UNIVERSIDADE FEDERAL DE LAVRAS

VICTOR SANTURBANO DA SILVA

PERFORMANCE AMBIENTAL DA PRODUÇÃO DE BIOGÁS DE MICROALGAS CULTIVADAS EM ESGOTO DOMÉSTICO

LAVRAS – MG
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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia Ambiental, área de concentração em Saneamento e Geotecnia Ambiental, para obtenção do título de Mestre.

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Orientadora

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ENVIRONMENTAL PERFORMANCE OF BIOGAS PRODUCTION FROM MICROALGAE CULTIVATED IN DOMESTIC SEWAGE

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APROVADO em 21 de dezembro de 2023
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RESUMO GERAL

O fenômeno de mudanças climáticas, impulsionado pelo uso de combustíveis fósseis e atividade manufatureira, resulta na emissão significativa de dióxido de carbono (CO_2) e outros gases de efeito estufa (GEE) provenientes de instalações industriais e demais atividades antrópicas. O aumento de consumo de energia, a iminente escassez de combustíveis fósseis e o crescente interesse em fontes renováveis motivam pesquisas em biocombustíveis. A produção de biogás através da digestão anaeróbia (DA) de microalgas, integrada ao tratamento de esgoto doméstico, emerge como uma técnica promissora para o gerenciamento de resíduos e redução da pegada de carbono. No entanto, desafios operacionais, políticos e de aceitação pública limitam o pleno potencial dessa abordagem. Nesse contexto, esforços de pesquisa devem ser dedicados na tentativa de aprimorar a eficiência e desempenho desse processo. Este estudo avalia a performance ambiental da produção de biogás a partir de microalgas cultivadas em esgoto doméstico, empregando análises de balanço de massa e energia, juntamente com avaliação do ciclo de vida. A modelagem, baseada em dados secundários, considerou um cenário base com cultivo em lagoa de alta taxa, colheita de biomassa por sedimentação gravitacional, DA líquida e melhoramento do biogás por *water scrubbing*. Cenários adicionais foram explorados visando menor consumo de insumos e impacto ambiental. A categoria de toxicidade humana cancerígena foi a mais afetada, destacando a importância do aquecimento do digestor anaeróbio e da água do cultivo. A etapa de DA foi a mais impactante, contribuindo com 35,28% dos potenciais impactos ambientais. O cenário S3, com técnicas como pré-tratamento térmico, recuperação de calor, co-digestão, melhoramento fotossintético do biogás e potencial uso do digestato como biofertilizante, demonstrou melhor desempenho ambiental. A análise de sensibilidade ressaltou a produtividade de metano como fator crucial na redução ou aumento dos potenciais impactos (por exemplo, 11% de redução dos potenciais impactos na categoria de radiação ionizante após acréscimo de 10% nesse parâmetro). Comparando à produção de gás natural, o S3 reduziu em até 6 vezes os danos aos recursos naturais, mas aumentou em 23 vezes os danos à saúde humana. Ao analisar as emissões de CO_2 equivalente (CO_{2-eq}) e a razão de energia líquida (REL) no cenário S3, observou-se emissões positivas (0.1795 kg C_{O2-eq}) e uma REL superior a 1 (1,71). A etapa de DA destacou-se como a principal contribuinte para esses resultados, representando 98% das emissões de CO_{2-eq} e 94% do consumo total de energia do sistema, sugerindo que esse cenário não é vantajoso para a produção de biogás, devido ao alto consumo de energia para aquecimento do digestor. Entretanto, após análise de sensibilidade, revelou-se que uma redução de 20% no parâmetro de temperatura de DA pode resultar em um cenário mais favorável para a produção de biogás. Nesse contexto, observou-se valores negativos de emissão de CO_{2-eq} (-0,1226 CO_{2-eq}) e um balanço energético positivo, com uma REL de 0,69. Esses resultados indicam que a DA a temperaturas ambientes pode impactar positivamente a eficiência ambiental e energética do processo. Este estudo fornece contribuições valiosas sobre a produção de biogás a partir da biomassa de microalgas, destacando a importância da otimização de parâmetros operacionais para alcançar desempenho ambiental e energético mais favorável. Essas descobertas têm o potencial de orientar futuras pesquisas e práticas no campo do aproveitamento de microalgas para a produção sustentável de biogás.

Palavras-chave: Biomassa algal. Digestão Anaeróbia. Avaliação do Ciclo de Vida. Águas Residuárias. Bioenergia. Análise de Sensibilidade.

ABSTRACT

The phenomenon of global warming, driven by the use of conventional fuels and manufacturing activities, results in significant emissions of carbon dioxide (CO_2) and other greenhouse gases (GHG) from industrial facilities and other anthropogenic activities. The increasing energy consumption, imminent fossil fuel scarcity, and growing interest in renewable sources motivate research in biofuels. Biogas production through anaerobic digestion (AD) of microalgae, integrated with domestic sewage treatment, emerges as a promising technique for waste management and reducing the carbon footprint. However, operational, political, and public acceptance challenges limit the full potential of this approach. In this context, research efforts should be dedicated to improving the efficiency and performance of this process. This study evaluates the environmental performance of biogas production from microalgae cultivated in domestic sewage, employing mass and energy balance analyses, along with a life cycle assessment. The modeling, based on secondary data, considered a baseline scenario with cultivation in a high-rate algal pond, biomass harvesting by gravitational sedimentation, liquid AD, and biogas upgrading by water scrubbing. Additional scenarios were explored to minimize input consumption and environmental impact. The carcinogenic human toxicity category was the most affected, highlighting the importance of AD heating and cultivation water. The AD step was the most impactful, contributing 35.28% to potential environmental impacts. Scenario S3, incorporating techniques such as thermal pretreatment, heat recovery, co-digestion, photosynthetic biogas upgrading, and potential use of digestate as biofertilizer, demonstrated better environmental performance. Sensitivity analysis emphasized methane productivity as a crucial factor in reducing or increasing potential impacts (e.g., 11% reduction in ionizing radiation potential impacts after a 10% increase in this parameter). Comparing to natural gas production, S3 reduced damages to natural resource by up to 6 times but increased damages to human health by 23 times. Analyzing carbon dioxide equivalent ($CO_{2\text{-eq}}$) emissions and the net energy ratio (NER) in scenario S3, positive emissions (0.1795 kg $CO_{2\text{-eq}}$) and a NER greater than 1 (1.71) were observed. The AD step stood out as the main contributor to these results, representing 98% of $CO_{2\text{-eq}}$ emissions and 94% of the total system energy consumption, suggesting that this scenario is not advantageous for biogas production due to high energy consumption for digester heating. However, sensitivity analysis revealed that a 20% reduction in AD temperature could result in a more favorable scenario for biogas production. In this context, negative $CO_{2\text{-eq}}$ emissions (-0.1226 $CO_{2\text{-eq}}$) and a positive energy balance with a NER of 0.69 were observed. These results indicate that AD at ambient temperatures can positively impact the environmental and energy efficiency of the process. This study provides valuable insights into biogas production from microalgae biomass, emphasizing the importance of optimizing operational parameters to achieve a more favorable environmental and energy performance. These findings have the potential to guide future research and practices in the field of harnessing microalgae for sustainable biogas production.

Keywords: Algae Biomass. Anaerobic Digestion. Life Cycle Assessment. Wastewater. Bioenergy. Sensitivity Analyses.

INDICADORES DE IMPACTO

O estudo aborda a produção de biogás a partir de microalgas cultivadas em esgoto doméstico, com ênfase nos impactos ambientais e energéticos, utilizando a ferramenta de avaliação do ciclo de vida (ACV). Conforme os resultados obtidos, embora o processo demonstre vantagens consideráveis, como a redução do consumo de água potável e nutrientes para o cultivo das microalgas, foram identificados desafios a serem superados para aprimorar sua sustentabilidade ambiental, particularmente relacionados ao consumo de eletricidade para o aquecimento dos biodigestores. No âmbito social, a implementação dessas práticas pode trazer benefícios às comunidades, ao reduzir a dependência de recursos fósseis e ao promover uma gestão mais eficaz dos resíduos e preservação dos recursos hídricos. Adicionalmente, a adoção de tecnologias mais sustentáveis pode contribuir para a melhoria da saúde pública, ao reduzir a poluição do ar e da água. Sob a perspectiva tecnológica, destacou-se a necessidade de desenvolver estratégias mais eficientes em termos energéticos, como a utilização de fontes renováveis, sobretudo a energia solar, no processo de produção de biogás. A etapa de aquecimento do digestor, por exemplo, foi identificada como responsável por uma parcela significativa das emissões de $CO_{2\text{-eq}}$ e do consumo total de energia do sistema, indicando que a transição para fontes sustentáveis de energia possui um considerável potencial de redução de impactos, corroborando com descobertas em estudos anteriores. Tal abordagem também pode estimular a inovação no setor de energia e estimular o desenvolvimento de novas tecnologias. No aspecto econômico, a produção de biogás a partir de microalgas pode representar uma oportunidade para o surgimento de novos empreendimentos e para a geração de empregos, especialmente em segmentos relacionados à biotecnologia e energia renovável. Do ponto de vista cultural, a disseminação dos resultados deste estudo pode contribuir para conscientizar sobre a importância da sustentabilidade ambiental, incentivando uma mudança de paradigma em relação ao uso dos recursos naturais. Em termos de alinhamento com os Objetivos de Desenvolvimento Sustentável (ODS) da ONU, este trabalho está diretamente relacionado aos ODS 6 (Água Potável e Saneamento), 7 (Energia Acessível e Limpa), 9 (Indústria, Inovação e Infraestrutura), 11 (Cidades e Comunidades Sustentáveis) e 13 (Ação Contra a Mudança Global do Clima). Portanto, contribui para promover práticas sustentáveis e para o cumprimento da Agenda 2030. Em síntese, este estudo oferece insights valiosos sobre os impactos sociais, tecnológicos, econômicos e culturais da produção de biogás a partir de microalgas, evidenciando a importância de prosseguir na busca por melhorias na sustentabilidade ambiental e energética desses processos.

LISTA DE SIGLAS E ABREVIATURAS

AD	*Anaerobic digestion*
APOS-S	*At the point of substitution - system*
C	Carbono
CH_4	Metano
CO_2	Dióxido de carbono
$CO_{2\text{-eq}}$	Dióxido de carbono equivalente
DA	Digestão Anaeróbia
DBO_5	Demanda bioquímica de oxigênio após período de incubação de 5 dias sob temperatura de °C
DQO	Demanda química de oxigênio
ECO-F	*Freshwater ecotoxicity*
ECO-M	*Marine ecotoxicity*
ECO-T	*Terrestrial ecotoxicity*
EUT-F	*Freshwater eutrophication*
EUT-M	*Marine eutrophication*
Fe(II)	Ferro II
FPMF	*Fine particulate matter formation*
FU	*Functional unit*
GEE	Gases de efeito estufa
GHG	*Greenhouse gases*
GW	*Global warming*
H_2S	Gás sulfídrico ou sulfeto de hidrogênio
H_2SO_4	Ácido sulfúrico
HRAP	*High Rate Algal Ponds*
HRT	*Hydraulic retention time*
HT-C	*Human carcinogenic toxicity*
HT-NC	*Human non-carcinogenic toxicity*
ICV	Inventário do ciclo de vida
IR	*Ionizing radiation*

LAT	Lagoas de Alta Taxa
LCA	*Life cycle assessment*
LCI	*Life Cycle Inventory*
LCIA	*Life cycle impact assessment*
LHT	Liquefação hidrotérmica
LHV	*Low heating value*
LU	*Land use*
N	Nitrogênio
N_{eq}	*Nitrogen equivalent*
N_2O	Óxido nitroso
NaOH	Hidróxido de sódio
NET	*Net energy ratio*
$N\text{-}NH_4^+$	*Ammonia nitrogen*
OD	*Stratospheric ozone depletion*
OF-H	*Ozone formation – human health*
OF-T	*Ozone formation – terrestrial ecosystems*
OLR	*Organic loading rate*
P	Fósforo
P_2O_5	*Phosphorus pentoxide*
pH	Potencial hidrogeniônico
RoW	*Rest of the world*
S1	*Scenario 1*
S2	*Scenario 2*
S3	*Scenario 3*
SCA-F	*Fossil resource scarcity*
SCA-M	*Mineral resource scarcity*
SDG	*Sustainable development goals*
SS	Sólidos suspensos
SST	Sólidos suspensos totais
SSV	Sólidos suspensos voláteis

TA	*Terrestrial acidification*
TDH	Tempo de detenção hidráulica
TN	*Total nitrogen*
VS	*Volatile solid*
WC	*Water consumption*

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Diante do avanço do crescimento populacional, vem a preocupação com a incapacidade dos recursos naturais de atender às necessidades emergentes da sociedade. Muitos países ao redor do mundo estão enfrentando o problema de escassez de água, mesmo aqueles que possuem um bom potencial hídrico se deparam com o problema da poluição de mananciais, em virtude do lançamento de substâncias em "excesso" ou indesejáveis, comprometendo sua qualidade e danificando os sistemas aquáticos (Abdelfattah et al. 2022).

Tendo em vista a preocupação com a proteção do meio ambiente e da saúde pública, surgiram os sistemas de tratamento de efluentes. As lagoas de alta taxa (LATs) foram introduzidas por volta de 50 anos atrás e são usadas desde então, não apenas para o crescimento de biomassa, mas também, para tratar uma ampla variedade de águas residuárias municipais e industriais (Craggs et al. 2014; Oswald and Golueke 1957). As LATs consistem em reatores em forma de canal de pequena profundidade, onde ocorre movimentação contínua do efluente por pás, na qual as microalgas assimilam nutrientes e produzem oxigênio, que é usado por bactérias heterotróficas para oxidar matéria orgânica, melhorando a qualidade da água (Craggs et al. 2014; Park, Craggs, and Shilton 2011). Por não necessitarem de aeração mecânica, o consumo de energia nas LATs é menor comparado a sistemas de tratamento convencionais, como lodos ativados, requerem áreas para implantação similares ou menores, praticamente eliminam o descarte de lodo, produzem menos odores, proporcionam maior remoção de nutrientes e matéria orgânica, e maior produção de algas quando comparadas com sistemas convencionais de lagoas (Craggs et al. 2014; Garfí, Flores, and Ferrer 2017; Passos et al. 2017; Young, Taylor, and Fallowfield 2017).

Ademais, atualmente, há uma necessidade urgente de mudar o paradigma do tratamento de águas residuárias para a recuperação de recursos a fim de mitigar os impactos ambientais negativos associados às atividades humanas, como poluição de água, emissões de gases de efeito estufa (GEE) e escassez de recursos minerais. Nesse contexto, as microalgas cultivadas em LATs podem ser colhidas e reutilizadas para obtenção de produtos valorados, devido sua taxa de crescimento rápido, alta capacidade de fixação de dióxido de carbono (CO_2), fornecimento de carboidratos, proteínas e lipídios, que podem ser convertidos em vários biocombustíveis e outros bioprodutos (Dasan et al. 2019; Zabed et al. 2020).

Muito estudos têm sido desenvolvidos durante os últimos anos para investigar o potencial das microalgas na produção de biocombustíveis (Arashiro et al. 2018a; Collet et al. 2011; Dasan et al. 2019; Marangon et al. 2021; Sun et al. 2019; Xiao et al. 2020). Esses estudos têm adotado a metodologia de Avaliação do Ciclo de Vida (ACV) para avaliar os impactos ambientais da produção de biocombustíveis a base de microalgas. Destaca-se que a metodologia da ACV é uma ferramenta apropriada para apoiar a pesquisa em estágio inicial e o desenvolvimento de novas tecnologias e processos (Pati 2023). Essa ferramenta leva em consideração e quantifica todas as trocas ambientais (ou seja, recursos, energia, emissões, resíduos) que ocorrem durante todo estágio do ciclo de vida da tecnologia (ISO, 2014). Apesar dos estudos desenvolvidos, são poucos os que avaliam os impactos ambientais da produção de biocombustíveis de biomassa cultivadas em LATs utilizando esgoto doméstico, sendo a maioria deles estudos que abordam as LATs sendo alimentadas por águas sintéticas com a suplementação de nutrientes, o que eleva os custos do sistema e os impactos ambientais relacionados a depleção de recursos minerais (Almeida et al. 2021; Schneider et al. 2018). Além disso o sistema de produção de biogás de microalgas cultivadas em esgoto doméstico carece de melhorias no processo para torná-lo ambiental e economicamente mais atrativo.

Nesse contexto, objetivou-se com o estudo avaliar os impactos ambientais decorrentes da produção de biogás via digestão anaeróbia (DA) líquida de microalgas cultivadas em LATs para tratar efluentes domésticos, utilizando a ferramenta de ACV. O propósito é unificar as incertezas provenientes das discrepâncias em cada etapa do processo de produção de biocombustíveis a partir de microalgas, estabelecendo assim um padrão comparativo entre os processos e destacando as etapas críticas que demandam melhorias ou estudos mais aprofundados. Além disso, este estudo busca ser um catalisador na tomada de decisões para indústrias, líderes e governo. Ele permite que os interessados avaliem o desempenho de um investimento ou projeto, comparando-os com outros em sua carteira. O objetivo é sempre buscar a segurança dos produtos, processos e minimizar os impactos ambientais.

2 HIPÓTESES

O cultivo de microalgas em esgoto doméstico, pode atuar como um mecanismo compensatório para mitigar os impactos negativos associados à produção de biomassa em lagoas de alta taxa e ao melhoramento do biogás por meio do *water scrubbing*, uma vez que essas etapas envolvem o uso significativo de água e nutrientes

No ciclo de vida da produção de biogás a partir de biomassa algal cultivada em lagoas de alta taxa, a etapa mais significativa em termos de impacto ambiental é a digestão anaeróbia mesofílica.

A otimização do balanço energético e a redução das emissões de gases de efeito estufa relacionadas à conversão da biomassa algal em biogás podem ser alcançadas pela redução da temperatura de aquecimento no reator anaeróbio.

3 OBJETIVOS

3.1 Objetivo geral

Realizar uma avaliação dos impactos ambientais associados à produção de biogás a partir de microalgas cultivadas em esgoto doméstico, utilizando a ferramenta de Avaliação do Ciclo de Vida (ACV).

3.2 Objetivos específicos

Identificar as categorias de impacto do método *Recipe Midpoint (H)* e *Recipe Endpoint (H)* mais impactadas pelo sistema de produção de biogás a partir de microalgas cultivadas em esgoto doméstico.

Realizar um balanço energético do sistema, a fim de avaliar a eficiência energética do processo.

Realizar análises de sensibilidade para avaliar o grau de influência de certos parâmetros, entradas ou saídas na quantificação dos potenciais impactos ambientais.

Modelar novos cenários de produção de biogás a partir de microalgas, considerando estratégias de melhor desempenho ambiental, e comparar os cenários propostos em termos de impactos ambientais.

Identificar oportunidades futuras para melhorar a performance ambiental do processo de digestão anaeróbia de biomassa algal produzida durante o tratamento de esgoto doméstico.

4 REFERENCIAL TEÓRICO

4.1 Cultivo de Microalgas

Nos últimos anos, o cultivo de microalgas tem atraído considerável atenção devido às suas diversas aplicações na produção de alimentos, rações, biomoléculas e na indústria farmacêutica (Olabi et al. 2023; Oliveira et al. 2022; Xu et al. 2023). Além disso, o processo fotossintético desses microrganismos oferece outras aplicações, como o sequestro de CO_2, o tratamento de águas residuárias e a produção de biocombustíveis (Acién et al. 2017; Chai et al. 2021; Meier et al. 2015).

Estima-se que existam entre 350.000 a 1.000.000 de espécies de algas. No entanto, até o momento, apenas cerca de 30.000 foram objeto de estudo e análise (Lee 2016). Dentre as espécies investigadas, a predominância é de organismos autotróficos, embora também demonstrem capacidade de crescimento sob condições mixotróficas ou heterotróficas (Rajesh Banu et al. 2020).

Os principais requisitos a serem satisfeitos no cultivo de microalgas fotoautotróficas são o fornecimento de luz, macronutrientes como nitrogênio (N), fósforo (P) e carbono (C); e diversos micronutrientes, que variam de acordo com a espécie (Lee 2016; de Souza et al. 2019). Nesse meio de cultivo, é necessário o uso de fotobiorreatores que devem ser adequadamente projetados, construídos e operados, para satisfazer as necessidades da microalga selecionada para cultivo (Li et al. 2019; Rajesh Banu et al. 2020).

