR is almost double. Although the adopted values are secondary data, the higher biomass production can explain this difference in the closed system. Thus, the photosynthetic activity is greater, which requires greater CO₂ consumption to maintain the pH in the desired range. Finally, it is noteworthy that for HRP, as the total biomass productivity is lower, it is necessary to treat a larger volume of effluent, so the values of water, nitrogen and phosphorus for this scenario are higher.

Table 6.3 - Life-cycle inventories.

<i>Parameters</i>	<i>PBR</i>	<i>HRP</i>
Concentration (mg L ⁻¹)	966.66	855.00
HRT (day)	3.00	10.00
System Volume (L)	45.00	1000.00
Biomass (kg)	1.00	1.00
Necessary Volume (L)	1034.49	1169.59
<i>Avoided Products</i>		
Water (kg)	1034.49	1169.59
NTK (g)	99.93	46.78
Ps (g)	1.66	5.38
<i>Input</i>		
<i>Material</i>		
CO ₂ (kg)	2.03	1.13
<i>Energy</i>		
Paddlewheel (kWh)	-	0.4000
CO ₂ Injection (kWh)	-	0.0251
Water Pump (kWh)	-	0.0046
Total (kWh)	177.40	0.4297
<i>Output</i>		
<i>Emission to Water</i>		
N-NH ₄ ⁺ (g)	0.00	9.71
N-NO ₃ ⁻ (g)	2.69	2.57

6.3.2. Environmental Impact Assessment

The results of environmental impacts are shown in Table 6.4. The impacts are presented by category, in terms of characterization (with the respective unit) and normalization. Comparing the environmental impacts of the two reactors, it is observed that the results for HRP were lower in 12 of the 13 categories, which gave it a better environmental performance. Also, HRP has negative environmental impacts in 7 of the 13 categories (except for Global Warming, Freshwater Eutrophication, Marine Eutrophication, Terrestrial Ecotoxicity, Human Non-Carcinogenic Toxicity, and Fossil Resource Scarcity), while PBR generates environmental impacts in all categories. Thus, the use of wastewater, which avoids the consumption of water and conventional fertilizers in the cultivation stage, and mainly the low energy consumption contributed to a reduction in the impact during HRP's operation in most categories. On the other hand, the higher productivity of the PBR was not enough to compensate for the environmental impacts caused by its high energy demand, which was the input that most impacted the system (Figure 6.3B).