Existem duas principais categorias de fotobiorreatores: abertos e fechados. Os sistemas de cultivo fechados, nos quais não há contato direto entre o meio de cultivo e a atmosfera, incluem colunas de bolhas, loops tubulares e painéis planos. Por outro lado, os sistemas de cultivo aberto, que mantém contato direto com o meio ambiente, incluem lagoas artificiais, tanques, plataformas de camada fina e as LATs (Acién et al. 2017). Os sistemas de cultivo aberto têm a capacidade de produzir grandes quantidades de biomassa a custos consideravelmente mais baixos do que os sistemas fechados (Benemann 2013). Entre suas vantagens estão a facilidade de limpeza, a exposição direta ao sol, o resfriamento automático por evaporação e a menor acumulação de oxigênio. No entanto, esses sistemas também apresentam desafios, como a forte dependência de condições climáticas, elevados riscos de contaminação microbiana, perdas significativas de CO_2 e uma demanda de área mais elevada em comparação com os sistemas fechados (Chisti 2016). Devido ao controle limitado das condições de cultivo e ao risco de contaminação, o uso de unidades de cultivo abertas é restrito a um número relativamente pequeno de espécies de microalgas. Essas unidades são adequadas para espécies robustas de microalgas, como as dos gêneros *Chlorella, Scenedesmus* e *Nannochloropsis*, que apresentam crescimento rápido, ou para aquelas que prosperam em condições muito seletivas, como *Arthospira e Dunaliella* (Acién et al. 2017).

O cultivo de microalgas em águas residuárias tem emergido como uma alternativa para a recuperação de nutrientes, ao mesmo tempo que produz um efluente clarificado (Morillas-España et al. 2022). Consórcios formados por microalgas e bactérias no tratamento de efluentes têm demonstrado eficácia na remoção de nutrientes, metais pesados, bactérias coliformes e compostos xenobióticos (Nagarajan, Lee, et al. 2020). Essa simbiose possibilita tratamento secundário e terciário parcial de águas residuárias, contribuindo para a remoção de DBO_5 e nutrientes (Craggs et al. 2014).

Nesse meio de cultivo, há uma favorável sinergia entre bactérias heterotróficas e microalgas: a matéria orgânica é degrada por bactérias heterotróficas, produzindo CO_2 que é consumido por microalgas autotróficas durante a fotossíntese, assimilando nutrientes durante esse processo e gerando oxigênio que as bactérias precisam para realizar a respiração aeróbia (Nagarajan et al. 2022). As microalgas convertem os nutrientes em biomassa e uma série de compostos orgânicos valiosos que são percussores de diferentes formas de bioenergia como biogás, biodiesel, bio-etanol e biobutanol (Robles et al. 2020).

A compreensão mais aprofundada de como as condições operacionais e ambientais impactam a composição do consórcio microalga-bactéria possibilita a maximização da recuperação de nutrientes e da produtividade de biomassa. Esse conhecimento é fundamental para otimizar o desempenho dos sistemas e promover a eficiência na utilização de águas residuárias como recurso no cultivo de microalgas.

Em sistemas abertos, a temperatura varia sazonalmente e ao longo do dia, e uma significante perda por evaporação pode ser observada. A temperatura ideal, medida sob condições de crescimento máximo, varia de espécie para espécie, entretanto, para muitas espécies se encontra na faixa entre 28 a 35 °C (de Carvalho Lopes et al. 2018). A variação no potencial hidrogeniônico (pH) desempenha um papel crucial no cultivo de microalgas, influenciando a solubilidade do CO_2 e minerais no meio, impactando diretamente esses organismos.

Mudanças no pH podem ocorrer devido a fatores como variações na temperatura, atividade metabólica das células, quantidade de CO_2 dissolvido, composição do meio, entre outros (Al-Muhtaseb et al. 2022). Manter o pH controlado é essencial para evitar a formação de amônia tóxica a partir de sais de amônio dissolvidos, o que pode inibir a produtividade das algas. Para isso, é recomendável manter o pH abaixo de oito, alcançando esse controle por meio da injeção de CO_2 (Chisti 2016). Dado que a absorção natural de CO_2 pela atmosfera é insuficiente, a injeção de gás se mostra mais eficiente para suprir as necessidades de carbono essenciais à fotossíntese e ao crescimento das microalgas.

Gases de combustão, em vez de cilindros de CO_2 puro, são comumente usados para reduzir os custos de produção e contribuir para a mitigação das emissões de GEE, aumentando assim a sustentabilidade do processo (Assis et al. 2019). Uma alternativa adicional é a utilização de biogás bruto proveniente do processo de DA, uma opção ambientalmente amigável e economicamente viável, que combina o fornecimento de CO_2 com o melhoramento do biogás em uma única etapa (Esther Posadas et al. 2017). Teoricamente, 1,8 g de CO_2 são necessárias para a produção de 1 g de microalga (Cavinato et al. 2017).

Os principais componentes da biomassa algal são carbono (30%-50% biomassa seca), oxigênio (30%-50%), hidrogênio (3%-7%), nitrogênio (4%-9%), fósforo (4%-9%), e outros elementos traços (Acién et al. 2017). A razão inicial de C/N/P na água residuária, na ausência de compostos inibitórios ou recalcitrantes, é um indicador de biodegradabilidade, sendo a relação ótima para biodegradabilidade de 100:18:2 (g/g/g/) (E. Posadas et al. 2017). A compreensão detalhada desses aspectos contribui para o desenvolvimento eficiente e sustentável do cultivo de microalgas em diversos cenários.

4.2 Lagoas de Alta Taxa

LATs foram desenvolvidas na Universidade da Califórnia em meados do século XX por Oswald e colegas de trabalho (Oswald and Golueke 1957), enquanto investigavam o uso de microalgas no tratamento de águas residuárias. Esses sistemas abertos consistem em reatores em forma de canais de pequena profundidade (0,25-0,30 m) onde ocorre movimentação contínua do afluente e biomassa algal por meio de uma pá (0,15-0,30 m s^{-1}), em um circuito fechado (Fig. 1). O conjunto do rotor com as pás causa turbulência ao meio, que fornece uma mistura vertical dentro da lagoa, fazendo com que a comunidade de algas seja intermitentemente exposta à luz solar, bem como contribui para a redução da estratificação térmica dentro da lagoa (Mehrabadi, Craggs, and Farid 2015).

A principal variável construtiva das LATs é a área total ocupada. LATs variando de 100 a 5.000 m^2 têm sido reportadas na literatura, sendo que instalações maiores são implementadas multiplicando o número de lagoas atualmente existentes (Acién et al. 2017). O comprimento dos canais é proporcional à largura, com razões de 10-20 geralmente aceitas, sendo preferível usar um menor número de curvas nos canais a fim de reduzir as perdas de carga e a formação de zonas mortas.

Fig. 1. LATs na Estação de Tratamento de Água de Melbourne, Austrália.

Fonte: Young et al., 2017.

Esses sistemas são considerados como sistemas de tratamento de águas residuárias de baixo custo quando comparados aos sistemas convencionais eletromecânicos, como os de lodos ativados, apresentando custos de construção tipicamente ~70% menores e custos de operação reduzidos, uma vez que exigem menor consumo de energia (Craggs et al. 2011; Young et al. 2017). Essa redução no consumo de energia não apenas reduz os custos, mas também reduz as emissões de GEE, tornando esses sistemas uma opção promissora para o tratamento de águas residuárias baseado em irradiação solar (Acién et al. 2016).

As principais variáveis de controle operacional em LATs incluem a taxa de carga orgânica, profundidade, tempo de detenção hidráulica (TDH) e a velocidade de mistura horizontal (Craggs et al. 2011). Dependendo do clima, a taxa máxima de aplicação de carga orgânica varia entre 100-150 kg DBO_5 ha^{-1} dia^{-1}, com o TDH apresentando variação sazonal (3-4 dias no verão e 7-9 dias no inverno) (Craggs et al. 2014). Outras variáveis de controle importantes que influenciam a produtividade de microalgas em LATs incluem temperatura, pH, concentração de CO_2 e a disponibilidade de nutrientes, conforme discutido na seção anterior.

As LATs tem sido empregadas no tratamento de diversas águas residuárias, incluindo esgoto doméstico (Arashiro et al. 2019a; Couto et al. 2021), de laticínios (Mark Ibekwe et al. 2017), e proveniente de atividades como avicultura, suinocultura e pecuária (Ferreira et al. 2018). Na abordagem do esgoto doméstico, a literatura reporta taxas de remoção de DQO na faixa de 26-86% (Alcántara et al. 2015; Couto et al. 2021; Santiago et al. 2013). Para o nitrogênio total, Park, Craggs e Shilton (2011) observaram remoções entre 36-59%, enquanto as remoção de amônia variaram de 74-84%. Arashiro et al. (2019) chegaram a reportar uma remoção de amônia de até 95%. Estudos indicam eficiências na remoção de fósforo solúvel, abrangendo uma faixa de 14-58,2% (Couto et al. 2021; Craggs, Sutherland, and Campbell 2012; Santiago et al. 2013). Conforme Young et al. (2017), os dois principais mecanismos de remoção de nitrogênio e fósforo são principalmente via incorporação na biomassa algal, volatilização de amônia e precipitação química do fósforo, todos dependentes do pH.

O poder calorífico típico da biomassa algal cultivada em LATs varia de 19 a 22 kJ g^{-1}, dependendo da espécie de microalga dominante e das condições climáticas sazonais (Park, Craggs, and Shilton 2013). O rendimento de energia da biomassa de microalgas (kJ m^{-2} d^{-1}) pode ser determinado pelo produto do teor de energia da biomassa em LATs (kJ g^{-1}) com a produtividade de biomassa colhida (g m^{-2} d^{-1}). Em um estudo sobre o cultivo de microalgas em LATs para o tratamento de esgoto doméstico, Park et al. (2013) relataram um rendimento de energia da biomassa de 118 kJ m^{-2} d^{-1}. Ao adotarem a estratégia de reciclar uma parte da biomassa colhida por sedimentação gravitacional de volta para a LAT, os autores observaram melhorias na produtividade de biomassa, eficiência de colheita e teor de energia da biomassa algal, resultando em um acréscimo de 66% no rendimento de energia da biomassa (195 kJ m^{-2} d^{-1}).

Uma LAT pode ser operada em batelada, ou em fluxo contínuo. No modo de cultivo em batelada, todos os nutrientes necessários são adicionados ao meio de cultivo antes do início do cultivo. O crescimento microbiológico cessa devido à depleção limitada de substrato ou à acumulação de produtos inibidores do crescimento, resultando em mudanças contínuas no ambiente que causam flutuações na produtividade. Por outro lado, o sistema de cultivo contínuo possui um ambiente e condições de cultivo controlados, permitindo a composição de biomassa em uma taxa fixa. Esses sistemas são abertos e são alimentados continuamente, com a remoção contínua do produto. O volume do meio dentro do biorreator permanece, mais ou menos, inalterado (Al-Muhtaseb et al. 2022; Chisti 2016).

A produtividade de biomassa em águas residuárias pode variar consideravelmente, dependendo dos fatores de cultivo mencionados anteriormente. Em uma LAT no tratamento de esgoto doméstico bruto, Arashiro et al. (2019b) relataram uma produtividade média de biomassa de 20 ± 7 g_{SSV} m^{-2} d^{-1}, 30% maior do que a produtividade média de biomassa no tratamento de esgoto doméstico após o tratamento primário. Park and Craggs (2010) operaram uma LAT com um TDH de 4 dias e relataram uma produtividade média de biomassa de 20,7 g V_{SS} m^{-2} d^{-1}, devido à adição de CO_2 para controlar o pH e evitar a limitação de carbono. Resultados semelhantes foram descritos por de Godos et al. (2016), com uma produtividade média de biomassa variando de 13,2 g V_{SS} m^{-2} d^{-1} (HRT de 5 dias na primavera) a 23,9 g V_{SS} m^{-2} d^{-1} (HRT de 3 dias no verão) em LATs sem injeção de CO_2.

4.3 Colheita de biomassa algal

A colheita de biomassa algal é uma operação unitária chave na produção de biocombustíveis e outros produtos. A seleção da tecnologia de colheita apropriada depende de fatores como o consumo de energia, custo de capital, requisitos de manutenção, eficiência, as especificidades da espécie de microalga e o uso final da biomassa (Zeng et al. 2016). Essa etapa pode consumir muita energia e representa cerca de 20-30% dos custos totais da produção de microalgas (Restrepo-Serna, Ortiz-Sánchez, and Cardona-Alzate 2018). Essa operação requer a remoção de água para que uma suspensão diluída de células de microalgas com cerca de 0,02-0,06% de sólidos suspensos totais (SST), seja concentrada a 5-25% de SST, dependendo do objetivo do processo de destino da biomassa (Uduman et al. 2010). Em alguns casos, a colheita ocorre em múltiplas etapas. Por exemplo, a biomassa pode ser concentrada a 2-7% de SST em uma etapa inicial, como a de sedimentação gravitacional, seguida de uma segunda colheita na qual se obtém uma concentração de 15-25% de sólidos, como a centrifugação.

Muitos processos estão disponíveis para colheita de biomassa algal. Esses incluem centrifugação, floculação, flotação, filtração, sedimentação, eletroforese, dentre outros. No tratamento de águas residuárias, a sedimentação por gravidade é o método mais comum de separação de sólidos, usado para clarificar grandes volumes de águas residuárias a custos razoáveis (<5% do custo total) (Metcalf & Eddy Inc 2014), enquanto a centrifugação é geralmente o método preferido em instalações comerciais de microalgas que visam a obtenção de produtos de alto valor (Molina Grima et al. 2003). Este estudo é focado em tecnologias eficientes e/ou de baixo custo de colheita de microalgas, e com perspectivas comerciais, assim a sedimentação gravitacional será mais bem discutida a seguir.

4.3.1 Sedimentação gravitacional

A sedimentação gravitacional é uma das técnicas mais baratas para separação sólido-líquido e é amplamente usada nos processos de tratamento de água. A biomassa cultivada em LATs para o tratamento de águas residuárias é constituída por uma mistura de populações de microalgas e bactérias heterotróficas as quais formam flocos espontâneos (diâmetro de 50–200 µm) que podem parcialmente sedimentar por gravidade, sem o consumo de energia ou adição de produtos químicos (Gutiérrez et al. 2016). Sem essa formação de agregados, a velocidade de sedimentação intrínseca de microalgas é muito baixa (~ 1 cm h^{-1}), devido ao seu pequeno diâmetro (<20 µm) e densidade próxima da água, o que acarreta uma deterioração da biomassa (Muylaert et al. 2017). A sedimentação gravitacional gera um lodo diluído e, portanto, estudos tem utilizado essa tecnologia como uma etapa primária de concentração de biomassa antes da colheita completa usando outra tecnologia, como a centrifugação, quando o objetivo posterior é a conversão da biomassa em biocombustíveis (Arashiro et al. 2018b; Sun et al. 2019; Xiao et al. 2020).

Ferreira et al. (2020), ao compararem o desempenho técnico e ambiental de diferentes métodos de colheita de biomassa algal, alcançaram valores de recuperação de biomassa de 87,44% e umidade final de 93,26% com a aplicação da sedimentação gravitacional. Fatores como a baixa concentração de microalga no meio de cultivo (geralmente <0,6 g L-1), as pequenas partículas das células das microalgas (2-50 µm), a carga superficial negativa (-7,5 a -40 mV) e a densidade celular (entre 1030 e 1140 kg m-3) foram citados pelos autores como contribuintes para a redução da velocidade da sedimentação gravitacional. Resultados semelhantes foram obtidos por Gutiérrez et al. (2016), com valores de recuperação de biomassa variando de 75,8% a 88,8%, para LATs na produção de biomassa algal cultivadas em esgoto doméstico.

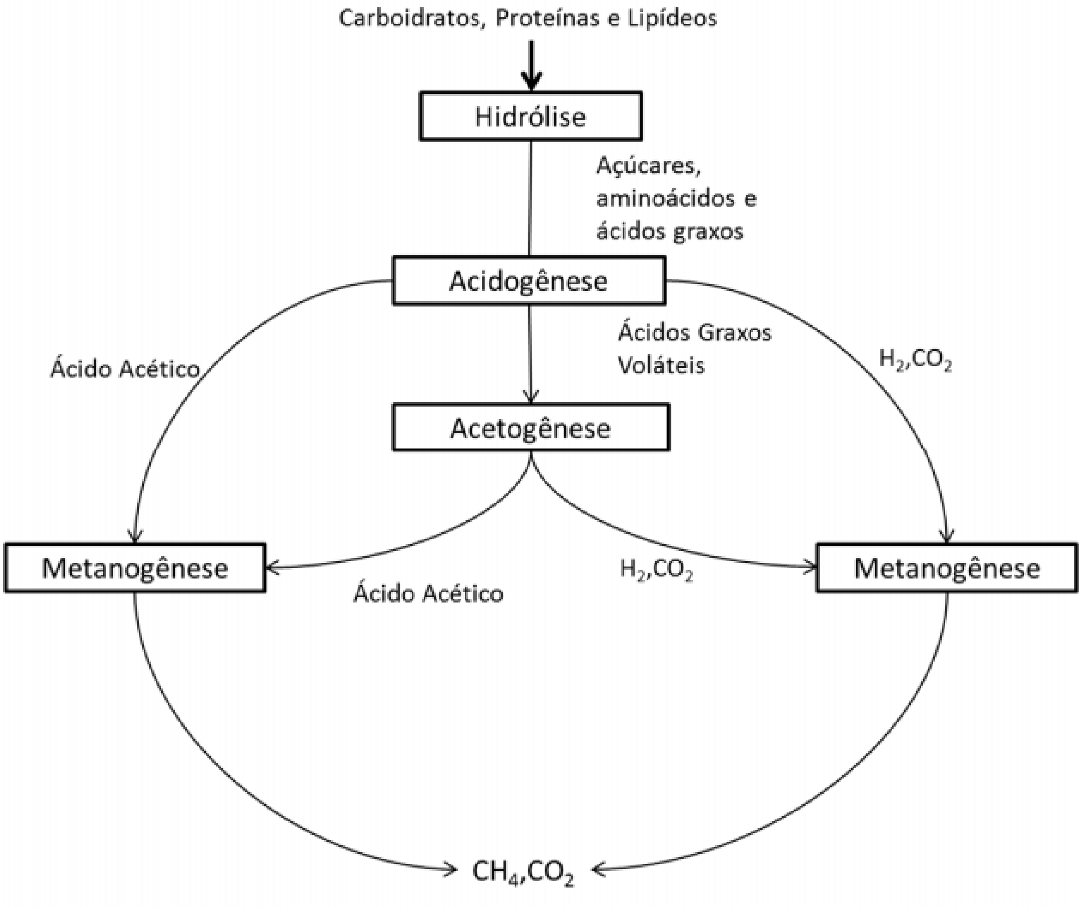
4.4 Digestão anaeróbia de microalgas

A produção de biogás a partir de microalgas tem sido amplamente estudada como uma tecnologia renovável verde e alternativa (Ganesh Saratale et al. 2018). A DA é uma aplicação promissora para a produção de metano (CH_4) a partir da biomassa. Este é um processo bioquímico no qual, na ausência de oxigênio, o carbono orgânico é convertido por oxidações e reduções, ao seu estado mais oxidado (CO_2) e seu estado mais reduzido (CH_4) (Uggetti et al.

2016), por meio de microrganismos especializados. Este processo ocorre via quatro etapas principais: hidrólise, acidogênese, acetogênese e metanogênese (Fig. 2).

Após a DA, o biogás gerado é composto por CH_4 (55-70%), CO_2 (30-45%) e pequenas quantidades de gás sulfídrico (H_2S) (50-2000ppm), vapor de água, oxigênio, e vários outros hidrocarbonetos traços (Braun 2007). Além do CH_4 como uma fonte de energia, a DA da biomassa algal gera outros dois subprodutos, CO_2 e digestato rico em nutrientes, que podem ser úteis para diversas finalidades. A variabilidade de produção de CH_4 na AD pode ser explicada pela composição da biomassa e pelas características da parede celular das diferentes espécies de microalgas (Cavinato et al. 2017), apesar que maiores impactos na produção têm sido observados devido a composição do meio de cultivo (Frigon et al. 2013).

Fig. 2. Degradação da matéria orgânica pela digestão anaeróbia.



Fonte: Adaptado de Li et al. (2011).

Os principais desafios da DA de microalgas incluem a baixa digestibilidade e biodegradabilidade do substrato, bem como o processo inibitório causado pela toxicidade da amônia (Choudhary et al. 2020). O problema associado ao excesso de proteína na biomassa algal, resultando em uma razão C/N desbalanceada e possível inibição devido à liberação de amônia, pode ser solucionado escolhendo um efluente rico em carboidratos (matéria orgânica) ajustado de acordo com o teor de proteína da biomassa ou a co-digestão da biomassa algal com outro substrato rico em carbono. Uma razão C/N de 15-30 têm sido reportada pela literatura como sendo benéfica para uma maior produtividade de CH_4 (Cavinato et al. 2017).

A baixa digestibilidade das microalgas está diretamente relacionada à composição e estrutura de suas paredes celulares, o que resulta em uma menor biodisponibilidade de matéria orgânica. Essa característica pode variar dependendo das condições ambientais de crescimento e do tipo de espécie (Qiu et al. 2020). Para contornar essa limitação, técnicas de pré-tratamento têm sido investigadas para aumentar a taxa de hidrólise da biomassa algal e aumentar tanto a biodisponibilidade quanto a biodegradabilidade das macromoléculas para a DA (Passos and Ferrer 2014).

Os métodos de pré-tratamento podem ser divididos em quatro categorias: mecânicos (como ultrassônico, micro-ondas, eletrocinético e homogeneização de alta pressão), químicos (envolvendo acidificação, alcalinização, ozonização, oxidação Fenton e persulfato ativado por Fe(II)), biológicos (incluindo DA em duas fases e células eletrolíticas microbianas) e térmicos (com baixas e altas temperaturas) (Kasinath et al. 2021; Passos et al. 2018).

O pré-tratamento mecânico consiste na redução do tamanho das microalgas por meio da aplicação da força física (Jankowska, Sahu, and Oleskowicz-Popiel 2017). Embora seja o método menos dependente da espécie de microalga, requer um grande fornecimento de energia (Uggetti et al. 2016).

O pré-tratamento químico envolve a adição de ácidos (como o ácido sulfúrico (H_2SO_4)) ou alcalinos (como o óxido nitroso (NaOH)) (Zabed et al. 2019). Esse método pode causar corrosão no digestor, formação de bioprodutos inibitórios para a metanogênese e alto consumo de energia quando combinado com o aquecimento do digestor (Passos et al. 2014).

O pré-tratamento biológico utiliza enzimas para danificar a parede celular das microalgas e hidrolisar biopolímeros (Uggetti et al. 2016). Apesar de ser um método mais atrativo em comparação com os métodos convencionais, sua implementação comercial ainda está em estágios iniciais, limitada pelo longo tempo de incubação, perda de carboidratos durante o pré-tratamento e altos custos para aquisição das enzimas (Jankowska et al. 2017; Passos et al. 2014; Zabed et al. 2019).

No pré-tratamento térmico, a biomassa algal é solubilizada pela aplicação de calor, sendo o método mais amplamente estudado (Uggetti et al. 2016). Temperaturas variando entre

50 e 270 °C tem sido comumente utilizadas, com a temperatura ótima de tratamento e o tempo dependendo das características específicas do substrato (Wang et al. 2017). Em um estudo conduzido por Passos e Ferrer (2014), foi realizada uma comparação da produtividade de biogás da DA de microalgas, sem pré-tratamento e pós pré-tratamento térmico. Os resultados revelaram um aumento significativo de 70% na produtividade de biogás após o pré-tratamento térmico a 75-95 °C por 10 horas (0,180 L_{CH4} g_{SV}^{-1}).

Como uma alternativa do pré-tratamento, a colheita de substratos em estágio avançado, pode melhorar a digestibilidade da biomassa (Grimm et al. 2015; Klassen et al. 2015). Klassen et al. (2015) demonstraram que mesmo espécies com parede celular rígidas, como a Scenedesmus obliquus, foram satisfatoriamente digeridas quando colhidas no estágio final de crescimento, quando a parede celular está naturalmente enfraquecida. De fato, rendimentos de biogás de 0,219–0,290 m^3_{CH4} kg_{SV}^{-1} foram obtidos usando diferentes biomassas de algas (espécies *Parachlorella kessleri*, *Scenedesmus obliquus* e *Chlamydomonas reinhardtii*) colhidas na fase inicial de crescimento. Os rendimentos de biogás aumentaram para 0,401–0,478 m^3_{CH4} kg_{SV}^{-1} quando as mesmas espécies foram colhidas durante a fase estacionária. Os autores reportaram maiores rendimentos de biogás na biomassa colhida após 8 dias de tratamento.

Diversos autores têm explorado o potencial da DA de microalgas. Arcila e Buitrón (2016) investigaram a influência do cultivo de microalgas em LAT alimentada com esgoto doméstico variando o TDH. Os resultados revelaram produtividades máximas de CH_4 de 329 mL_{CH4} g_{SV}^{-1} (TDH = 2 dias), 290 mL_{CH4} g_{SV}^{-1} (TDH = 6 dias) e 347 mL_{CH4} g_{SV}^{-1} (TDH = 10 dias). Esse estudo corrobora com as conclusões de Klassen et al. (2015), os quais afirmaram que um tempo de cultivo mais longo da biomassa antes da colheita, ou seja, um TDH maior no fotobiorreator, resulta em maior produtividade de biogás, devido ao enfraquecimento da parede celular das microalgas.

Outro estudo realizado por Carrillo-Reyes et al. (2021) examinou, para a mesma água residuária, o cultivo de microalgas em LAT com TDH de 10 dias, seguido pela DA sem pré-tratamento da biomassa, com diferentes TDH (30 e 15 dias) e temperaturas (37 e 55 °C). Nas condições mesofílicas (35 °C), foram obtidas produtividades de CH_4 de 0,26 m^3_{CH4} kg_{SV}^{-1} (TDH = 30 dias) e 0,25 m^3_{CH4} kg_{SV}^{-1} (TDH = 15 dias). Já nas condições termofílicas (55 °C), a produtividade foi de 0,41 m^3_{CH4} kg_{SV}^{-1} (TDH = 30 dias) e 0,17 m^3_{CH4} kg_{SV}^{-1} (TDH = 15 dias). Os resultados sugerem que o TDH teve um impacto significativo no desempenho da digestão termofílica em comparação com a digestão mesofílica. Além disso, o aumento da temperatura de mesofílica para termofílica, para digerir a biomassa de agregado de microalgas e bactérias sem pré-tratamento, favoreceu a produtividade e o rendimento de CH_4.

4.5 Avaliação do Ciclo de Vida

O número e a variedade de tecnologias disponíveis para a produção de biocombustíveis à base de microalgas exigem um meio holístico e imparcial para se comparar diferentes métodos de produção. A avaliação do ciclo de vida (ACV) permite uma avaliação do desempenho ambiental da produção de biomassa de microalgas à conversão a biocombustíveis.

A ACV aborda os aspectos ambientais e os impactos ambientais ao longo do ciclo de vida de um produto, desde a aquisição de matérias-primas, produção, uso, tratamento pós-uso, reciclagem até a disposição final (ISO, 2014a). A ACV padrão é composta por: (1) objetivo e definição do escopo, (2) inventário do ciclo de vida (ICV), (3) avaliação de impactos do ciclo de vida (AICV), e (4) interpretação. A Figura 5 mostra as etapas da ACV e suas interações.

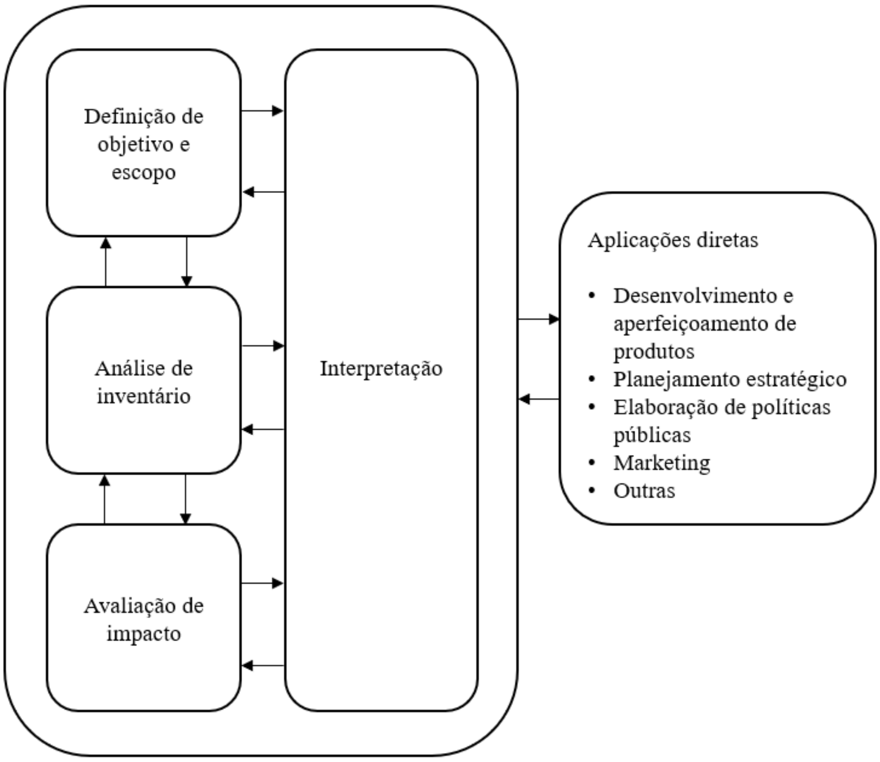
No objetivo e definição do escopo, uma "unidade funcional" (UF) será definida para o produto em questão. Todos os materiais e emissões geradas por um produto serão dadas em quantidades da UF (por exemplo, quantidade de biomassa para geração de 1 MJ de energia). Os limites do sistema também serão definidos, qual será o escopo do estudo. Embora uma ACV normalmente implique que o escopo seja definido do "berço-ao-túmulo", ou seja, da origem da matéria-prima até a disposição final, há alguns casos em que pode não ser prático adotar um escopo dessa magnitude (Culaba et al. 2020). Para biocombustíveis, a abordagem "berço-ao-túmulo" pode ser adotada, começando desde a extração da matéria-prima até a preparação, o processamento, a utilização e o descarte, porém impraticável, dado que a energia pode ser usada para propósitos múltiplos (Culaba et al. 2020).

No ICV, todos os materiais de entrada e saída e todas as emissões são identificadas e quantificadas de acordo com a UF. Nesse sentido, os materiais e emissões devem-se somar à massa real do próprio produto. Assim, os elementos do ICV serão atribuídos as categorias de impacto por meio da classificação. Nessa etapa são calculados os impactos ambientais e quantificadas as cargas ambientais por meio da caracterização, na qual cada resultado será multiplicado por um fator de caracterização, formando as emissões equivalentes que diferenciam de acordo com cada categoria de impacto ambiental. Na última etapa, serão interpretados os resultados, o que leva à identificação do gargalo ambiental e às recomendações, limitações do estudo e conclusões (ISO, 2014b).

Collet et al. (2011) foram os primeiros autores a aplicar a ACV usando dados experimentais e de literatura para avaliar os potenciais impactos ambientais da produção de biogás à base de microalgas da espécie *Chlorella Vulgaris* e comparar os resultados com a produção de biodiesel à base de microalgas e biodiesel de primeira geração. A UF foi 1 MJ produzida por um motor de combustão interna com vazões diárias baseadas em 100 ha de área cultivada (LAT localizada no sul da Europa tratando água sintética) e 23.000 m^3 de volume digerido. O esquema proposto envolveu o cultivo da espécie *Chlorella vulgaris*, a sedimentação natural, a centrifugação, obtendo concentrações da biomassa de 50 kg m^{-3} antes da DA. Para essa análise, 30% do biogás gerado foi usado para aquecimento do processo e o restante foi purificado para obtenção de biometano. O CO2 recuperado da DA foi redissolvido em água e utilizado como fonte de carbono no cultivo das microalgas. O digestor anaeróbio trabalhou com TDH de 46 dias com taxa de carga orgânica de 1,4 g_{DQO} L^{-1} dia^{-1}. O efluente anaeróbio foi separado em sólido e líquido, sendo a fração sólida utilizada para correção de solos e a fração líquida reciclada para a LAT como suplementação de nutrientes.

Como resultado, os autores observaram que ambos os biocombustíveis à base de algas (biometano e biodiesel) foram melhores na categoria de depleção da camada de ozônio (42,9% para o biometano e 34,3% para o biodiesel) e que o CH_4 a base de algas tem menores impactos em acidificação e eutrofização (52,9% e 9,9%, respectivamente) devido a reciclagem do digestato líquido nas LATs. A combustão do CH_4 teve grandes impactos na categoria de aquecimento global (94,3%), porém o impacto foi mitigado pela absorção de CO_2 no cultivo das microalgas, uma vez que os autores consideraram a suplementação de CO_2 nas LATs via gases de combustão de indústrias. Além disso, a análise realizada destacou alguns gargalos relacionados ao consumo de energia (pás, sistema de bombeamento de água, mistura da DA e sistema de aquecimento), que contribuíram fortemente nas categorias de radiação ionizante, depleção abiótica e acidificação terrestre (89,9; 72,7 e 67%, respectivamente). Para lidar com esse gargalo, os autores propuseram algumas estratégias para melhorar a produção de CH_4, como a troca de equipamentos para outros com menor consumo de energia, diminuir o consumo de energia pela adoção de um TDH menor, aumentar a concentração da biomassa antes da AD, ou usar uma espécie de microalga com maior potencial de geração de biogás ou ser codigerida com outro substrato a fim de aumenta a razão C:N.

Fig. 3. Fases de uma ACV.



Fonte: Adaptado de ISO (2014a).

Em outro estudo de ACV, Marangon et al. (2022) avaliaram os impactos ambientais da produção de bio-óleo à base de biomassa algal, cultivada em esgoto doméstico, via liquefação hidrotérmica (LHT) e sua transformação à diesel renovável. Os autores consideraram a abordagem “portão-ao-portão” na obtenção de 1 MJ de energia como UF. Nessa abordagem os autores consideraram as etapas de concentração da biomassa, LHT, fase de separação (bio-óleo, fase sólida e fase líquida), e conversão do bio-óleo a diesel renovável. A fase de cultivo e colheita da biomassa não foram incluídas no limite do sistema. Foram avaliadas 18 categorias de impacto, porém após normalização dos dados, os autores interpretaram os resultados de 6 categorias que tiveram maiores destaques. De acordo com os resultados, os impactos da produção de bio-óleo foram maiores na categoria de eutrofização marinha devido as emissões de N da fase líquida. Para as outras categorias de impacto, a etapa mais impactante foi a LHT devido à alta demanda de energia para aquecimento do reator, exceto para a categoria de mudanças climáticas, na qual a etapa responsável pela maioria dos impactos foi a etapa de conversão do bio-óleo e diesel renovável. Os autores propuseram um novo cenário no qual foram considerados a redução da demanda aquecimento do reator de LHT, reciclagem da fase aquosa após diluição e maiores concentrações da biomassa antes da aplicação aos reatores, e com isso os resultados mostraram que essas foram medidas eficazes para diminuir os impactos ambientais negativos do processo (redução de 45 % em média).

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - ENHANCING ENVIRONMENTAL PERFORMANCE IN BIOGAS PRODUCTION FROM WASTEWATER-GROWN MICROALGAE: A LIFE CYCLE ASSESSMENT PERSPECTIVE

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ABSTRACT

The production of biogas from microalgae has gained attention due to their rapid growth, CO2 sequestration, and minimal land use. This study uses life cycle assessment to assess the environmental impacts of biogas production from wastewater-grown microalgae through anaerobic digestion within an optimized microalgae-based system. Using SimaPro® 9 software, 3 scenarios were modeled considering the ReCiPe v1.13 midpoint and endpoint methods for environmental impact assessment in different categories. In the baseline scenario (S1), a hypothetical system for biogas production was considered, consisting of a high rate algal pond (HRAP), a settling, an anaerobic digester, and a biogas upgrading unit. The second scenario (S2) included strategies to enhance biogas yield, namely co-digestion and thermal pre-treatment. The third scenario (S3), besides considering the strategies of S2, proposed the biogas upgrading in the HRAP and the digestate recovery as a biofertilizer. After normalization, human carcinogenic toxicity was the most positively affected category due to water use in the cultivation step, accounted as avoided product. However, this category was also the most negatively affected by the impacts of the digester heating energy. Anaerobic digestion was the most impactful step, constituting on average 60.37% of total impacts. Scenario S3 performed better environmentally, primarily due to the integration of biogas upgrading within the cultivation reactor and digestate use as a biofertilizer. Sensitivity analysis highlighted methane yield's importance, showing potential for an 11.28% reduction in ionizing radiation impacts with a 10% increase. Comparing S3 biogas with natural gas, the resource scarcity impact was reduced sixfold, but the human health impact was 23 times higher in S3.

KEYWORDS: Wastewater-grown microalgae**,** Biogas**,** Life Cycle Assessment**,** Sensitivity Analysis, High Rate Algal Pond, Biomass

1 INTRODUCTION

The need for alternative energy sources to replace fossil fuels has become increasingly evident in recent years. The finite nature of fossil fuel reserves and their detrimental environmental impact, including the release of large amounts of greenhouse gases (GHG) such as CO_2, nitrogen oxide, methane, and sulfur dioxide, has prompted a global search for sustainable and renewable energy options (Arutselvan et al., 2022). Exploring alternative energy sources is crucial for addressing climate change, reducing dependence on fossil fuels, and ensuring a secure energy source for future generations.

Biofuels from agricultural resources may be a sustainable option for bioenergy production. However, the production of biofuels from conventional crops raises substantial environmental apprehensions, including watercourse eutrophication, resource depletion, deforestation, water scarcity, and competition with food crops (Collet et al., 2011). Therefore, there is a pressing need to explore alternative sources that are environmentally friendly.

Among the various potential energy sources, algal biomass has emerged as a particularly promising candidate for biofuel production, including hydrogen (Nagarajan et al., 2020), biodiesel (Arutselvan et al., 2022), biogas (Xiao et al., 2020), bioethanol (Kusmiyati et al., 2023), and bio-oil (Couto et al., 2020). Algae's ability to capture large amounts of CO_2 during photosynthesis makes them attractive for carbon sequestration and reducing GHG emissions (Marangon et al., 2021). These microscopic organisms offer several advantages, including high growth rates, high biomass productivity surpassing other traditional energy crops (Zabed et al., 2019), and the ability to be cultivated using non-arable land and various water sources, including seawater, brackish water, and even wastewater (Kumar et al., 2020).

Anaerobic digestion (AD) allows the microbial degradation of organic matter in the absence of oxygen, resulting in the production of biogas and digestate, which is rich in nutrients and is usually used as a fertilizer (Ganesh Saratale et al., 2018; Vargas-Estrada et al., 2022). AD presents an advantage as it can utilize wet biomass with low solid contents (5-8%) for biogas production and does not require the extraction of specific macromolecules such as lipids, proteins, or carbohydrates (Mehrabadi et al., 2015). However, the composition of the biogas needs to be optimized by reducing CO_2 content through a biogas upgrading process to maximize its energy content (Miyawaki et al., 2021).

While AD is a promising waste management solution, it is crucial to thoroughly analyze its environmental impacts to ensure sustainability. Among the several methodologies used to estimate and quantify the environmental impacts of products or processes, life cycle assessment (LCA) can be highlighted. LCA has been widely used to assess biofuel production pathways and address environmental impacts (Arashiro et al., 2022; Magalhães et al., 2021).

Although AD is a wide and well-known process used to treat a great variety of wastes, limited studies have focused on the LCA of biogas from wastewater-grown microalgae. Arashiro et al. (2022) undertook a comprehensive analysis wherein they compared the environmental implications of diverse microalgae-based systems for wastewater treatment and bioproduct recovery. Their investigation unveiled discernible variations predicated on the nature of the wastewater source. Specifically, the authors observed that the scenario involving the treatment of urban wastewater yielded more pronounced environmental impacts when juxtaposed with the scenario entailing the treatment of wastewater from the food industry. This discrepancy emanated from several key factors, namely, diminished biogas generation, elevated atmospheric emissions, and augmented concentrations of heavy metals in the former scenario. Previous studies have mainly considered microalgae from freshwater sources (Collet et al., 2011; Mediboyina et al., 2020; Sun et al., 2019). Therefore, more research is needed to understand the environmental implications of biogas recovery from wastewater-grown microalgae and identify potential bottlenecks for sustainable industrial-scale facilities (Arashiro et al., 2022; Magalhães et al., 2021).

The objective of this study is to pioneer an innovative LCA of AD biogas production from microalgae cultivated in domestic wastewater. Our unique contribution lies in comprehensively examining various scenarios, including integrating pretreatment methods, heat recovery, and biogas upgrading processes, besides considering the inclusion of long-term emissions with a more comprehensive perspective of the product's impact in a longer timeline. This study not only identifies potential areas for improvement but also advances existing knowledge by exploring the environmental impacts of biogas recovery from wastewater-grown microalgae. Our findings are expected to be instrumental for policymakers, industry stakeholders, and researchers who are keen on developing sustainable biogas production pathways.

2 MATERIALS AND METHODS

2.1 Life Cycle Assessment

The study followed the technical framework for the LCA methodology standardized by ISO standards (ISO, 2006a, 2006b). According to ISO 14040, LCA consists of four steps: definition of the objective and scope, inventory analysis, evaluation of impacts, and interpretation of results.

2.2 Objective and scope of the study

This study aimed to demonstrate the environmental performance of biogas production from microalgal biomass grown in domestic sewage and identified factors that could be improved. The FU used as a basis for comparison was 1 MJ of biofuel (Collet et al., 2011; Marangon et al., 2021; Mediboyina et al., 2020; Sun et al., 2019), and the system limit was set to "gate-to-gate." Steps of construction, transport of inputs and materials, end of life of equipment and infrastructure, and combustion/end use of biogas were not considered in any of the scenarios. In the baseline scenario (S1), a hypothetical system for biogas production was considered, consisting of a High Rate Algal Pond (HRAP), a settling, an anaerobic digester, and a biogas upgrading unit. Besides biogas, the system produced only emissions to both soil and air. Therefore, the allocation of outputs was deemed unnecessary (ISO, 2014a).