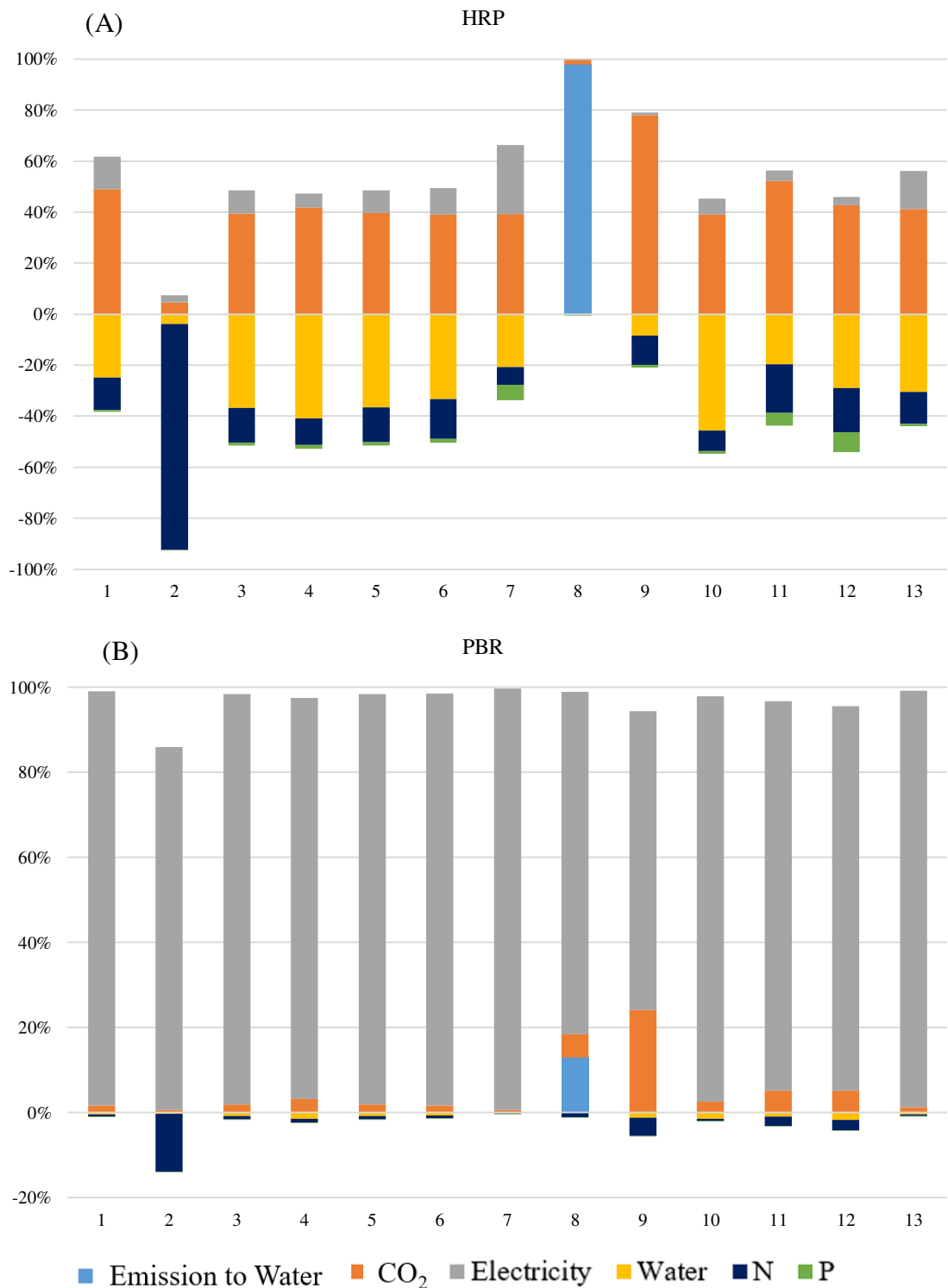
Table 6.4 - Results of environmental impacts: characterization and normalization.

Impact category	Characterization			Normalization	
	Unit	HRP	PBR	HRP	PBR
Global warming	kg CO ₂ eq	5.24E-01	1.18E + 02	6.56E-05	1.47E-02
Stratospheric ozone depletion	kg CFC-11 eq	-4.74E-06	5.67E-05	-7.92E-05	9.47E-04
Ozone formation, Human health	kg NO _x eq	-1.03E-04	1.29E-01	-5.02E-06	6.28E-03
Fine particulate matter formation	kg PM _{2.5} eq	-1.79E-04	7.22E-02	-6.98E-06	2.82E-03
Ozone formation, Terrestrial ecosystems	kg NO _x eq	-9.86E-05	1.31E-01	-5.55E-06	7.35E-03
Terrestrial acidification	kg SO ₂ eq	-6.18E-05	2.64E-01	-1.51E-06	6.45E-03
Freshwater eutrophication	kg P eq	5.35E-05	1.85E-02	8.24E-05	2.84E-02
Marine eutrophication	kg N eq	2.44E-03	1.33E-03	5.30E-04	2.89E-04
Terrestrial ecotoxicity	kg 1,4-DCB	5.07E + 00	4.36E + 01	4.89E-03	4.21E-02
Human carcinogenic toxicity	kg 1,4-DCB	-1.35E-03	3.86E-01	-4.88E-04	1.39E-01
Human non-carcinogenic toxicity	kg 1,4-DCB	1.51E-02	2.03E + 00	1.01E-04	1.36E-02
Mineral resource scarcity	kg Cu eq	-5.42E-04	9.11E-02	-4.52E-09	7.59E-07
Fossil resource scarcity	kg oil eq	5.54E-02	2.83E + 01	5.65E-05	2.89E-02

The only category in which the open system was more impactful than the closed one was Marine Eutrophication (kg N eq). This was because the efficiency of removing ammoniacal nitrogen and nitrate in the HRP was lower, contributing to a residual that was considered as “emission to water” during the cultivation stage.

Toxicity categories are measured in terms of the emissions of 1,4-dichlorobenzene-equivalents (1,4DCB-eq) in kg. In the normalization results, the Human Carcinogenic Toxicity category was the most impactful for PBR. The emission of Chromium VI for water was the main substance responsible for this result (91.7% of the total impact). Also regarding toxicity, Terrestrial Ecotoxicity was the most impactful category for HRP and the second most impactful for PBR. The impact on the ponds was over 8 times smaller than in the closed bioreactor in this category.