After evaluating the environmental impacts of S1, two new scenarios were proposed based on changes in S1 to improve the environmental performance of biogas production (Fig.1). The second scenario (S2) included strategies to enhance biogas yield, namely co-digestion and thermal pre-treatment. The third scenario (S3), besides considering the strategies of S2, proposed the biogas upgrading in the HRAP and the digestate recovery as a biofertilizer. The new scenarios are described in more detail in section 2.1.4.

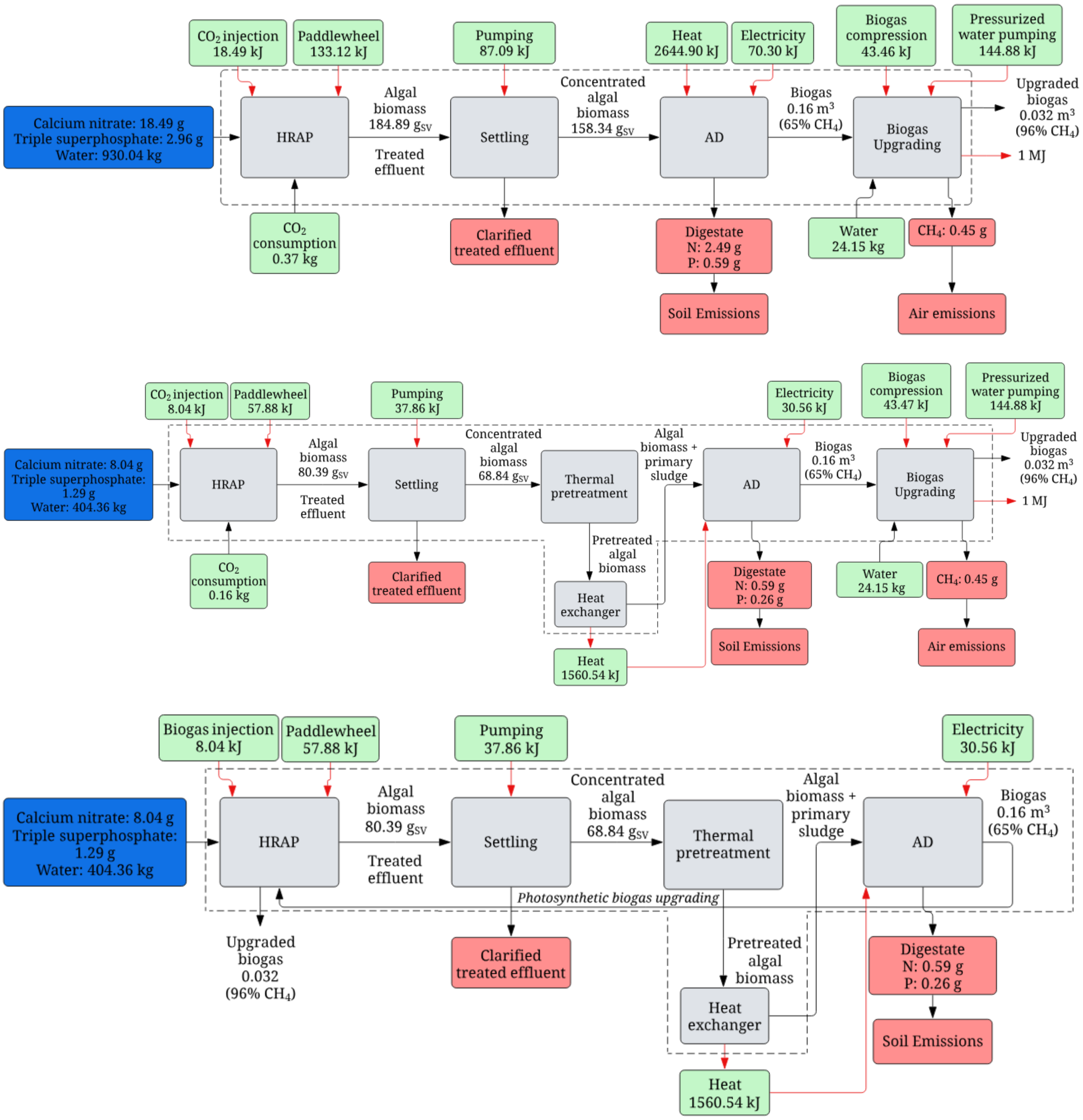

Fig. 3. System boundary and mass and energy flow for scenarios (a) S1, (b) S2, and (c) S3 of biogas production from microalgae.

The environmental impacts assessment was conducted using SimaPro® 9 software.

2.3 Life Cycle Inventory (LCI)

The *background* data were obtained from the Ecoinvent version 3.9.1 database using the allocation method at the point of substitution - system (APOS-S) (Sonderegger & Stoikou, 2022). Data for the LCA model were gathered from studies involving microalgae cultivation in domestic sewage and biogas recovery through liquid AD. This specificity aimed to obtain data on biomass productivity in HRAP and methane yield in the digester, given the unique characteristics of algal biomass from domestic sewage compared to other media (Chiu et al., 2015). However, operational parameters for biomass harvesting and biogas upgrading units were sourced from studies using different media due to data scarcity and considering that both processes are not directly influenced by biomass characteristics, as the biomass and biogas production stages are. Data from the literature were compiled and averaged to create the modeling scenarios, with parameter values provided in Table S1 in the supplementary material.

- Microalgae cultivation step

The microalgae growth system was modeled using an HRAP. CO_2 supplementation in the HRAP was considered to come from gas cylinders containing 99% CO_2 (T. C. de Assis et al., 2019; Couto et al., 2021; L. R. de Assis et al., 2017; de Godos et al., 2016; Posadas et al., 2015). The fertilizer supplements calcium nitrate and triple superphosphate, as well as water, were considered avoided products, as wastewater meet the microalgae demand for nutrients and water (Magalhães et al., 2021), which means they were no longer produced and were considered environmental credits, offsetting the overall negative impact of the system (Arashiro et al., 2022). The system expansion method was used following the ISO guidelines to consider when these conventional products were not produced (ISO, 2006b). The electricity consumption of the cultivation process was calculated based on the requirements of the paddlewheels and CO_2 injector.

The treated and clarified effluent was not considered a polluting source; therefore, its potential environmental impacts were not included in the LCA. Data from studies using HRAPs treating secondary domestic effluents demonstrated a strong performance in macronutrient and organic matter removal, meeting effluent discharge standards (T. C. de Assis et al., 2019; L. R. de Assis et al., 2017; de Godos et al., 2016).

- Biomass harvesting step

The biomass was harvested through gravitational settling with a final moisture content of 93.26% (Ferreira et al., 2020). The energy consumption of the biomass harvesting step was calculated based on the electricity requirements of the pumps.

- Anaerobic Digestion Step

This study utilized liquid AD of algal biomass, characterized by a biomass solids content limited to 15% (Zabed et al., 2019). The volume of biogas (65% CH_4 and 35% CO_2)

corresponding to the FU was calculated, considering a lower biogas calorific value of 34.37 MJ m^{-3} CH_4 (Sun et al., 2019) and an energy conversion efficiency of 90% (Passos et al., 2017; Passos & Ferrer, 2014).

The digestate after AD was considered as a soil emission. The digestate was subjected to characterization regarding its nitrogen and phosphorus composition, using as reference the molar mass of these elements present in the avoided products, calcium nitrate and triple superphosphate, respectively. These parameters were determined based on information provided by Ecoinvent, considering a proportion of 79% for calcium nitrate and 46% for triple superphosphate in relation to total production.

The energy demand for biomass mixing and pumping and the heat demand for AD were calculated according to Ferrer et al. (2009) and Passos et al. (2017). Mesophilic temperature condition (35 °C) was considered for AD. All parameters used for the energy flow calculations of the system are presented in Table S2.

For the anaerobic reactor, the heat input was calculated as the energy required to heat the influent biomass from ambient temperature (Ta) to the digestion temperature (Td), according to Eq. (1). The density (ρ) and specific heat (γ) of algal biomass were considered to be equal to those of water, 1000 kg m^{-3} and 4.18 kJ kg^{-1} $°C^{-1}$, respectively. The heat losses through the reactor wall were calculated assuming a heat transfer coefficient (k) of 1 W m^{-2} $°C^{-1}$ (Metcalf and Eddy, 2004). The surface area of the reactor wall was calculated from the useful volume of the reactor, considering a diameter/height ratio of 2:1 (Passos & Ferrer, 2014), while the areas of the bottom and top of the reactor were not accounted for.

$$E_{input,heat} = [Q\rho\gamma(T_d - T_a) + kA(T_d - T_a)86.4] * HRT \quad (1)$$

where $E_{input,heat}$: input heat (kJ); Q: flow rate (m^3 d^{-1}); ρ: density (kg m^{-3}); γ: specific heat (kJ kg^{-1} $°C^{-1}$); Td: temperature of AD (35 °C); Ta: ambient temperature (25 °C); k: heat transfer coefficient (W m^{-2} $°C^{-1}$); A: surface area of the reactor wall (m^2); and HRT: hydraulic retention time of the reactor (d).

The input energy for AD was calculated as the energy required for pumping the biomass and mixing it into the reactor, which was assumed to be 1800 kJ m^{-3} and 300 kJ m^3, respectively (LU et al., 2008) (Eq. (2)).

$$E_{input,energy} = (Q\theta + V\omega) * HRT \quad (2)$$

where $E_{input,energy}$: input energy (kJ); θ: energy consumption for pumping (kJ m^3); V: useful volume (m^3); ω: energy consumption for mixing (kJ m^3 reactor d^{-1}) and HRT: hydraulic retention time of the reactor (d).

- Biogas upgrading step

While biogas can be directly used for electricity generation, its efficiency is significantly affected by a high proportion of CO_2. This impact results in a reduced heating value and increased expenses for compression and transportation (Zabed et al., 2020). Therefore, biogas washing with pressurized water, also known as water scrubbing, was adopted because it is the simplest, most economical, and environmentally friendly method among existing biogas upgrading technologies, representing approximately 41% of the market share (Kapoor et al., 2019). In this step, approximately 2% of the biogas was lost and treated as air emissions (Muñoz et al., 2015). The energy consumption in this process mainly comes from the compression of raw biogas at 8-10 bar and water pumping (Rotunno et al., 2017).

2.4 Impact assessment

The recommendations of ISO 14044 (ISO, 2006b) were followed for the classification, characterization, and normalization of the Life Cycle Impact Assessment (LCIA) results. The ReCiPe v1.13 midpoint and endpoint methods were used to characterize the LCIA impacts (Huijbregts et al., 2016). Among other factors, the ReCiPe method was chosen due to the broadest set of midpoint impact categories, and its impact mechanisms that have global scope (Pre-Sustainability, 2016). Long-term emissions that occur over large time frames of substantially more than 100 years were included. The characterization phase of this study considered all 18 midpoint impact categories: global warming (GW), stratospheric ozone depletion (OD), ionizing radiation (IR), fine particulate matter formation (FPMF), ozone formation – human health (OF-H), ozone formation – terrestrial ecosystems (OF-T), terrestrial acidification (TA), freshwater eutrophication (EUT-F), marine eutrophication (EUT-M), human carcinogenic toxicity (HT-C), human non-carcinogenic toxicity (HT-NC), terrestrial ecotoxicity (ECO-T), freshwater ecotoxicity (ECO-F), marine ecotoxicity (ECO-M), land use (LU), water consumption (WC), mineral resource scarcity (SCA-M) and fossil resource scarcity (SCA-F). After obtaining the LCIA results, only the categories with the most significant environmental impacts were selected for further analyses. Through normalization, the impact categories were expressed on the same scale (ecopoints) and compared. As a reference, the average global pressure applied on the environment by an individual in 2010 was considered (RIVM, 2016).

In the endpoint assessment (H), impact categories are grouped into three damage categories: human health, ecosystem quality, and resource scarcity, respectively measured in DALYs, species.year, and \$. These categories are subsequently normalized and weighted into a single value in points (Pt) or millipoints (mPt). These damage categories in the endpoint approach (H) serve as foundations for sustainable development decisions and policymaking (Alyaseri & Zhou, 2017; Huijbregts et al., 2016).

2.5 Improved alternative scenarios

After identifying the critical factors in the potential environmental impacts of S1, two novel new scenarios were formulated. In S2, a thermal pre-treatment of algal biomass, emphasizing heat recovery, was introduced before the AD process, along with codigestion with primary sludge from domestic sewage treatment, following Solé-Bundó et al. (2018). S3, based on S2, involves utilizing digestate as a biofertilizer and implementing biogas upgrading within the HRAP system.

For LCI in scenarios S2 and S3, the average parameters from S1 served as the baseline, except for the methane yield parameter, which came from Solé-Bundó et al. (2018). In S3, the digestate impacts on the soil were not considered, so its emissions weren't quantified as LCA outputs due to its use as a biofertilizer. The choice to treat digestate as a biofertilizer was justified based on its potential environmental impacts and benefits. Also, for biogas upgrading within the HRAP, the impacts of the water scrubbing process were disregarded. The photosynthetic biogas upgrading is a novel technology addressed in some recent studies (Rodero et al., 2018; Marín et al., 2018) and here, some simplifications were assumed, such as no CH_4 losses and no energy consumption were considered during the process.

The energy demand for mixing and pumping the biomass in scenarios S2 and S3 was calculated according to Eq. (2). The heat demand was calculated as the energy required to heat the influent biomass (Ta) to the pretreatment temperature (Tp), subtracted by the heat recovered when the biomass was cooled from Tp to Td (Equation 3). Heat recovery was assumed to occur using a heat exchanger, with an efficiency φ of 85%.

$$E_{input,heat} = \left[Q\rho\gamma\left(T_p - T_a\right) - Q\rho\gamma\left(T_p - T_d\right)\varphi + kA(T_d - T_a)86.4\right] * HRT \qquad \text{Eq. (3)}$$

where $E_{\text{input,heat}}$: input heat (kJ); Q: flow rate (m^3 d^{-1}); ρ: density (kg m^{-3}); γ: specific heat (kJ kg^{-1} $°C^{-1}$); Td: temperature of AD (35 °C); Ta: ambient temperature (25 °C); Tp: pretreatment temperature (75 °C); φ: heat recovery efficiency (85%); k: heat transfer coefficient (W m^{-2} $°C^{-1}$); A: surface area of the reactor wall (m^2) and HRT: hydraulic retention time of the reactor (d). All parameters used for the energy flow calculations of the systems are presented in Table S2.

2.6 Interpretation of Results

After the assessment of the magnitude and significance of the potential environmental impacts of S1, analyses of contribution, and sensitivity were conducted following ISO standards (ISO, 2006ab). The contribution analysis identified the processes that played a significant role in the results, revealing the processes with the greatest negative and positive impacts.

The potential environmental impacts of S2 and S3 were compared to those of S1 through results normalization. For these new scenarios, sensitivity analysis was conducted with respect to key parameters, namely: methane yield, pretreatment temperature, and heat exchanger efficiency, considering variations of ± 10% with respect to the mean values. Sensitivity analysis was performed to assess the effect of possible variations in model inputs on the model response (Marangon et al., 2021; Pérez-López et al., 2018; Porcelli et al., 2020; Sun et al., 2019).

The evaluation of damages in the categories of human health, ecosystem quality, and resource depletion by the Endpoint method (H) was applied to the scenario that presented the best environmental performance at the midpoint level and compared to the production data of 1 MJ of natural gas obtained through the library of Ecoinvent 3.9.1 (Sonderegger & Stoikou, 2022).

3 RESULTS AND DISCUSSION

3.1 Potential environmental impacts of the biogas life cycle

Fig. 2 shows the normalized values for the 18 environmental impact categories in the S1 LCIA. The 6 categories with the greatest positive environmental impacts (negative values) and the 6 with the greatest negative impacts (positive values) were selected, with 8 categories identified as most significant (in order of relevance): HT-C, WC, EUT-F, ECO-F, ECO-M, SCA-F, OF-T and IR. The characterization and normalization values for each impact category are presented in Tables S3 and S4.

The total normalized environmental impacts of the system in the selected impact categories (Table 1) showed that in most of the 8 categories, negative impacts outnumbered the positive impacts, except for WC and IR. Thus, the categories with negative impacts exceeded the positive impacts by approximately 38.59%.

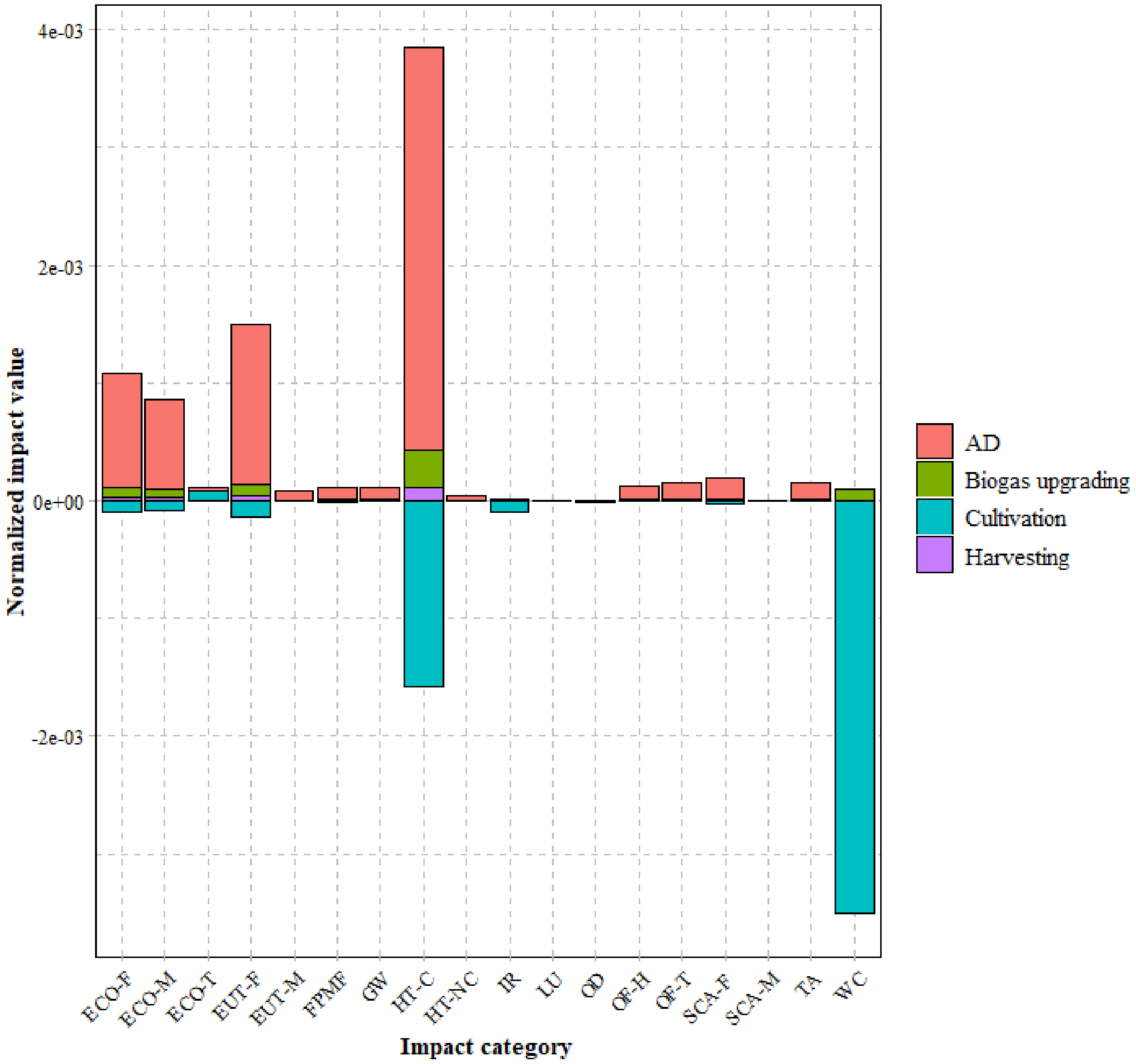

Fig. 4. Normalized values of the potential environmental impacts of the life cycle of microalgae biogas via AD in the 18 impact categories. ECO-F: freshwater ecotoxicity, ECO-M: marine ecotoxicity, ECO-T: terrestrial ecotoxicity, EUT-F: freshwater eutrophication, EUT-M: marine eutrophication, FPMF: fine particulate matter formation, GW: global warming, HT-C: human carcinogenic toxicity, HT-NC: human non-carcinogenic toxicity, IR: ionizing radiation, LU: land use, OD: stratospheric ozone depletion, OF-H: ozone formation – human health, OF-T: ozone formation – terrestrial ecosystem, SCA-F: fossil resource scarcity, SCA-M: mineral resource scarcity, TA: terrestrial acidification, WC: water consumption.

Table 1 - Total normalized environmental impacts of the system in the 8 most significant impact categories in Scenario 1.