Figure 6.3 shows the contribution analysis of inputs, avoided products, and emissions to water for each scenario. For HRP (Figure 6.3A), the CO₂ supplement was the most impactful process (up to 80% for Terrestrial Ecotoxicity), followed by energy (up to 25% for Freshwater Eutrophication). A proposal to reduce the impacts associated with CO₂ consumption is the use of CO₂ emissions gases recovered from industrial processes. In the context of the meat processing industry, reusing boiler gases can be an option (Van Den Hende et al., 2012). The applicability of the proposal must be evaluated mainly in (i) biochemical terms, considering the gas composition and its influence on algal growth; (ii) economically, considering the cost associated with system implementation. Assis et al. (2019) studied the feasibility of using exhaust gas from gasoline combustion for carbon supplementation. In comparison with an HPR supplemented with industrial CO₂, the study showed that the CO₂ source did not interfere with treatment efficiency, yield, or composition of the biomass produced.



(1) Global warming; (2) Stratospheric ozone depletion; (3) Ozone formation, Human health; (4) Fine particulate matter formation; (5) Ozone formation, Terrestrial ecosystems; (6) Terrestrial acidification; (7) Freshwater eutrophication; (8) Marine eutrophication; (9) Terrestrial ecotoxicity; (10) Human carcinogenic toxicity; (11) Human non-carcinogenic toxicity; (12) Mineral resource scarcity; (13) Fossil resource scarcity.
 Figure 6.3 - Percentage contribution of inputs by impact category, for (A) HRP and (B) PBR.

For PBR (Figure 6.3B) energy consumption was the most impactful input in all categories (over 75%). This input is pointed out as the major contrast in the feasibility of

applying closed systems when compared to open systems (Abu-Ghosh et al., 2015; Dasan et al., 2019; Jorquera et al., 2010; SundarRajan et al., 2019). Studies have investigated strategies to reduce the impacts associated with the energy consumption during the cultivation stage, such as the use of photovoltaic panels (Abu-Ghosh et al., 2015; Castro et al., 2020; Souza et al., 2019). Castro et al. (2020) studied a 100% photovoltaic power supply scenario. The results showed a reduction of up to 51% in the impacts of the evaluated categories. However, the authors emphasize carcinogenic heavy metals in the panels' production chain as a challenge in this proposal. Also aiming at reducing energy demand, Yang et al. (2016) proposed a closed system with water circulation, to reduce bubble formation time and mixing time. The authors reduced energy demand by 21.2% but lost productivity by 12.7%. Future studies can evaluate the applicability of improvements systems' performance for wastewater.

6.3.2 Energy Balance

Given energy was one of the most impactful cultivation processes, the NER calculation was presented as a complement in system comparison. The results of the calculation of the energy efficiency analysis are shown in Table 6.5.

Table 6.5 - Comparative analysis of the energy balance of the photobioreactor and high-rate pond.

Parameter	PBR	HRP
Total biomass annual yield (kg.year ⁻¹)	1.57	15.39
Energetic Yield (kWh kg ⁻¹ biomass)	177.40	0.43
Energy (kWh.year ⁻¹)	277.81	6.61
Yearly Energetic Yield (kJ.year ⁻¹)	1.00E+06	2.38E+04
Total Annual Energy Consumption (GJ.year-1)	1.00	0.02
Total energy content in biomass (GJ / kg ⁻¹ biomass) ^a	1.70E-02	1.70E-02
Total annual energy yield (GJ.year⁻¹)	2.66E-02	2.61E-01
NER biomass	0.03	10.98

^aCastro et al., 2021

NER values above 1 indicate economically feasible processes (Hulatt and Thomas, 2011). Once again, HRP proved to be more favorable than PBR, even though the last presented higher productivity. The results found here are consistent with studies in the literature, such as Jorquera et al. (2010) who reported NER of 8.34 for ponds, and Suparmaniam et al. (2019) with

0.05-0.25 for tubular reactors. For the closed system to become viable (with $NER = 1$), a reduction in energy demand to the range of 4.7 kWh per kg of biomass would be necessary (-97.35%).

6.3.4 Comparison of the technical and environmental performance of bioreactors and future perspectives

During the elaboration of wastewater treatment projects, some criteria are widely used to study the feasibility of the type of reactor/treatment, such as the desired efficiency, energy consumption, and required area. When considering microalgae biotechnology, achieving higher biomass production is desirable, especially within the context of bio-economy, considering the potential by-products. With advances in the quest to design more sustainable treatment plants, life cycle and energy efficiency analysis are presented as parameters of choice with an environmental perspective and, in the case of NER , also an economic one.

The bubble column PBR offers the advantages of higher productivity and lower area requirements. However, the high energy consumption to operate this reactor results not only in negative energy efficiency but also in higher environmental impacts. Strategies for optimizing energy consumption, whether through renewable sources or more efficient systems, are the major challenges of this system. Still, its applicability may be best suited for high-value-added products such as fine chemicals, human nutrition and food technology in general. This type of production requires controlled cultivation conditions and higher biomass yield for feasibility, which is the case for PBR (Kothari et al., 2017).

Thus, as the treatment efficiencies of HRP and PBR are similar, it is believed that HRP is the most favorable system for large-scale applications. Using of HRP is recommended not only when the biomass produced is used in an energy route but also for other purposes. Thus, studies aimed at increasing the productivity of these reactors should be encouraged. The increase in HRP productivity can still contribute to improved removal and recovery of nutrients (N and P). Considering the effluent from meat processing industry, the recovery of emission gases from the industrial processes can be studied for carbon supplementation, aiming at reducing system impacts.

Using bubble column PBR in hybrid cultivation systems is encouraged to overcome its disadvantages (SundarRajan et al., 2019). Integration of a PBR and HRP, in a hybrid system,

offered greater production of microalgae rich in lipids (Narala et al., 2016). Microalgae were grown in the PBR and, once sufficient growth was achieved, they were transferred to HRP. The authors reported that the average growth rate in the hybrid system was higher than that of the individual systems. However, the economic and environmental benefits of this strategy still need to be better clarified.

On the other hand, hybrid systems that integrate suspended cultivation with attached growth to biofilm reactors have attracted attention and proved to be a promising option (Assis et al., 2017; Assis et al., 2020; Barlow et al., 2016; Ferreira et al., 2020; Morales et al., 2020). Given attached growth is a new field of research, there is still no consensus in the literature whether it achieves greater productivity against suspended cultivation (Mantzorou and Ververidis, 2019). However, some studies show it offers greater productivity against HRP operated alone (Assis et al., 2020). Besides that, Assis et al. (2017) observed, when coupling a vertical biofilm reactor (operated 10 hours a day) to an HRP, that indirect contact with atmospheric air and solar radiation does not require the use of additional CO₂ supplementation. These results point to a possibility to further reduce the impacts found in HRP cultivation. Considering the cultivation and harvesting stages (the suspended biomass is harvested by decantation and the attached biomass by hand scraping) of biomass grown in domestic sewage, the hybrid system offered higher production, biomass recovery efficiency, and lower environmental impacts than HRP without CO₂ supplementation (Ferreira et al., 2020). Thus, the results found here and in the literature encourage the use of HRP and reveal new trends to optimize HRP.

6.4 CONCLUSIONS

Although the PBR offers higher productivity of total biomass, its high energy consumption causes most of the environmental impacts. HRP resulted in lower environmental performance in 12 of the 13 categories analyzed. The only exception was the Marine Eutrophication category, mainly due to the lower efficiency in nitrogen recovery during cultivation in HRP. Still, PBR's energy demand was not offset by the use of wastewater as a culture media, with energy demand responsible for over 75% of the environmental impacts in all categories. The energy balance shows HRP as a feasible process (NER = 10.68) and a need for a 97% reduction in energy demand for PBR (NER = 0.03) to be economically feasible.

Still, the potential of using microalgae cultivation coupled with the treatment of agro-industrial effluents is a promising field. Ample further research can still be performed in the area to reuse resources from industrial processes, aiming at the sustainability of biorefineries and circular economy.