Impact Category	Total	Anaerobic digestion	Harvesting	Cultivation	Biogas upgrading
Ionizing radiation	-8.25226E-05	4.45232E-06	1.42804E-07	-9.04258E-05	3.30806E-06
Ozone formation, Terrestrial ecosystems	0.000148954	0.000137209	4.40086E-06	-3.02981E-06	1.03736E-05
Freshwater eutrophication	0.001354953	0.001359886	4.06845E-05	-0.000142349	9.67317E-05
Freshwater ecotoxicity	0.000982068	0.000964994	3.09512E-05	-9.71071E-05	8.32302E-05
Marine ecotoxicity	0.000777086	0.000772761	2.47856E-05	-8.66042E-05	6.61433E-05
Human carcinogenic toxicity	0.002257199	0.003415334	0.000109544	-0.001583363	0.000315684
Fossil resource scarcity	0.000174878	0.000177405	5.69011E-06	-2.27703E-05	1.45527E-05
Water consumption	-0.003408632	5.69139E-06	1.82546E-07	-0.003506056	9.15494E-05

The most significant impact categories of the study were similar to those found in other LCA studies on the production of biofuels from microalgae that used the Recipe midpoint (H) method to evaluate potential impacts. Branco-Vieira et al. (2020) conducted an environmental assessment of the industrial production of biodiesel from microalgae in south-central Chile. They found that GW, TA, EUT-F, LU, WC, and SCA-F were the most significant categories. Arashiro et al. (2022) evaluated the potential environmental impacts of two wastewater treatment systems (food industry and domestic sewage) using microalgae and resource recovery (natural pigments, biofertilizers, and biogas). The categories selected by the authors were GW, OD, TA, EUT-M, EUT-F, TH-C, SCA-M, SCA-F, and FPMF. The authors claimed that these categories are most relevant regarding wastewater treatment for resource recovery.

Despite the similarity in the results, LCA studies are difficult to compare because they can vary in scope, methodology, data, and assumptions. For example, studies may evaluate different products or processes, use other FU or system boundaries, apply different impact assessment methods, and use diverse data and assumptions to model product life cycle (Branco-Vieira et al., 2020).

This study differs from others by considering long-term emissions to assess potential environmental impacts. Long-term emissions account for greenhouse gas (GHG) emissions associated with a product or system's life cycle over an extended period (ten Hoeve et al., 2019). This approach provides a more comprehensive view of the product's climate impact, potentially resulting in a different impact category compared to an analysis focused solely on direct emissions during the usage phase.

3.2 Contribution analysis

Table 2 presents the percentage contribution of the life cycle steps in S1 to each of the 8 impact categories. Fig. 3 shows the percentage contribution of inputs and outputs in S1 for each category.

Table 2 – Contribution (%) of the life cycle steps in Scenario 1 to each of the 8 most significant impact categories.

Impact Category	Anaerobic Digestion	Harvesting	Cultivation	Biogas Upgrading
Ionizing radiation	5%	0%	-92%	3%
Ozone formation, Terrestrial ecosystems	89%	3%	-2%	7%
Freshwater eutrophication	83%	2%	-9%	6%
Freshwater ecotoxicity	82%	3%	-8%	7%
Marine ecotoxicity	81%	3%	-9%	7%
Human carcinogenic toxicity	63%	2%	-29%	6%
Fossil resource scarcity	80%	3%	-10%	7%
Water consumption	0%	0%	-97%	3%

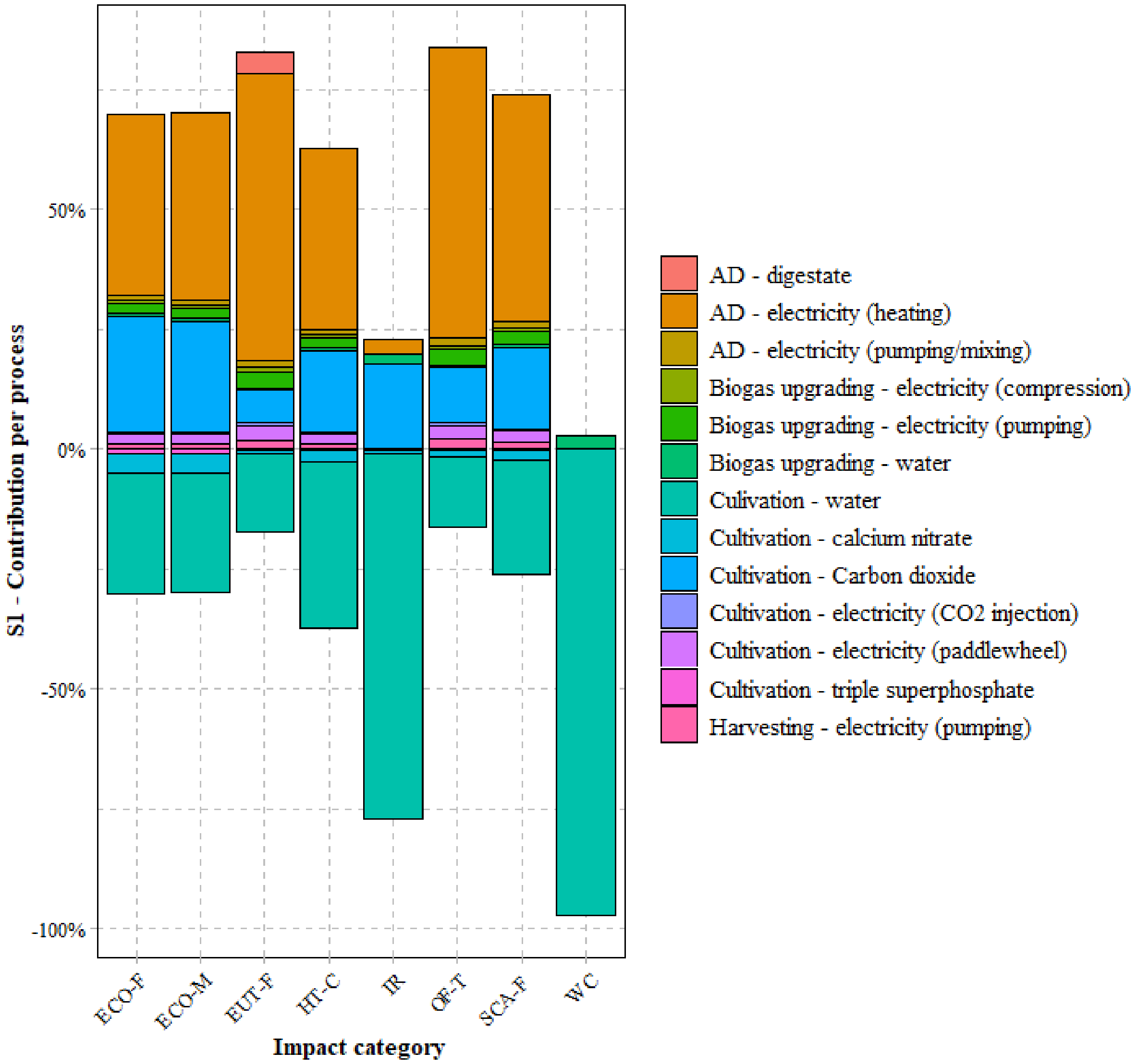

Fig. 5. Percentage contribution of the processes modeled in the life cycle of the baseline scenario in the eight impact categories in which the environment was most affected. ECO-F: freshwater ecotoxicity, ECO-M: marine ecotoxicity, EUT-F: freshwater eutrophication, EUT-M: marine eutrophication, HT-C: human carcinogenic toxicity, IR: ionizing radiation, SCA-F: fossil resource scarcity, WC: water consumption.

In S1, the AD step was responsible for the highest negative impacts in 7 out of 8 categories (an average of 60.37% of the total impact), except for the WC category, where the biogas upgrading step had the greatest contribution to potential negative impacts. The AD step had the most contribution in the impacts of OF-T category, accounting for approximately 88.51% of the generated impacts. The harvesting step had the lowest contribution to potential impacts (an average of 1.91% of the total impact) compared to the other steps, except for the category of OF-T, where the cultivation step had a lower contribution. The energy consumption for anaerobic digester heating was the input that contributed the most to potential negative environmental impacts (an average of 35.28% of the total impact), except for the category of

IR, where the carbon dioxide from the cultivation step contributed approximately 17.43% of the total impact, and in the WC category, where the water consumption of the biogas upgrading step had the greatest contribution (2.52% of the total impact). As observed in other studies on the production of biofuels from microalgae (Collet et al., 2011; Marangon et al., 2021), heat and electricity sources were the primary environmental bottlenecks of these systems, and in this study, they contributed approximately 39.77 % of the total impact of S1.

The cultivation step yielded favorable outcomes in all chosen categories due to the utilization of fertilizers and water as avoided products. On average, the impacts were mitigated by 44.39% using water, calcium nitrate, and triple superphosphate as avoided products. In WC and IR categories, these products accounted for 97.11 and 77.2%of the total potential impacts, respectively. Water was the most effective avoided product for reducing environmental impacts in all 8 categories analyzed, representing an average of 39.12% of the total impacts. Therefore, using domestic sewage in microalgae cultivation for biogas production offers significant environmental benefits.

3.3 Comparison between the life cycle of the baseline scenario and other proposed scenarios

After identifying that the AD step was primarily responsible for the negative impacts of the system, mainly due to its electricity demand for digester heating, two new scenarios were proposed to mitigate environmental impacts and enhance the overall environmental performance of the biogas production system, as detailed in Section 2.1.4. The potential environmental impacts associated with scenarios S1, S2, and S3 are shown in Fig. 4.

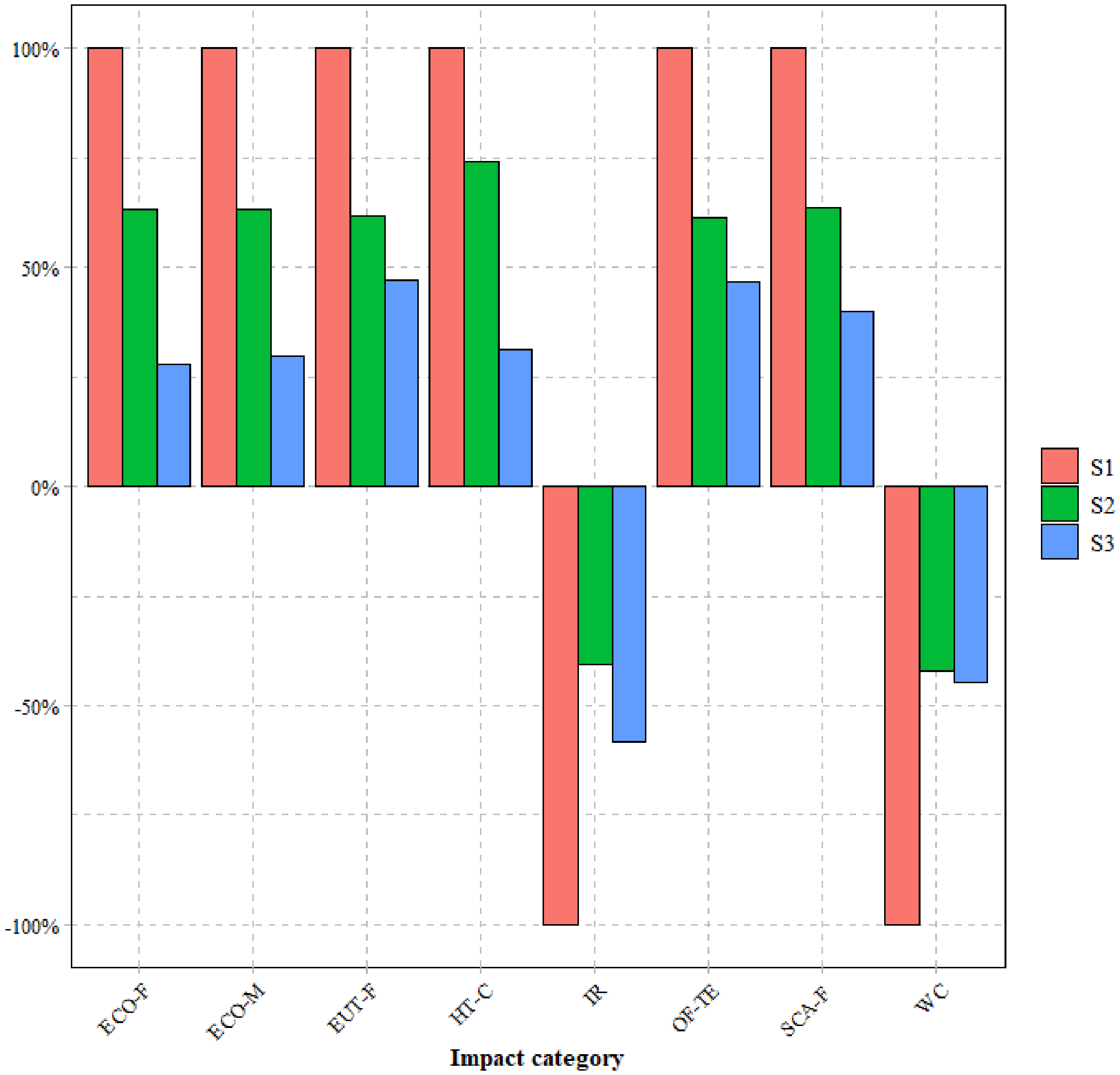

Fig. 4. Potential environmental impacts associated with scenarios S1, S2, and S3 in the eight most significant impact categories. ECO-F: freshwater ecotoxicity, ECO-M: marine ecotoxicity, EUT-F: freshwater eutrophication, EUT-M: marine eutrophication, HT-C: human carcinogenic toxicity, IR: ionizing radiation, SCA-F: fossil resource scarcity, WC: water consumption.

Scenario S3 performed better than the other scenarios in 6 of the 8 impact categories (reduction in negative impacts of 77.46 and 46.09% in relation to S1 and S2, respectively). This was mainly because the water scrubbing process for the biogas upgrading step was excluded in S3 and replaced by the photosynthetic upgrading, which resulted in no water consumption in this phase. Also, no emission of digestate to the soil occurred as the digestate was considered a biofertilizer. In addition, strategies such as adopting a higher methane yield in the AD step and heat recovery provided S3 with better performance than the other scenarios.

These results indicate that biogas upgrading in the HRAP may be a promising strategy to reduce the environmental impact of biogas production while generating two products:

upgraded biogas and improved biomass production (Meier et al., 2015). However, a deeper analysis of the process within the LCA objective is needed, as some simplifications were assumed. For example, possible CH_4 losses during the process should be accounted for, and the need for an absorption column prior to the HRAP, replacing the carbonation column, should be better evaluated (Rodero et al., 2018), in addition to any energy or input requirement. Porcelli et al. (2020) conducted a comparative LCA of microalgae cultivation for non-energy purposes using residual CO_2 and synthetic CO_2 as carbon dioxide sources. Results support the statement that using residual CO_2 instead of synthetic CO_2 offers significant environmental benefits, especially regarding reduced GHG emissions. This improvement can be attributed to the elimination of the synthetic CO_2 production process and slightly higher productivity in the cultivation phase.

Fig. 5 and 6 shows the contribution of each input, emission, and avoided product in the impact categories for scenarios S2 and S3. In scenarios S2 and S3, there was a reduction of approximately 69.95% in electricity consumption for digester heating compared to S1. The improvement in methane yield resulted in reduced digester volume and a higher methane production rate to reach the FU of 1 MJ, resulting in a considerable reduction in environmental impacts.

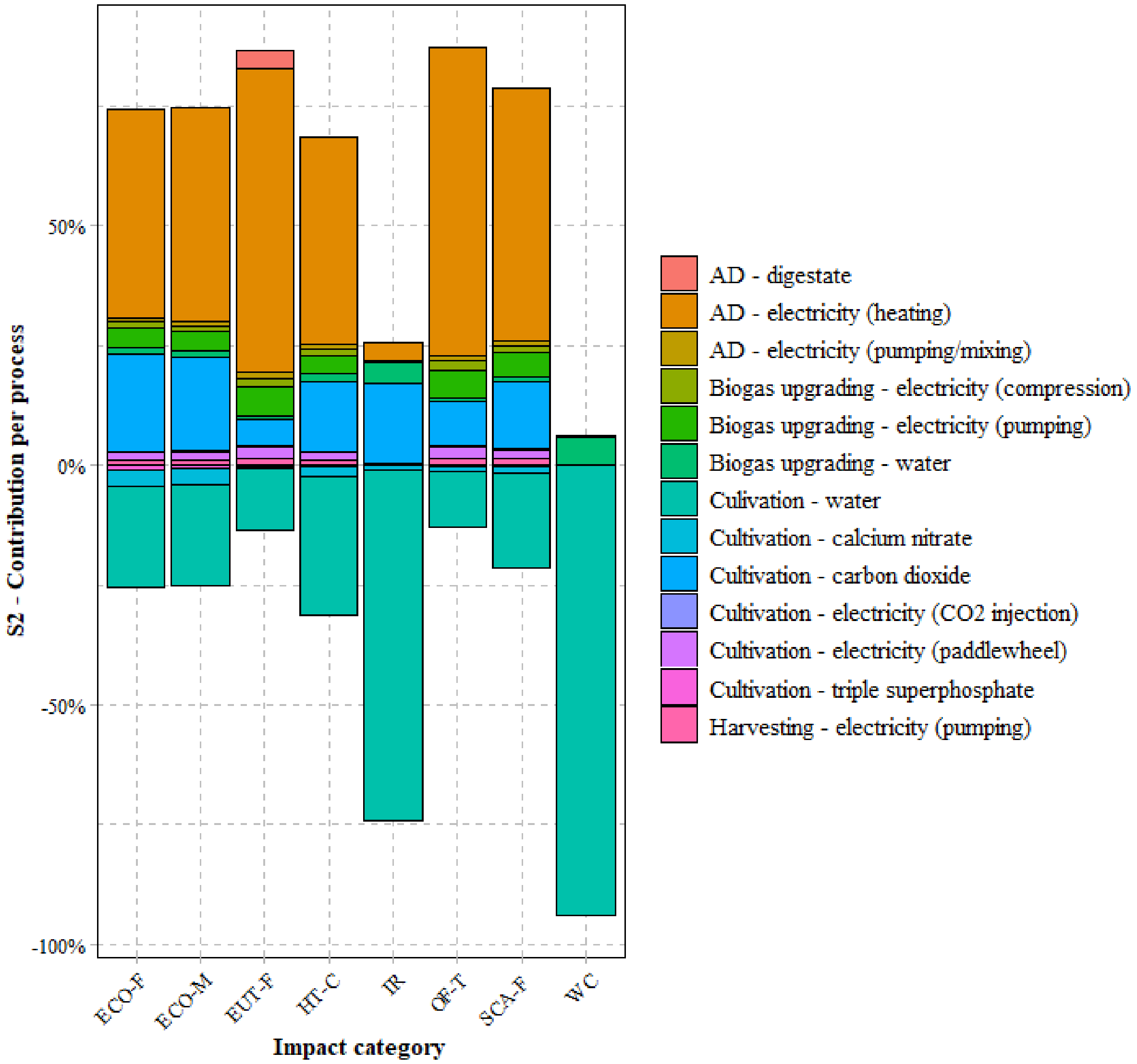

Fig. 6. Percentage contribution of the processes modeled in the life cycle of scenario S2 in the eight impact categories in which the environment was most affected. ECO-F: freshwater ecotoxicity, ECO-M: marine ecotoxicity, EUT-F: freshwater eutrophication, EUT-M: marine eutrophication, HT-C: human carcinogenic toxicity, IR: ionizing radiation, SCA-F: fossil resource scarcity, WC: water consumption.