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7 CONCLUSÃO GERAL

Os resultados da avaliação de ciclo de vida demonstraram os benefícios do cultivo de microalgas em águas residuárias. Para as tecnologias de aumento de produtividade em LATs, os impactos ambientais foram reduzidos em pelo ao menos 30% pelo uso de água, nitrogênio e fósforo recuperados do efluente. A utilização da suplementação de carbono industrial foi observada como um dos principais gargalos do processo. O uso de gás recuperado de emissões, entretanto, se provou atrativo como solução técnica (pela produtividade) e ambiental (pelos impactos associados), reduzindo impactos em pelo ao menos 56% comparado ao cenário de cultivo base. O uso da desinfecção UV do efluente não apresentou compensação ambiental favorável, principalmente em indicadores de toxicidade. O uso do sistema híbrido apresentou impactos associados ao material de suporte do crescimento aderido, principalmente na cadeia produtiva do tecido do reator biofilme (algodão). Em geral, a associação de tecnologias de aumento de produtividade ao cultivo é viabilizada mais pela recuperação de recursos do que pelo incremento de biomassa produzida.

Na comparação dos sistemas abertos e fechados, a LAT se demonstrou como sistema mais viável do ponto de vista energético e ambiental. Mesmo com maiores produtividades, o gasto energético do PBR gerou maiores impactos que cultivo em lagoas em 12 das 13 categorias avaliadas. Assim como os impactos ambientais associados ao cultivo, o balanço energético confirmou a maior viabilidade da lagoa ($NER = 10.68$) sobre o PBR ($NER = 0.03$).

A utilização da avaliação de ciclo de vida se provou eficaz como ferramenta de análise da sustentabilidade do cultivo de microalgas. Da identificação dos processos impactantes à proposta de soluções aos sistemas, a avaliação de impactos permitiu direcionar os avanços no campo do cultivo em águas residuárias. Considerando os impactos ambientais associados, a perspectiva da economia circular é o melhor cenário para difundir a produção sustentável das microalgas.

8 SUGESTÕES PARA PESQUISAS FUTURAS

Os resultados aqui obtidos indicam que trabalhos futuros podem se beneficiar do uso da Avaliação de Ciclo de Vida para estudos aprofundados no cultivo de microalgas em águas residuárias. O uso de águas residuárias como fonte de água e nutrientes foi eficaz na redução dos impactos do cultivo, atingindo impactos negativos pela recuperação de resíduos.

O estudo de diferentes fontes de suplementação de carbono, como gases aproveitados de processos industriais, foi um dos processos críticos com base nos impactos obtidos. Estudos de viabilidade da aplicação de gases da indústria de processamento de carnes para a suplementação de carbono durante o cultivo devem ser avaliados em termos da influência na produtividade e nos impactos ambientais gerados.

Quanto ao uso de sistemas híbridos, novos estudos podem se dirigir a ACV e avaliação econômica do uso de diferentes materiais de suporte para o crescimento aderido. Principalmente, recomenda-se o estudo de materiais com maior durabilidade e menor utilização de pesticidas na cadeia produtiva.

Em termos de sistemas abertos e fechados, a principal pesquisa futura indicada pelos resultados é a redução da demanda energética do sistema fechado. O uso de fontes energéticas mais sustentáveis e a proposição de sistemas com menor gasto energético devem ser avaliados afim de obter tecnologias viáveis ambiental e economicamente. Assim, recomendam-se considerações sobre a ampliação dos sistemas em escala real e suas implicações econômicas e produtivas. Estudos futuros considerando os custos de investimento e operação desse tipo de tecnologia podem ser perspectivas de pesquisas no tópico, buscando solucionar a questão do balanço custo-energia-ambiente.

O cultivo de microalgas em águas residuárias é um passo à implantação de sistemas sustentáveis, com amplo potencial para inovação. Viabilizar técnica, ambiental e economicamente os processos é o principal ponto para difusão da perspectiva de economia circular na área.

9 APENDICES

9.1 APPENDIX A

Supplementary Material for Technologies for improving microalgae biomass production coupled to effluent treatment: a life cycle approach

Table S1 presents the processes from Ecoinvent 3 database used to model the scenarios.

Table S1

Database processes: Ecoinvent 3 - allocation at point of substitution – system | APOS, S

Process	Unit	Description
Carbon dioxide, liquid {RoW} production APOS, S	kg	This dataset represents the production of 1 kg of liquid carbon dioxide out of waste gases from different production processes.
Electricity, high voltage {DE} production mix APOS, S	kWh	Dutch electricity mix. The shares of electricity technologies on this market are valid for the year 2014. Basic source: IEA. 2017. IEA World Energy Statistics and Balances.
Nitrogen fertilizer, as N {RoW} urea ammonium nitrate production APOS, S	g	Refers to 1 kg N, resp. 3.13 kg urea ammonium nitrate with a N-content of 32.0%
Phosphate fertilizer, as P2O5 {RoW} single superphosphate production APOS, S	g	Refers to 1 kg N, resp. 1 kg P2O5 in monoammonium phosphate with a N-content of 11.0% and a P2O5-content of 52.0%.
Tap water {RoW} tap water production, conventional treatment APOS, S	kg	This dataset represents production of 1 kg of tap water under pressure at facility gate, ready for distribution in network. It represents average operation of conventional with biological filtration treatment for production of tap water.
Textile, woven cotton {GLO} production APOS, S	g	This dataset covers all relevant input and output flows of the represented gate to gate unit process with a good overall data quality.
Ultraviolet lamp {GLO} ultraviolet lamp production, for water disinfection APOS, S	p	This dataset represents production of 1 ultraviolet lamp for water treatment.

In order to standardize data presented in the reference studies for the production of 1 kg of biomass, some calculation steps were required. Starting from the productivity reported in Table 2 from the main text, the following equations were used to define the amount of effluent needed for each production (Eq. S1 to Eq.S3).

$$\text{Surface Productivity (g m}^{-2}\text{)} = \text{Productivity (g m}^{-2} \text{ day}^{-1}\text{)} \times \text{HTR (days)} \quad (\text{S1})$$

$$\text{Area (m}^2\text{)} = \frac{1000 \text{ g}}{\text{Surface Productivity (g m}^{-2}\text{)}} \quad (\text{S2})$$

$$\text{Volume (m}^3\text{)} = \text{Area (m}^2\text{)} \times \text{HRP depth (m)} \quad (\text{S3})$$

Results are presented in Table S2.

Table S2. Parameters for system standardization.

<i>Parameters</i>	<i>Assemany, 2015</i>		<i>Assis, 2019</i>		<i>de Assis, 2017</i>		
	<i>C1</i>	<i>C2</i>	<i>C3</i>	<i>C4</i>	<i>C5</i>	<i>C6</i>	<i>C7</i>
Surface Production (g m ⁻²)	45.72	37.20	48.00	48.96	33.95	31.00	31.35
Area (m ²)	21.87	26.88	20.83	20.42	29.46	32.26	31.90
Volume (m ³)	6.56	8.06	6.25	6.13	8.84	9.68	9.57

With these data it was possible to find the necessary software inputs. through Eq. S4 to Eq. S13.

$$\text{Paddlewheel (kWh)} = \frac{\text{Area (m}^2\text{)} \times (2.7 \text{ kW ha}^{-1} \times 24 \text{ h day}^{-1}\text{)} \times \text{HTR (day)}}{10000 \text{ m}^2\text{ha}^{-1}} \quad (\text{S4})$$

$$\text{Water Pump (kWh)} = \frac{\text{Area (m}^2\text{)} \times (0.5 \text{ kW ha}^{-1} \times 24 \text{ h day}^{-1}\text{)} \times \text{HTR (day)}}{10000 \text{ m}^2\text{ha}^{-1}} \quad (\text{S5})$$

$$\text{CO}_2 \text{ Inj. (kg)} = \frac{\text{Area (m}^2\text{)} \times (293 \text{ kg day}^{-1} \text{ ha}^{-1}\text{)} \times \text{HTR (day)}}{10000 \text{ m}^2\text{ha}^{-1}} \quad (\text{S6})$$

$$\text{CO}_2 \text{ Inj. (kWh)} = \text{CO}_2 \text{ Inj. (kg)} \times (22.2 \text{ Wh (kg CO}_2\text{)}^{-1}) \quad (\text{S7})$$

$$\text{Biofilm Recirculation (kWh)} = \frac{\text{HRT (day)} \times (10 \text{ h day}^{-1}\text{)} \times (13 \text{ W})}{1000} \quad (\text{S8})$$

$$\text{UV Reactor (kWh)} = 5.64 \text{ Wh m}^{-3} \times \text{Volume (m}^3\text{)} \quad (\text{S9})$$

$$\text{Proportional BR Area (m}^2\text{)} = \frac{\text{Area (m}^2\text{)}}{\text{Hybrid System Area (m}^2\text{)}} \quad (\text{S10})$$

$$\begin{aligned} \text{Biofilm Cotton (kg)} & \quad (\text{S11}) \\ & = \frac{\text{Proportional BR Area (m}^2\text{)} \times \text{Textile Density (gm}^{-2}\text{)} \times \text{HTR (day)}}{\text{Operation (day)} \times} \end{aligned}$$

$$\text{Water (kg)} = \text{Volume (m}^3\text{)} \times (1000 \text{ kg m}^{-3}\text{)} \quad (\text{S12})$$

$$N \text{ and } P \text{ (g)} = \text{Volume (m}^3\text{)} \times \text{Concentration (mg L}^{-1}\text{)} \quad (\text{S13})$$