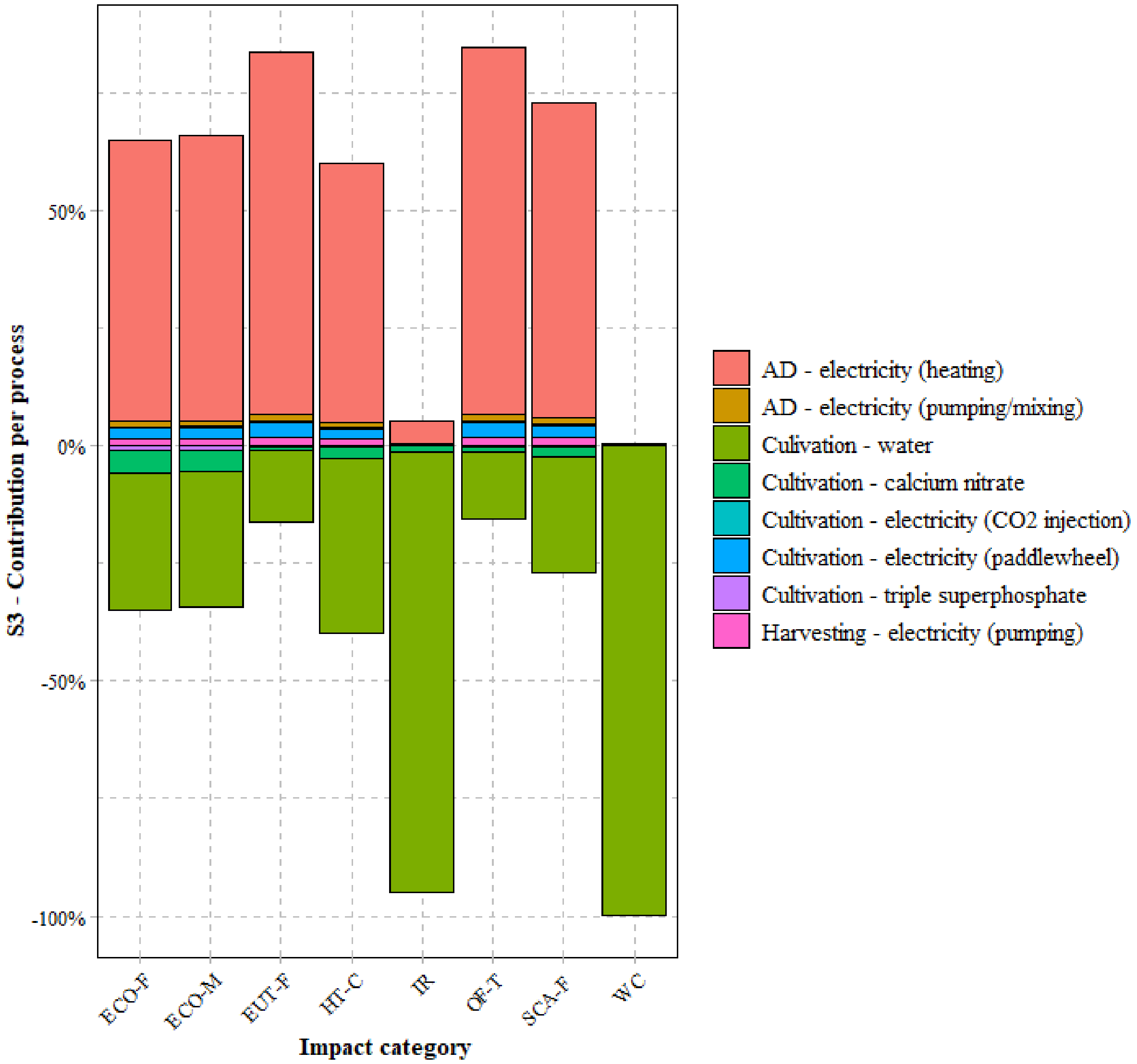

Fig. 6. Percentage contribution of the processes modeled in the life cycle of scenario S3 in the eight impact categories in which the environment was most affected. ECO-F: freshwater ecotoxicity, ECO-M: marine ecotoxicity, EUT-F: freshwater eutrophication, EUT-M: marine eutrophication, HT-C: human carcinogenic toxicity, IR: ionizing radiation, SCA-F: fossil resource scarcity, WC: water consumption.

S1 had greater positive impacts compared to S2 and S3. This was due to the greater consumption of water, triple superphosphate, and calcium nitrate in S1. This positively contributed to the greater quantities of avoided products, reducing the total environmental impact. The amounts of water, triple superphosphate, and calcium nitrate in S1 were 2.30 times greater than in scenarios S2 and S3, resulting in greater potential positive environmental impacts. By adopting a higher methane yield in scenarios S2 and S3, the calculated HRAP and digester volumes required to obtain 1 MJ of biogas were lower than those in scenario S1, resulting in lower amounts of digestate and avoided products.

These results are consistent with the findings of Passos and Ferrer (2014), who evaluated the influence of thermal pretreatment on algal biomass in an AD system for algal biomass cultivated in domestic sewage. The results showed that preheating at low temperatures (75-95 °C) increased methane yield by 70% and contributed to a positive energy balance, with an average energy gain of 2.7 GJ d^{-1}. Solé-Bundó et al. (2018) also observed an increase in methane yield and a positive energy balance in AD through the adoption of codigestion of primary sludge with pretreated algal biomass. An average of 339 mL_{CH4} g_{SV}^{-1} was observed when 75% primary sludge was added to the pre-treated algal biomass, and an energy ratio of 3.5-4 was reported when the digester was operated with a 20-day HRT.

3.4 Sensitivity analysis

The electricity consumption for heating the anaerobic digester was the input that most contributed to the potential negative environmental impacts in scenarios S2 and S3. Therefore, as proposed in section 2.1.5, some parameters used to calculate the demand for digester heating during the AD step (Eq. (3)) were varied by ± 10% in scenarios S2 and S3.

Table 3 shows the variation in potential environmental impacts of the biogas life cycle resulting from the sensitivity analysis. 5 out of 8 categories were more sensitive to methane yield variations than the other parameters in scenarios S2 and S3. The most affected category was IR, which may increase or decrease environmental impacts in this category by up to 11.28% and 9.22%, respectively. These results indicated that slight variations in the methane yield parameter can significantly impact the environmental impact assessment of the biogas life cycle.

Table 3 - Variation (%) in potential environmental impacts of the biogas life cycle resulting from the sensitivity analysis for Scenarios 2 and 3.

	Methane yield				**Pretreatment temperature**				**Heat exchanger efficiency**			
	-10%		**10%**		**-10%**		**10%**		**-10%**		**10%**	
Impact category	**S2**	**S3**	**S2**	**S3**	**S2**	**S3**	**S2**	**S3**	**S2**	**S3**	**S2**	**S3**
Ionizing radiation	12.47%	11.28%	-10.17%	-9.22%	0.09%	0.06%	-0.08%	-0.06%	-0.25%	-0.18%	0.25%	0.18%
Ozone formation, Terrestrial ecosystems	7.05%	7.46%	-5.92%	-6.30%	-0.95%	-1.24%	0.95%	1.24%	2.86%	3.74%	-2.86%	-3.74%
Freshwater eutrophication	7.00%	7.41%	-5.88%	-6.26%	-0.96%	-1.25%	0.96%	1.25%	2.89%	3.79%	-2.89%	-3.79%
Freshwater ecotoxicity	6.71%	4.60%	-5.66%	-4.11%	-0.98%	-2.21%	0.98%	2.20%	2.96%	6.66%	-2.96%	-6.66%
Marine ecotoxicity	6.69%	4.91%	-5.64%	-4.34%	-0.99%	-2.10%	0.99%	2.10%	2.99%	6.35%	-2.99%	-6.35%
Human carcinogenic toxicity	5.22%	2.16%	-4.49%	-2.25%	-1.28%	-3.03%	1.28%	3.03%	3.88%	9.16%	-3.88%	-9.16%
Fossil resource scarcity	6.68%	6.37%	-5.63%	-5.47%	-1.01%	-1.61%	1.01%	1.61%	3.04%	4.85%	-3.04%	-4.85%
Water consumption	11.83%	11.12%	-9.68%	-9.10%	0.00%	0.00%	0.00%	0.00%	-0.01%	-0.01%	0.01%	0.01%

3.5 Damage assessment

In Scenario S3 and natural gas, human health damage was higher (7.02mPt and 0.28 mPt, respectively), while ecosystems damage was lower (-0.94 mPt and 0.01 mPt, respectively). Ecosystems and resources damage in S3 exhibited negative values, indicating benefits. Simulating natural gas production through the Ecoinvent process "Natural gas, high pressure {RoW}| natural gas production | APOS, S," (Weidema,2013), S3 had about 6 times less impact on resources compared to natural gas (-0.01 mPt and 0.06 mPt, respectively). However, the human health category had an impact 24 times greater in S3 than in natural gas. Analysis of the emissions in the midpoint categories classified as harmful to human health (as shown in Table S5) revealed that FPMF, GW, HT-NC, and HT-C were the most significant contributors to human health damage. The HT-C midpoint category accounted for 3.86% of the total impacts, primarily due to electricity demand for digester heating, while FPMF, GW, and HT-NC categories accounted for 39.72, 18.99 and 7.29%, respectively. Ecosystem benefits were mainly due to the water consumption category, accounting for 63.10% of total impacts.

In Alyaseri and Zhou (2017) LCA study on wastewater treatment and sludge incineration, the highest damage was also observed in the human health damage category, while ecosystems had the lowest damage. Human health impacts were linked to electricity, emissions, and natural gas. Marangon et al. (2021) also found similar results in their study on bio-oil production through hydrothermal liquefaction, with human health being most affected and ecosystems least affected. In the present research, fine particulate matter emissions played a significant role in human health damage. Thus, considering that the electricity demand for digester heating most contributed to fine particulate matter formation, global warming, and human non-carcinogenic, careful studies on this process are vital to reduce human health impacts and enhance the environmental performance of the biogas life cycle.

4 CONCLUSIONS

Based on the findings, key factors related to the life cycle of biogas production, and its associated environmental impacts have been identified. From 18 impact categories, 8 were highlighted as the most significant (in order of importance): human carcinogenic toxicity, water consumption, freshwater eutrophication, freshwater ecotoxicity, marine ecotoxicity, fossil resource scarcity, ozone formation – terrestrial ecosystem, and ionizing radiation. Adverse impacts were predominant in most categories, except for water consumption and ionizing radiation, where beneficial impacts outweighed the detrimental ones. This was primarily due to the utilization of materials and resources that were avoided in the cultivation step.

In the baseline scenario (S1), the AD step emerged as the primary source of negative impacts, with an average of 60.73% of the total impact associated with the energy demand for the digester heating. The carbon dioxide used in the microalgae cultivation and the water needed in the biogas upgrading stage were other inputs with major impacts on ionizing radiation and water consumption categories, respectively. In contrast, the use of domestic sewage in microalgae cultivation for biogas production was recognized as a more sustainable and environmentally friendly alternative, effectively reducing the environmental footprint associated with potable water consumption.

Improved scenarios showed that in scenarios S2 and S3, there was a reduction of approximately 69.95% in electricity consumption for digester heating compared to S1. Additionally, scenario S3 performed better than the other scenarios in 6 of the 8 impact categories. Sensitivity analysis highlighted that a 10% increase in methane yield could provide an 11.28% reduction in ionizing radiation impacts. Therefore, strategies to improve methane yield from microalgae AD, such as codigestion and thermal pretreatment, should be applied. Also, biogas upgrading in the HRAP and digestate valorization as biofertilizers can contribute to a reduction in environmental impacts and improve the environmental and energy performance of the biogas production system.

In conclusion, this study's LCA comprised the modeling of improvement scenarios and sensitivity analysis, being able to identify critical strategies that, when applied in future studies, could help to improve the environmental performance of the process. Another important aspect is regarding the use of wastewater for biomass cultivation in the LCA analysis, allowing us to calculate the beneficial effects of such practice beyond the production of renewable energy. These findings can guide decision-making and the implementation of more sustainable practices in the production of biogas from algal biomass.

CREDIT AUTHOR STATEMENT

V. Santurbano: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review and Editing, Visualization; B. Marangon: Software, Writing – Review and Editing, Visualization; J. Castro:

Software, Writing – Review and Editing, Visualization; M. L. Calijuri: Resources, Writing – Review and Editing, Visualization, Funding Acquisition; M. Leme: Conceptualization, Methodology, Software, Resources, Writing – Review and Editing, Visualization, Supervision; P. Assemany: Conceptualization, Resources, Writing – Review and Editing, Visualization, Supervision, Project Administration, Funding Acquisition.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLMENTARY MATERIALS

Enhancing environmental performance in biogas production from wastewater-cultivated microalgae: a life cycle assessment perspective

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Process systems from the ecoinvent library v3.9.1 (Table S5), used to model the inputs (heat, electricity, chemical inputs), which are the “known input from the technosphere”.

For the choice of the ecoinvent v3.9.1 process system, rest of the world (code {RoW}) was prioritized for modeling. This term is used to categorize or represent data that applies to the entire world or doesn't pertain to a specific geographical region The electricity production volume represents the total amount of electricity from coal used globally for aluminium production (electrolysis and ingot castin

Table S1. Parameters considered for obtaining the life cycle inventory of biogas production in scenarios S1, S2, and S3.

Parameters	Unit	Base scenario average value	S2 and S3 scenario	Reference
Cultivation				
HRT	days	6	6	Assis et al 2017; Assis et al 2019; de Godos et al 2016; Park et al 2013
Biomass productivity	g_{VS} m^{-2} d^{-1}	9.94	9.94	Assis et al 2017; Assis et al 2019; de Godos et al 2016; Park et al 2013
CO_2 consumption	L min^{-1}	5.6	5.6	Assis et al 2017; Assis et al 2019; Pousadas et al 2015; Couto et al 2021; de Godos et al 2016
CO_2 specific mass	kg m^{-3}	1.83	1.83	
Harvesting				
Recovery efficiency	%	85.64	85.64	Ferreira et al 2020; Gutiérrez et al 2016; Gutiérrez et al 2016b
Humidity	%	93.26	93.26	Ferreira et al 2020
Anaerobic digestion				
HRT	Dias	22	22	Solé-Bundó et al 2018; Carrillo-Reyes et al 2021; Passos et al 2014; Passos et al 2014b; Arashiro et al 2019
Organic loading rate	g_{VS} L^{-1} d^{-1}	0.86	0.86	Solé-Bundó et al 2018; Carrillo-Reyes et al 2021; Passos et al 2014; Passos et al 2014b
Methane production rate	L_{CH4} L^{-1} d^{-1}	0.12	0.53	Solé-Bundó et al 2018;; Passos et al 2014; Passos et al 2014b
Methane yield	L_{CH4} $g^{-1}{}_{VS}$	0.2	0.46	Solé-Bundó et al 2018; Arashiro et al 2019; Carrillo-Reyes et al 2021; Passos et al 2014; Passos et al 2014b

Methane lower heating value	MJ m^{-3}_{CH4}	22.4	22.4	Metcalf & Eddy 2014
Methane content in biogas	% CH_4	65	65	Metcalf & Eddy 2014
CH_4 specific mass	kg m^{-3}	0.72	0.72	
Biogas upgrading				
Methane lower heating value	MJ m^{-3}_{CH4}	34.37	34.37	Sun et al 2019
Water flow rate	m^3_{water} Nm^{-3}_{biogas}	0.15	0.15	Muñoz et al 2015
Methane content in upgraded biogas	% CH_4	96	96	Sun et al 2019
CH_4 loss	% CH_4	2	2	Muñoz et al 2015

Table S2. Parameters considered for calculating the electricity and heat consumption in the anaerobic digestion stage of algal biomass in the scenarios.

Parameter	**Unit**	**Base scenario value**	**S2 and S3 value**	**Reference**
Density of water (ρ)	kg m^{-3}	1000	1000	Metcalf e Eddy, 2003
Flow rate (Q)	m^3 d^{-1}	0.0004	0.0002	Calculated
Specific heat of water (γ)	kJ kg^{-1} $°C^{-1}$	4.18	4.18	Metcalf e Eddy, 2003
Anaerobic digestion temperature (T_d)	°C	35	35	This study
Ambient temperature (T_a)	°C	25	25	This study
Pretreatment temperature (T_p)	°C	-	75	Solé-Bundó et al 2018
Heat transfer coefficient (k)	W m^{-2} C^{-1}	1	1.00	Metcalf e Eddy, 2003
Surface area of the reactor wall (A)	m^2	0.12	0.07	Calculated

Useful volume (V)	m^3	0.008	0.004	Calculated
Heat recovery by heat exchanger (ϕ)	%	-	85	Passos e Ferrer, 2014
Electricity consumption for pumping (θ)	kJ m^{-3}	1800	1800	Passos e Ferrer, 2014
Electricity consumption rate for mixing (ω)	kJ m^{-3} d^{-1}	300	300	Passos e Ferrer, 2014
Lower heating value of methane (ξ)	kJ m^{-3}	34370	34370	Metcalf e Eddy, 2007
Methane production rate (P_{CH4})	m^3_{CH4} $m^{-3}_{digester}$ d^{-1}	0.12	0.53	Solé-Bundó et al 2018; Passos et al 2014; Passos et al 2014b
Energy conversion efficiency (η)	%	90	90	Passos e Ferrer, 2014

Table S3. Life Cycle Impact Assessment results of characterization for scenarios S1, S2, and S3, associated to the production of 1 MJ of biogas.

S1																
Impact Category	Unit	Cultivation						Harvesting	Anaerobic Digestion			Biogas Upgrading				Total
		Carbon dioxide	Electricity (Paddlewheel)	Electricity (CO2 injection)	Calcium Nitrate	Triple Superphosphate	Water	Electricity (Pumping)	Electricity (Pumping/ Mixing)	Electricity (Heating)	Digestate	Electricity (Compression)	Electricity (Pumping)	Water	Biogas Losses (CH4)	
Global warming	kg CO2 eq	0,35271549	3,86E-02	5,36E-03	-5,30E-02	-2,22E-03	-3,14E-01	2,53E-02	2,04E-02	7,67E-01	0,00E+00	1,26E-02	4,20E-02	8,16E-03	1,64E-02	0,918935057
Stratospheric ozone depletion	kg CFC 11 eq	7,47302E-08	6,67E-09	9,26E-10	-8,29E-07	-9,57E-10	-1,46E-07	4,36E-09	3,52E-09	1,33E-07	0,00E+00	2,18E-09	7,26E-09	3,80E-09	0,00E+00	-7,40724E-07

Ionizing radiation	kBq Co-60 eq	0,012709984	1,05E-04	1,46E-05	-6,86E-04	-8,04E-05	-5,55E-02	6,87E-05	5,54E-05	2,09E-03	0,00E+00	3,43E-05	1,14E-04	1,44E-03	0,00E+00	-0,039674316
Ozone formation, Human health	kg NOx eq	0,000447278	1,19E-04	1,66E-05	-5,02E-05	-7,32E-06	-5,77E-04	7,80E-05	6,30E-05	2,37E-03	0,00E+00	3,89E-05	1,30E-04	1,50E-05	0,00E+00	0,00264328
Fine particulate matter formation	kg PM2.5 eq	0,000408573	1,33E-04	1,84E-05	-4,22E-05	-9,37E-06	-7,32E-04	8,67E-05	7,00E-05	2,63E-03	0,00E+00	4,33E-05	1,44E-04	1,90E-05	0,00E+00	0,002772656
Ozone formation, Terrestrial ecosystems	kg NOx eq	0,000454434	1,19E-04	1,66E-05	-5,14E-05	-7,45E-06	-5,85E-04	7,82E-05	6,31E-05	2,37E-03	0,00E+00	3,90E-05	1,30E-04	1,52E-05	0,00E+00	0,002645719
Terrestrial acidification	kg SO2 eq	0,000769328	2,72E-04	3,78E-05	-1,51E-04	-2,56E-05	-1,08E-03	1,78E-04	1,44E-04	5,40E-03	0,00E+00	8,88E-05	2,96E-04	2,80E-05	0,00E+00	0,005961779
Freshwater eutrophication	kg P eq	9,23246E-05	4,04E-05	5,61E-06	-8,80E-06	-3,31E-06	-2,19E-04	2,64E-05	2,13E-05	8,02E-04	5,94E-05	1,32E-05	4,40E-05	5,68E-06	0,00E+00	0,00087984
Marine eutrophication	kg N eq	1,8883E-05	1,85E-06	2,57E-07	-4,25E-07	-7,60E-08	-1,45E-05	1,21E-06	9,79E-07	3,68E-05	3,14E-04	6,05E-07	2,02E-06	3,76E-07	0,00E+00	0,000362279
Terrestrial	kg 1,4-	2,5586465	2,38E-02	3,30E-03	-2,36E-01	-6,96E-02	-1,06E+00	1,55E-02	1,25E-02	4,72E-01	0,00E+00	7,76E-03	2,59E-02	2,74E-02	0,00E+00	1,78532108

ecotoxicity	DCB															
Freshwater ecotoxicity	kg 1,4-DCB	0,015115841	1,19E-03	1,66E-04	-2,52E-03	-5,93E-04	-1,58E-02	7,80E-04	6,29E-04	2,37E-02	0,00E+00	3,89E-04	1,30E-03	4,10E-04	0,00E+00	0,024737226
Marine ecotoxicity	kg 1,4-DCB	0,019426119	1,65E-03	2,29E-04	-3,29E-03	-7,81E-04	-2,10E-02	1,08E-03	8,70E-04	3,27E-02	0,00E+00	5,38E-04	1,79E-03	5,45E-04	0,00E+00	0,033786333
Human carcinogenic toxicity	kg 1,4-DCB	0,015408967	1,72E-03	2,40E-04	-2,26E-03	-1,71E-04	-3,12E-02	1,13E-03	9,11E-04	3,43E-02	0,00E+00	5,63E-04	1,88E-03	8,11E-04	0,00E+00	0,023246122
Human non-carcinogenic toxicity	kg 1,4-DCB	0,28896761	6,53E-02	9,07E-03	-4,29E-02	-1,14E-02	-3,90E-01	4,27E-02	3,45E-02	1,30E+00	0,00E+00	2,13E-02	7,11E-02	1,01E-02	0,00E+00	1,3964324
Land use	m2a crop eq	0,005579054	3,99E-04	5,54E-05	-7,13E-04	-7,16E-04	-7,39E-03	2,61E-04	2,11E-04	7,92E-03	0,00E+00	1,30E-04	4,34E-04	1,92E-04	0,00E+00	0,006362188
Mineral resource scarcity	kg Cu eq	0,001074093	8,99E-06	1,25E-06	-2,12E-04	-1,50E-04	-1,78E-03	5,88E-06	4,75E-06	1,79E-04	0,00E+00	2,94E-06	9,78E-06	4,61E-05	0,00E+00	-0,000805131
Fossil resource scarcity	kg oil eq	0,06060567	8,53E-03	1,18E-03	-7,14E-03	-6,67E-04	-8,48E-02	5,58E-03	4,50E-03	1,69E-01	0,00E+00	2,78E-03	9,28E-03	2,20E-03	0,00E+00	0,171448942
Water consumption	m3	0,001800217	7,44E-05	1,03E-05	-4,67E-04	-1,32E-04	-9,36E-01	4,87E-05	3,93E-05	1,48E-03	0,00E+00	2,43E-05	8,10E-05	2,43E-02	0,00E+00	-0,908968624