Results for these calculations are presented in the main text. in Table 4.

System inventory for sensitivity analysis is presented in Table S3.

Table S3. Sensitivity analysis inventory.

Analysis	Parameter	Scenario		
		-	Original	+
1	Cotton lifespan (days)	20	40	60
	Cotton (g)	179.81	89.91	59.94
	CO ₂ supply (kg day ⁻¹ ha ⁻¹)	175.80	293.00	410.20
2	CO ₂ (kg)	1.99	4.32	4.64
	CO ₂ Injection (kWh)	0.44	0.96	1.03
	Total Energy (kWh)	2.18	2.6	2.77

9.2 APENDICE B

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Technologies for improving microalgae biomass production coupled to effluent treatment: A life cycle approach

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ABSTRACT

Algal biomass production in wastewater is a promising value-adding alternative that should be coupled to the treatment system. The technical aspects of cultivation systems for improved biomass yield have been widely reported in the literature; yet, their environmental performance can still be further studied and compared. This study evaluated the environmental impacts associated with the production of 1 kg of biomass grown in high-rate ponds, with domestic effluent as culture media. Seven systems with three approaches to cultivation were modeled through the life cycle assessment method: using effluent pre-treated with ultraviolet disinfection; with supplementation from industrial CO₂ and with exhaust gas from gasoline combustion; coupled with a biofilm reactor for biomass attached growth. Environmental impacts were also compared to a base cultivation using the ReCiPe method, for 13 impact categories. The environmental impacts were reduced at least 30% by using wastewater as a water and nutrient source, with systems reaching negative impact values. The only exception was eutrophication categories, with the highest normalized impacts along with toxicity-related categories. The system using CO₂ supply from exhaust gas from gasoline combustion had the best performance in 11 categories, reducing impacts in at least 56% compared to a base cultivation scenario. The most impactful process was industrial CO₂ supplementation, followed by biofilm. Coupling industrial CO₂ supply and hybrid systems for increased biomass productivity did not compensate environmentally, increasing impacts up to 227%. Scenario evaluations were performed for increased and worsened performance of CO₂ supply rates ($\pm 40\%$) and biofilm reactor lifespan (± 20 days). Opportunities to improve lie in the use of recovered gas from different industries and different support materials for the attached growth of biomass in hybrid systems.

1. Introduction

The search for environmentally friendly technologies to supply the growing energy demand stimulates the study of renewable sources. Microalgae have been identified as a promising feedstock, being autotrophic organisms that use solar energy, carbon dioxide, and nutrients (such as nitrogen and phosphorus) to grow in aquatic media. They could require low-quality freshwater such as wastewater, brackish, or even highly saline water, also able to generate rich biomass in terms of carbohydrates, proteins, and lipids [1,2]. Today, there is a commercial application of many microalgae products considered being of high value (pigments, cosmetics, and pharmaceuticals). However, other products are not economically feasible yet, such as biofuels, biogas, biofertilizer and animal feed, given their low commercial value with investments

higher than the final profit. Low productivity, high installation, and operating costs are barriers that still major drawbacks [3].

In this sense, the use of wastewater as culture media emerges as an economically attractive option, which integrates the potential of biomass production with effluent bioremediation [1,4,5]. Since wastewater treatment is a necessary step before discharge into water bodies, associating the production of a biomass rich in nutrients and organic matter (applicable to various recovery routes) to the process would be the ideal scenario [2]. For this reason, the cultivation of microalgae biomass has been evaluated in different wastewater, such as domestic sewage [6–9] and agro-industrial effluents [8,10,11].

Using wastewater as a culture medium, however, can result in lower algal biomass yield when compared to synthetic media, given productivity depends on factors inherent to the culture, such as pH, nutrient

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