S2

Impact Category	Unit		Cultivation					Harvesting	Anaerobic Digestion			Biogas Upgrading				Total
		Carbon dioxide	Electricity (Paddlewheel)	Electricity (CO2 injection)	Calcium Nitrate	Triple Superphosphate	Water	Electricity (Pumping)	Electricity (Pumping/ Mixing)	Electricity (Heating)	Digestate	Electricity (Compression)	Electricity (Pumping)	Water	Biogas Losses (CH4)	
Global warming	kg CO2 eq	0,15336277	1,68E-02	2,33E-03	-2,31E-02	-9,65E-04	-1,37E-01	1,10E-02	8,87E-03	4,53E-01	0,00E+00	1,26E-02	4,20E-02	8,16E-03	1,64E-02	0,563375958
Stratospheric ozone depletion	kg CFC11 eq	3,24931E-08	2,90E-09	4,03E-10	-3,61E-07	-4,16E-10	-6,36E-08	1,90E-09	1,53E-09	7,82E-08	0,00E+00	2,18E-09	7,26E-09	3,80E-09	0,00E+00	-2,94003E-07
Ionizing radiation	kBq Co-60 eq	0,005526376	4,56E-05	6,34E-06	-2,98E-04	-3,49E-05	-2,41E-02	2,99E-05	2,41E-05	1,23E-03	0,00E+00	3,43E-05	1,14E-04	1,44E-03	0,00E+00	-0,016026573
Ozone formation, Human health	kg NOx eq	0,000194479	5,19E-05	7,20E-06	-2,18E-05	-3,18E-06	-2,51E-04	3,39E-05	2,74E-05	1,40E-03	0,00E+00	3,89E-05	1,30E-04	1,50E-05	0,00E+00	0,001620978
Fine particulate matter formation	kg PM2.5 eq	0,00017765	5,76E-05	8,01E-06	-1,83E-05	-4,07E-06	-3,18E-04	3,77E-05	3,04E-05	1,55E-03	0,00E+00	4,33E-05	1,44E-04	1,90E-05	0,00E+00	0,001731126
Ozone formation, Terrestrial	kg NOx eq	0,000197591	5,20E-05	7,22E-06	-2,24E-05	-3,24E-06	-2,55E-04	3,40E-05	2,74E-05	1,40E-03	0,00E+00	3,90E-05	1,30E-04	1,52E-05	0,00E+00	0,001622998

ecosystems																
Terrestrial acidification	kg SO2 eq	0,000334508	1,18E-04	1,64E-05	-6,56E-05	-1,11E-05	-4,68E-04	7,73E-05	6,24E-05	3,19E-03	0,00E+00	8,88E-05	2,96E-04	2,80E-05	0,00E+00	0,003664034
Freshwater eutrophication	kg P eq	4,01433E-05	1,76E-05	2,44E-06	-3,82E-06	-1,44E-06	-9,51E-05	1,15E-05	9,27E-06	4,73E-04	2,58E-05	1,32E-05	4,40E-05	5,68E-06	0,00E+00	0,000542594
Marine eutrophication	kg N eq	8,21046E-06	8,06E-07	1,12E-07	-1,85E-07	-3,30E-08	-6,30E-06	5,27E-07	4,25E-07	2,17E-05	1,37E-04	6,05E-07	2,02E-06	3,76E-07	0,00E+00	0,000164919
Terrestrial ecotoxicity	kg 1,4-DCB	1,1125145	1,03E-02	1,43E-03	-1,03E-01	-3,03E-02	-4,59E-01	6,76E-03	5,46E-03	2,79E-01	0,00E+00	7,76E-03	2,59E-02	2,74E-02	0,00E+00	0,884068257
Freshwater ecotoxicity	kg 1,4-DCB	0,006572457	5,18E-04	7,20E-05	-1,10E-03	-2,58E-04	-6,87E-03	3,39E-04	2,74E-04	1,40E-02	0,00E+00	3,89E-04	1,30E-03	4,10E-04	0,00E+00	0,015616301
Marine ecotoxicity	kg 1,4-DCB	0,008446591	7,16E-04	9,95E-05	-1,43E-03	-3,40E-04	-9,13E-03	4,69E-04	3,78E-04	1,93E-02	0,00E+00	5,38E-04	1,79E-03	5,45E-04	0,00E+00	0,021396263
Human carcinogenic toxicity	kg 1,4-DCB	0,00669991	7,50E-04	1,04E-04	-9,84E-04	-7,42E-05	-1,36E-02	4,91E-04	3,96E-04	2,02E-02	0,00E+00	5,63E-04	1,88E-03	8,11E-04	0,00E+00	0,017263826
Human non-carcinoge	kg 1,4-DCB	0,12564482	2,84E-02	3,95E-03	-1,87E-02	-4,96E-03	-1,70E-01	1,86E-02	1,50E-02	7,66E-01	0,00E+00	2,13E-02	7,11E-02	1,01E-02	0,00E+00	0,866624243

nic toxicity																
Land use	m2a crop eq	0,002425805	1,73E-04	2,41E-05	-3,10E-04	-3,11E-04	-3,21E-03	1,13E-04	9,15E-05	4,67E-03	0,00E+00	1,30E-04	4,34E-04	1,92E-04	0,00E+00	0,004423415
Mineral resource scarcity	kg Cu eq	0,000467022	3,91E-06	5,43E-07	-9,20E-05	-6,52E-05	-7,72E-04	2,56E-06	2,06E-06	1,05E-04	0,00E+00	2,94E-06	9,78E-06	4,61E-05	0,00E+00	-0,000289046
Fossil resource scarcity	kg oil eq	0,026351702	3,71E-03	5,15E-04	-3,11E-03	-2,90E-04	-3,69E-02	2,43E-03	1,96E-03	1,00E-01	0,00E+00	2,78E-03	9,28E-03	2,20E-03	0,00E+00	0,108909312
Water consumption	m3	0,000782745	3,24E-05	4,49E-06	-2,03E-04	-5,74E-05	-4,07E-01	2,12E-05	1,71E-05	8,72E-04	0,00E+00	2,43E-05	8,10E-05	2,43E-02	0,00E+00	-0,381171648

S3												
Impact Category	Unit		Cultivation					Harvesting	Anaerobic Digestion			Total
		Carbon dioxide	Electricity (Paddlewheel)	Electricity (CO2 injection)	Calcium Nitrate	Triple Superphosphate	Water	Electricity (Pumping)	Electricity (Pumping/Mixing)	Electricity (Heating)	Digestate	
Global warming	kg CO2 eq	0	1,68E-02	2,33E-03	-2,31E-02	-9,65E-04	-1,37E-01	1,10E-02	8,87E-03	4,53E-01	0,00E+00	3,31E-01
Stratospheric ozone depletion	kg CFC11 eq	0	2,90E-09	4,03E-10	-3,61E-07	-4,16E-10	-6,36E-08	1,90E-09	1,53E-09	7,82E-08	0,00E+00	-3,40E-07

Ionizing radiation	kBq Co-60 eq	0	4,56E-05	6,34E-06	-2,98E-04	-3,49E-05	-2,41E-02	2,99E-05	2,41E-05	1,23E-03	0,00E+00	-2,31E-02
Ozone formation, Human health	kg NOx eq	0	5,19E-05	7,20E-06	-2,18E-05	-3,18E-06	-2,51E-04	3,39E-05	2,74E-05	1,40E-03	0,00E+00	1,24E-03
Fine particulate matter formation	kg PM2.5 eq	0	5,76E-05	8,01E-06	-1,83E-05	-4,07E-06	-3,18E-04	3,77E-05	3,04E-05	1,55E-03	0,00E+00	1,35E-03
Ozone formation, Terrestrial ecosystems	kg NOx eq	0	5,20E-05	7,22E-06	-2,24E-05	-3,24E-06	-2,55E-04	3,40E-05	2,74E-05	1,40E-03	0,00E+00	1,24E-03
Terrestrial acidification	kg SO2 eq	0	1,18E-04	1,64E-05	-6,56E-05	-1,11E-05	-4,68E-04	7,73E-05	6,24E-05	3,19E-03	0,00E+00	2,92E-03
Freshwater eutrophication	kg P eq	0	1,76E-05	2,44E-06	-3,82E-06	-1,44E-06	-9,51E-05	1,15E-05	9,27E-06	4,73E-04	0,00E+00	4,14E-04
Marine eutrophication	kg N eq	0	8,06E-07	1,12E-07	-1,85E-07	-3,30E-08	-6,30E-06	5,27E-07	4,25E-07	2,17E-05	0,00E+00	1,71E-05
Terrestrial ecotoxicity	kg 1,4-DCB	0	1,03E-02	1,43E-03	-1,03E-01	-3,03E-02	-4,59E-01	6,76E-03	5,46E-03	2,79E-01	0,00E+00	-2,89E-01
Freshwater ecotoxicity	kg 1,4-DCB	0	5,18E-04	7,20E-05	-1,10E-03	-2,58E-04	-6,87E-03	3,39E-04	2,74E-04	1,40E-02	0,00E+00	6,95E-03
Marine ecotoxicity	kg 1,4-DCB	0	7,16E-04	9,95E-05	-1,43E-03	-3,40E-04	-9,13E-03	4,69E-04	3,78E-04	1,93E-02	0,00E+00	1,01E-02

Human carcinogenic toxicity	kg 1,4-DCB	0	7,50E-04	1,04E-04	-9,84E-04	-7,42E-05	-1,36E-02	4,91E-04	3,96E-04	2,02E-02	0,00E+00	7,31E-03
Human non-carcinogenic toxicity	kg 1,4-DCB	0	2,84E-02	3,95E-03	-1,87E-02	-4,96E-03	-1,70E-01	1,86E-02	1,50E-02	7,66E-01	0,00E+00	6,38E-01
Land use	m2a crop eq	0	1,73E-04	2,41E-05	-3,10E-04	-3,11E-04	-3,21E-03	1,13E-04	9,15E-05	4,67E-03	0,00E+00	1,24E-03
Mineral resource scarcity	kg Cu eq	0	3,91E-06	5,43E-07	-9,20E-05	-6,52E-05	-7,72E-04	2,56E-06	2,06E-06	1,05E-04	0,00E+00	-8,15E-04
Fossil resource scarcity	kg oil eq	0	3,71E-03	5,15E-04	-3,11E-03	-2,90E-04	-3,69E-02	2,43E-03	1,96E-03	1,00E-01	0,00E+00	6,83E-02
Water consumption	m3	0	3,24E-05	4,49E-06	-2,03E-04	-5,74E-05	-4,07E-01	2,12E-05	1,71E-05	8,72E-04	0,00E+00	-4,06E-01

Table S4. Life Cycle Impact Assessment results of normalization for scenarios S1. S2. and S3. associated to the production of 1 MJ of biogas.

S1															
Impact Category	Total	Cultivation						Harvesting	Anaerobic Digestion			Biogas Upgrading			
		Carbon dioxide	Electricity (Paddle wheel)	Electricity (CO2 injection)	Calcium Nitrate	Triple Superphosphate	Water	Electricity (Pumping)	Electricity (Pumping/ Mixing)	Electricity (Heating)	Digestate (Methane)	Electricity (Compression)	Electricity (Pumping)	Water	Biogas Losses (Methane)
Global warming	0,000114867	4,40894E-05	4,83E-06	6,70E-07	-6,63E-06	-2,78E-07	-3,93E-05	3,16E-06	2,55E-06	9,59E-05	0,00E+00	1,58E-06	5,25E-06	1,02E-06	2,04E-06

Stratospheric ozone depletion	-1,23701E-05	1,24799E-06	1,11E-07	1,55E-08	-1,39E-05	-1,60E-08	-2,44E-06	7,29E-08	5,88E-08	2,21E-06	0,00E+00	3,64E-08	1,21E-07	6,34E-08	0,00E+00
Ionizing radiation	-8,25226E-05	2,64368E-05	2,18E-07	3,03E-08	-1,43E-06	-1,67E-07	-1,16E-04	1,43E-07	1,15E-07	4,34E-06	0,00E+00	7,13E-08	2,38E-07	3,00E-06	0,00E+00
Ozone formation, Human health	0,000128463	2,17377E-05	5,80E-06	8,05E-07	-2,44E-06	-3,56E-07	-2,80E-05	3,79E-06	3,06E-06	1,15E-04	0,00E+00	1,89E-06	6,31E-06	7,28E-07	0,00E+00
Fine particulate matter formation	0,000108411	1,59752E-05	5,18E-06	7,20E-07	-1,65E-06	-3,66E-07	-2,86E-05	3,39E-06	2,74E-06	1,03E-04	0,00E+00	1,69E-06	5,64E-06	7,44E-07	0,00E+00
Ozone formation, Terrestrial ecosystems	0,000148954	2,55846E-05	6,73E-06	9,34E-07	-2,90E-06	-4,19E-07	-3,30E-05	4,40E-06	3,55E-06	1,34E-04	0,00E+00	2,20E-06	7,32E-06	8,56E-07	0,00E+00
Terrestrial acidification	0,000145467	1,87716E-05	6,63E-06	9,22E-07	-3,68E-06	-6,24E-07	-2,63E-05	4,34E-06	3,50E-06	1,32E-04	0,00E+00	2,17E-06	7,22E-06	6,83E-07	0,00E+00
Freshwater eutrophication	0,001354953	0,00014218	6,22E-05	8,64E-06	-1,35E-05	-5,10E-06	-3,37E-04	4,07E-05	3,28E-05	1,24E-03	9,14E-05	2,03E-05	6,77E-05	8,74E-06	0,00E+00
Marine eutrophication	7,86146E-05	4,09762E-06	4,02E-07	5,59E-08	-9,23E-08	-1,65E-08	-3,14E-06	2,63E-07	2,12E-07	7,99E-06	6,82E-05	1,31E-07	4,38E-07	8,16E-08	0,00E+00

Terrestrial ecotoxicity	0,000117474	0,000168359	1,56E-06	2,17E-07	-1,55E-05	-4,58E-06	-6,95E-05	1,02E-06	8,26E-07	3,11E-05	0,00E+00	5,10E-07	1,70E-06	1,80E-06	0,00E+00
Freshwater ecotoxicity	0,000982068	0,000600099	4,73E-05	6,57E-06	-1,00E-04	-2,35E-05	-6,27E-04	3,10E-05	2,50E-05	9,40E-04	0,00E+00	1,54E-05	5,15E-05	1,63E-05	0,00E+00
Marine ecotoxicity	0,000777086	0,000446801	3,79E-05	5,26E-06	-7,56E-05	-1,80E-05	-4,83E-04	2,48E-05	2,00E-05	7,53E-04	0,00E+00	1,24E-05	4,12E-05	1,25E-05	0,00E+00
Human carcinogenic toxicity	0,002257199	0,001496211	1,67E-04	2,33E-05	-2,20E-04	-1,66E-05	-3,03E-03	1,10E-04	8,84E-05	3,33E-03	0,00E+00	5,47E-05	1,82E-04	7,88E-05	0,00E+00
Human non-carcinogenic toxicity	4,46858E-05	9,24696E-06	2,09E-06	2,90E-07	-1,37E-06	-3,65E-07	-1,25E-05	1,37E-06	1,10E-06	4,15E-05	0,00E+00	6,83E-07	2,28E-06	3,24E-07	0,00E+00
Land use	1,03067E-06	9,03807E-07	6,46E-08	8,97E-09	-1,16E-07	-1,16E-07	-1,20E-06	4,23E-08	3,41E-08	1,28E-06	0,00E+00	2,11E-08	7,03E-08	3,11E-08	0,00E+00
Mineral resource scarcity	-6,70674E-09	8,9472E-09	7,49E-11	1,04E-11	-1,76E-09	-1,25E-09	-1,48E-08	4,90E-11	3,95E-11	1,49E-09	0,00E+00	2,45E-11	8,15E-11	3,84E-10	0,00E+00
Fossil resource scarcity	0,000174878	6,18178E-05	8,70E-06	1,21E-06	-7,28E-06	-6,80E-07	-8,65E-05	5,69E-06	4,59E-06	1,73E-04	0,00E+00	2,84E-06	9,47E-06	2,25E-06	0,00E+00

Water consumption	-0,003408632	6,75081E-06	2,79E-07	3,88E-08	-1,75E-06	-4,95E-07	-3,51E-03	1,83E-07	1,47E-07	5,54E-06	0,00E+00	9,11E-08	3,04E-07	9,12E-05	0,00E+00

S2															
Impact Category	Total							Harvesting	Anaerobic Digestion			Biogas Upgrading			
		Carbon dioxide	Electricity (Paddlewheel)	Electricity (CO2 injection)	Calcium Nitrate	Triple Superphosphate	Water	Electricity (Pumping)	Electricity (Pumping/Mixing)	Electricity (Heating)	Digestate	Electricity (Compression)	Electricity (Pumping)	Water	Biogas Losses (Methane)
Global warming	7,0422E-05	1,91703E-05	2,10E-06	2,91E-07	-2,88E-06	-1,21E-07	-1,71E-05	1,37E-06	1,11E-06	5,66E-05	0,00E+00	1,58E-06	5,25E-06	1,02E-06	2,04E-06
Stratospheric ozone depletion	-4,90985E-06	5,42635E-07	4,84E-08	6,73E-09	-6,02E-06	-6,95E-09	-1,06E-06	3,17E-08	2,56E-08	1,31E-06	0,00E+00	3,64E-08	1,21E-07	6,34E-08	0,00E+00
Ionizing radiation	-3,33353E-05	1,14949E-05	9,49E-08	1,32E-08	-6,21E-07	-7,27E-08	-5,02E-05	6,21E-08	5,01E-08	2,56E-06	0,00E+00	7,13E-08	2,38E-07	3,00E-06	0,00E+00
Ozone formation, Human health	7,87795E-05	9,45169E-06	2,52E-06	3,50E-07	-1,06E-06	-1,55E-07	-1,22E-05	1,65E-06	1,33E-06	6,80E-05	0,00E+00	1,89E-06	6,31E-06	7,28E-07	0,00E+00
Fine particulate matter formation	6,7687E-05	6,94611E-06	2,25E-06	3,13E-07	-7,17E-07	-1,59E-07	-1,25E-05	1,47E-06	1,19E-06	6,08E-05	0,00E+00	1,69E-06	5,64E-06	7,44E-07	0,00E+00

Ozone formation, Terrestrial ecosystems	9,13748E-05	1,11244E-05	2,92E-06	4,06E-07	-1,26E-06	-1,82E-07	-1,43E-05	1,91E-06	1,54E-06	7,89E-05	0,00E+00	2,20E-06	7,32E-06	8,56E-07	0,00E+00
Terrestrial acidification	8,94024E-05	8,162E-06	2,88E-06	4,01E-07	-1,60E-06	-2,71E-07	-1,14E-05	1,89E-06	1,52E-06	7,78E-05	0,00E+00	2,17E-06	7,22E-06	6,83E-07	0,00E+00
Freshwater eutrophication	0,000835594	6,18206E-05	2,70E-05	3,76E-06	-5,89E-06	-2,22E-06	-1,46E-04	1,77E-05	1,43E-05	7,29E-04	3,97E-05	2,03E-05	6,77E-05	8,74E-06	0,00E+00
Marine eutrophication	3,57875E-05	1,78167E-06	1,75E-07	2,43E-08	-4,01E-08	-7,17E-09	-1,37E-06	1,14E-07	9,23E-08	4,71E-06	2,96E-05	1,31E-07	4,38E-07	8,16E-08	0,00E+00
Terrestrial ecotoxicity	5,81717E-05	7,32035E-05	6,80E-07	9,44E-08	-6,75E-06	-1,99E-06	-3,02E-05	4,45E-07	3,59E-07	1,83E-05	0,00E+00	5,10E-07	1,70E-06	1,80E-06	0,00E+00
Freshwater ecotoxicity	0,000619967	0,000260927	2,06E-05	2,86E-06	-4,35E-05	-1,02E-05	-2,73E-04	1,35E-05	1,09E-05	5,55E-04	0,00E+00	1,54E-05	5,15E-05	1,63E-05	0,00E+00
Marine ecotoxicity	0,000492114	0,000194272	1,65E-05	2,29E-06	-3,29E-05	-7,81E-06	-2,10E-04	1,08E-05	8,70E-06	4,44E-04	0,00E+00	1,24E-05	4,12E-05	1,25E-05	0,00E+00
Human carcinogenic toxicity	0,001676317	0,000650561	7,28E-05	1,01E-05	-9,56E-05	-7,20E-06	-1,32E-03	4,76E-05	3,84E-05	1,96E-03	0,00E+00	5,47E-05	1,82E-04	7,88E-05	0,00E+00

Human non-carcinogenic toxicity	2,7732E-05	4,02063E-06	9,09E-07	1,26E-07	-5,97E-07	-1,59E-07	-5,43E-06	5,95E-07	4,80E-07	2,45E-05	0,00E+00	6,83E-07	2,28E-06	3,24E-07	0,00E+00
Land use	7,16593E-07	3,9298E-07	2,81E-08	3,90E-09	-5,02E-08	-5,05E-08	-5,21E-07	1,84E-08	1,48E-08	7,57E-07	0,00E+00	2,11E-08	7,03E-08	3,11E-08	0,00E+00
Mineral resource scarcity	-2,40775E-09	3,89029E-09	3,26E-11	4,52E-12	-7,67E-10	-5,44E-10	-6,43E-09	2,13E-11	1,72E-11	8,78E-10	0,00E+00	2,45E-11	8,15E-11	3,84E-10	0,00E+00
Fossil resource scarcity	0,000111088	2,68787E-05	3,78E-06	5,25E-07	-3,17E-06	-2,96E-07	-3,76E-05	2,47E-06	2,00E-06	1,02E-04	0,00E+00	2,84E-06	9,47E-06	2,25E-06	0,00E+00
Water consumption	-0,001429394	2,93529E-06	1,21E-07	1,69E-08	-7,62E-07	-2,15E-07	-1,53E-03	7,94E-08	6,41E-08	3,27E-06	0,00E+00	9,11E-08	3,04E-07	9,12E-05	0,00E+00

S3											
Impact Category	Total	Cultivation						Harvesting	Anaerobic Digestion		
		Carbon dioxide	Electricity (Paddlewheel)	Electricity (CO2 injection)	Calcium Nitrate	Triple Superphosphate	Water	Electricity (Pumping)	Electricity (Pumping/Mixing)	Electricity (Heating)	Digestate
Global warming	4,13574E-05	0	2,10E-06	2,91E-07	-2,88E-06	-1,21E-07	-1,71E-05	1,37E-06	1,11E-06	5,66E-05	0

Stratospheric ozone depletion	-5,6735E-06	0	4,84E-08	6,73E-09	-6,02E-06	-6,95E-09	-1,06E-06	3,17E-08	2,56E-08	1,31E-06	0
Ionizing radiation	-4,81382E-05	0	9,49E-08	1,32E-08	-6,21E-07	-7,27E-08	-5,02E-05	6,21E-08	5,01E-08	2,56E-06	0
Ozone formation, Human health	6,03986E-05	0	2,52E-06	3,50E-07	-1,06E-06	-1,55E-07	-1,22E-05	1,65E-06	1,33E-06	6,80E-05	0
Fine particulate matter formation	5,2664E-05	0	2,25E-06	3,13E-07	-7,17E-07	-1,59E-07	-1,25E-05	1,47E-06	1,19E-06	6,08E-05	0
Ozone formation, Terrestrial ecosystems	6,98768E-05	0	2,92E-06	4,06E-07	-1,26E-06	-1,82E-07	-1,43E-05	1,91E-06	1,54E-06	7,89E-05	0
Terrestrial acidification	7,11706E-05	0	2,88E-06	4,01E-07	-1,60E-06	-2,71E-07	-1,14E-05	1,89E-06	1,52E-06	7,78E-05	0
Freshwater eutrophication	0,000637295	0	2,70E-05	3,76E-06	-5,89E-06	-2,22E-06	-1,46E-04	1,77E-05	1,43E-05	7,29E-04	0,00E+00
Marine eutrophication	3,70553E-06	0	1,75E-07	2,43E-08	-4,01E-08	-7,17E-09	-1,37E-06	1,14E-07	9,23E-08	4,71E-06	0
Terrestrial ecotoxicity	-1,90479E-05	0	6,80E-07	9,44E-08	-6,75E-06	-1,99E-06	-3,02E-05	4,45E-07	3,59E-07	1,83E-05	0
Freshwater ecotoxicity	0,00027581	0	2,06E-05	2,86E-06	-4,35E-05	-1,02E-05	-2,73E-04	1,35E-05	1,09E-05	5,55E-04	0

Marine ecotoxicity	0,000231699	0	1,65E-05	2,29E-06	-3,29E-05	-7,81E-06	-2,10E-04	1,08E-05	8,70E-06	4,44E-04	0
Human carcinogenic toxicity	0,000710073	0	7,28E-05	1,01E-05	-9,56E-05	-7,20E-06	-1,32E-03	4,76E-05	3,84E-05	1,96E-03	0
Human non-carcinogenic toxicity	2,04291E-05	0	9,09E-07	1,26E-07	-5,97E-07	-1,59E-07	-5,43E-06	5,95E-07	4,80E-07	2,45E-05	0
Land use	2,01136E-07	0	2,81E-08	3,90E-09	-5,02E-08	-5,05E-08	-5,21E-07	1,84E-08	1,48E-08	7,57E-07	0
Mineral resource scarcity	-6,78806E-09	0	3,26E-11	4,52E-12	-7,67E-10	-5,44E-10	-6,43E-09	2,13E-11	1,72E-11	8,78E-10	0
Fossil resource scarcity	6,9656E-05	0	3,78E-06	5,25E-07	-3,17E-06	-2,96E-07	-3,76E-05	2,47E-06	2,00E-06	1,02E-04	0
Water consumption	-0,001523878	0	1,21E-07	1,69E-08	-7,62E-07	-2,15E-07	-1,53E-03	7,94E-08	6,41E-08	3,27E-06	0

Table S5. Damage assessment of scenario S3 and natural gas.

Damage Category	Unit	S3	Natural gas. high pressure {RoW}| natural gas production | APOS. S
Total	mPt	6,0693493	0,35450201
Human health	mPt	7,0197487	0,28316278
Ecosystems	mPt	-0,94124669	0,009825401
Resources	mPt	-0,009152793	0,061513828

Damage Category	Unit	S3	Natural gas. high pressure {RoW}| natural gas production | APOS. S
Human health	DALY	4,21E-07	1,70E-08
Ecosystems	species.yr	-3,48E-09	3,63E-11
Resources	USD2013	-0,001281904	0,008615382

Impact Category	Unit	S3	Natural gas. high pressure {RoW}| natural gas production | APOS. S
Global warming. Human health	DALY	3,07E-07	8,01E-09
Global warming. Terrestrial ecosystems	species.yr	9,26E-10	2,42E-11
Global warming. Freshwater ecosystems	species.yr	2,53E-14	6,60E-16
Stratospheric ozone depletion	DALY	-1,80E-10	2,78E-13
Ionizing radiation	DALY	-1,96E-10	8,31E-13
Ozone formation. Human health	DALY	1,13E-09	9,63E-12
Fine particulate matter formation	DALY	8,46E-07	7,57E-09
Ozone formation. Terrestrial ecosystems	species.yr	1,60E-10	1,74E-12
Terrestrial acidification	species.yr	6,18E-10	8,09E-12
Freshwater eutrophication	species.yr	2,78E-10	5,77E-13
Marine eutrophication	species.yr	2,90E-14	2,59E-16
Terrestrial ecotoxicity	species.yr	-3,31E-12	1,56E-14
Freshwater ecotoxicity	species.yr	4,82E-12	7,80E-14
Marine ecotoxicity	species.yr	1,06E-12	1,52E-14
Human carcinogenic toxicity	DALY	2,43E-08	4,68E-10
Human non-carcinogenic toxicity	DALY	1,46E-07	9,08E-10
Land use	species.yr	1,10E-11	1,57E-12
Mineral resource scarcity	USD2013	-0,000188427	1,27E-06
Fossil resource scarcity	USD2013	-0,001093477	0,00861411

Water consumption. Human health	DALY	-9,03E-07	6,75E-12
Water consumption. Terrestrial ecosystem	species.yr	-5,48E-09	4,77E-14
Water consumption. Aquatic ecosystems	species.yr	-2,44E-13	8,68E-18

ARTIGO 2 – REDUCING CARBON FOOTPRINT: A STUDY ON GREENHOUSE GAS EMISSIONS AND ENERGY BALANCE IN BIOGAS PRODUCTION FROM WASTEWATER-GROWN MICROALGAE

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ABSTRACT

Traditional fuel use and manufacturing significantly contribute to global warming by releasing substantial CO_2 and greenhouse gases (GHG). Biogas and methane combustion in various energy processes have a high global warming potential of 94.3%, which can be offset by CO_2 uptake during algal growth. In this context, strategies for integrating biogas production and microalgae cultivation during wastewater treatment need a thorough evaluation. The study aimed to assess the GHG emissions and net energy ratio of biogas production from wastewater-grown microalgae in two scenarios, including changes for a less consuming system. The results showed that the total GHG emissions from biogas production was 0.1795 kg $CO_{2\text{-eq}}$, with 98% of the potential environmental impact stemming from the energy needed to heat the digester. The cultivation step contributed to a minimum 63% GHG emissions reduction by the use of calcium nitrate, triple superphosphate, water, and CO_2 as avoided products. The process NER was calculated at 1.71, with the AD step constituting 94% of the total energy requirement. Sensitivity analysis showed that digestion temperature exerted the most significant impact on NER. Scenario 2, with the energy requirement for heating the digester being the most significantly affected process and so decreased from 35 to 28 °C, provided a 64% GHG emissions reduction. In this scenario, a negative $CO_{2\text{-eq}}$ emissions (-0.1226 CO2-eq) and a positive energy balance with a NER of 0.69 were observed. This study provides valuable insights into biogas production from microalgae biomass, highlighting the importance of optimizing operational parameters to achieve more favorable environmental and energy performance.

KEYWORDS: Microalgae, Domestic Sewage, Biofuel, Life Cycle Assessment, Sensitivity Analysis, High Rate Algal Pond, Biomass

1 INTRODUCTION

Human activities driven by greenhouse gas emissions (GHG) have raised global temperatures by 0.8 – 1.2 °C above pre-industrial levels (IPCC, 2018). Despite efforts outlined in the Paris Agreement, the United Nations Environment Programme warns of a projected 3.2°C increase by 2100, even with full implementation of mitigation measurements (Olhoff & Christensen, 2018). Over 60% of the world faces record-high temperatures, primarily due to excessive CO_2 emissions, reaching 36.4 Gt in 2021, surpassing natural carbon sequestration (21 Gt) (Xu et al., 2023). CO_2 emissions from energy use rose by 2% in 2020 compared to 2019, driven by the economic rebound following the COVID-19 pandemic (Daneshvar et al., 2022). To mitigate this crisis, prioritizing efficient and renewable energy sources capable of removing CO_2 and achieving negative emissions is crucial (Kusmiyati et al., 2023; You et al., 2022).

Biogas production via anaerobic digestion (AD) is a well-established, eco-friendly technique with minimal sludge and cost advantages (Adarme et al., 2017). Burning methane from biogas generates lower CO_2 and more heat per unit mass than other hydrocarbon fuels (Shuba & Kifle, 2018). Biogas production aligns with 12 of 17 Sustainable Development Goals (SDGs), fostering renewable energy growth and contributing to climate change mitigation through improved waste management processes (Archana et al., 2024). However, optimizing biogas composition requires reducing CO_2 content through a biogas upgrading process for maximum energy content (Miyawaki et al., 2021).

In this context, biogas upgrading using algal-bacterial processes emerges as a cost-competitive and environmentally friendly platform, removing CO_2 and H_2S in a single step while integrated with wastewater treatment (Meier et al., 2015; Rodero et al., 2019). Microalgae, rich in volatile solids, presents an attractive source for the low-carbon footprint biogas production (Abusweireh et al., 2023). Combining microalgae and AD is a key component in a biorefinery strategy, demonstrating significant industrial potential (Bele et al., 2023). However, microalgae's tough cell wall hinders AD hydrolysis (Passos et al., 2014), necessitating pretreatment. Thermal pretreatment at relatively low temperatures (<100 °C) proves promising for energy balance and cost-effectiveness (Kasinath et al., 2021; Passos & Ferrer, 2014).

Moreover, the high protein content in microalgae results in a low C/N ratio and ammonium toxicity during AD (González-Fernández et al., 2011; Uggetti et al., 2016; Zabed et al., 2020a). To address this challenge, co-digesting microalgae with a carbon-rich co-substrate, such as primary sludge, can enhance methane production cost-effectively. The codigestion with primary sludge, rich in lipids (Serna-García et al., 2020), eliminates transportation costs (Solé-Bundó et al., 2018), increases the organic loading rate (OLR) in digesters, and improves methane productivity (Chrispim et al., 2021), all while maintaining control over ammonia levels.

Positive life cycle assessment (LCA) results are crucial for environmentally responsible biogas production from microalgae. LCA, a quantitative method for measuring the life-cycle impacts of a product or process, is usually used to calculate net energy ratio (NER) and GHG emissions for the production of biofuels. Wei et al. (2023) undertook a comparative analysis of microalgae freshwater-grown harvesting methods with a focus on their implications for biogas production. The study revealed a notable variation in NER, ranging from 0.83 to 2.92, and GHG emissions spanning from 21.86 to 551.37 g $CO_{2\text{-eq}}$. Centrifugation emerged as the least sustainable harvesting method, while methods involving flocculation with cationic starch and electroflotation were identified as more environmentally sound. Extending the investigation to other biofuels, the study highlighted concerning NER outcomes but promising reductions in GHG emissions. Sun et al. (2019) conducted a comparative assessment of NER and GHG emissions derived from diverse bioenergy conversion systems. Biodiesel production through transesterification exhibited a NER of 1.52 and GHG emissions of -10.37 g $CO_{2\text{-eq}}$. Bio-oil production via hydrothermal liquefaction demonstrated a NER of 1.25 and GHG emissions of -6.77 g $CO_{2\text{-eq}}$. In contrast, bio-oil production from pyrolysis yielded a NER of 2.46 and GHG emissions of 256.26 g $CO_{2\text{-eq}}$.

Addressing environmental concerns in biofuel production, this study focuses on the significance of examining biogas derived from microalgae cultivated in domestic sewage. Despite the AD's widespread use for waste treatment, the process still needs improvements to optimize its energy performance, mainly when microalgae biomass produced during wastewater treatment is used as a substrate to be digested. So, the manuscript aims to assess the environmental impacts of AD biogas production, utilizing an optimized system to enhance methane production. Emphasis is placed on evaluating NER and GHG emissions as critical metrics. Additionally, a sensitivity analysis explores key factors such as digestion temperature, AD hydraulic retention time (HRT), methane yield, OLR, and pretreatment temperature to understand their impact on energy conversion and GHG emissions. This comprehensive analysis is crucial for evaluating the technical and environmental performance of innovative technologies, identifying potential bottlenecks, and optimizing energy efficiency and carbon footprint. The manuscript introduces a multifaceted and innovative approach to sustainable energy production, with potential implications for renewable energy, environmental science, and wastewater treatment.

2 MATERIALS AND METHODS

The environmental aspects of two biogas production scenarios were evaluated following the ISO 14040 and 14044 standards (ISO 14040, 2006; ISO 14044, 2006).

2.1 LCA goal and scope of study

This study evaluated the NER and GHG emissions of biogas production from microalgae biomass grown in domestic sewage for a hypothetical scenario using data from Ecoinvent 3.9.1 database relevant literature and information collected directly from literature (hereafter called ‘secondary data’) across the system boundary considering “gate-to-gate”. Data from the literature were compiled to ensure compatibility among the units, and mean values were used to model the scenarios. 1 MJ of microalgae biofuel energy was considered as the functional unit (FU), which has also been used in other studies (Collet et al., 2011; Marangon et al., 2021; Mediboyina et al., 2020; Sun et al., 2019). The system boundary is shown in Fig. (1).

The process chain for biogas production involves unit operations and processes such as cultivation of microalgae in high-rate algal ponds (HRAP), harvesting by gravitational settling, liquid AD of algal biomass, and photosynthetic biogas upgrading in the HRAP. Thermal pretreatment of algal biomass and codigestion of algal biomass with primary sludge from domestic sewage treatment were considered for the AD process, focusing on heat recovery and increasing methane yield (Solé-Bundó et al., 2018). The produced biogas after AD was recirculated to the HRAP, where it was sparged into the algal-bacterial cultivation, thus allowing for an integral biogas upgrading (Bahr et al., 2014). The low heating value (LHV) of microalgae-based biogas after photosynthetic upgrading was considered to be 34.37 MJ m^{-3}_{biogas} (96% CH_4) (Sun et al., 2019). Scenario 2 was modeled after identifying the parameters that had the greatest influence on variations in the NER in Scenario 1 through sensitivity analysis in order to propose a novel scenario with better energy and environmental performance (section 2.6).

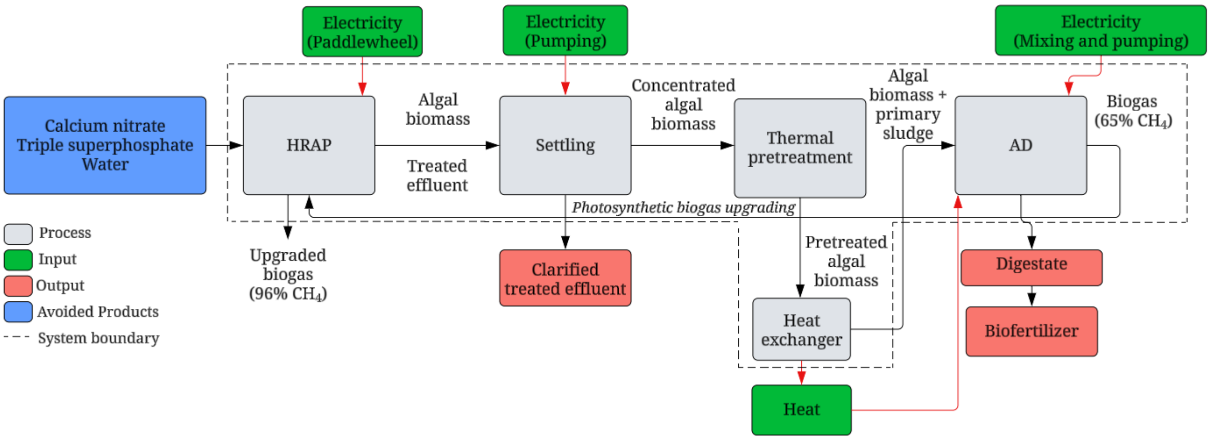

Fig. 7. System boundary of biogas production from wastewater-grown microalgae.

The characteristics and design parameters of Scenarios 1 is listed in Table 1. Methodological details and assumptions of each process stage are described in the following sections.

Parameters	Unit	Value	Reference
Cultivation			
HRT	d	6	Assis et al. (2017); Assis et al. (2019); de Godos et al. (2016); Park et al. (2013)
Water depth	m	0.30	
Biomass productivity	g_{VS} m^{-2} d^{-1}	9.94	Assis et al. (2017); Assis et al. (2019); de Godos et al. (2016); Park et al. (2013)
CO_2 consumption	Kg kg_{DW}^{-1}	2.02	Assis et al. (2017); Assis et al. (2019);

			Pousadas et al. (2015); Couto et al. (2021); de Godos et al. (2016)
Electricity consumption for biogas injection	kJ g_{VS}^{-1}	0.10	Collet et al. (2011)
Electricity consumption for paddlewheels	kJ g_{VS}^{-1}	0.72	Collet et al. (2011)
Harvesting			
Recovery efficiency	%	0.86	Ferreira et al. (2020); Gutiérrez et al. (2016); Gutiérrez et al. (2016b)
Humidity	%	0.93	Ferreira et al. (2020)
Electricity consumption for pumping (θ)	kJ g_{VS}^{-1}	0.55	Collet et al. (2011)
Anaerobic digestion			
Hydraulic Retention Time	d	22	Solé-Bundó et al. (2018); Carrillo-Reyes et al. (2021); Passos et al. (2014); Passos et al. (2014b); Arashiro et al. (2019)
Organic loading rate	g_{VS} L^{-1} d^{-1}	0.86	Solé-Bundó et al. (2018); Carrillo-Reyes et al. (2021); Passos et al. (2014); Passos et al. (2014b)
Methane yield	L_{CH4} g_{VS}^{-1}	0.46	Solé-Bundó et al. (2018); Arashiro et al. (2019); Carrillo-Reyes et al. (2021); Passos

et al. (2014);
Passos et al.
(2014b)

2.2 Cultivation in high-rate algal pond

A HRAP was used for microalgae cultivation in this study. The microalgae species were not considered when obtaining the biomass productivity data, as wastewater cultivation usually implies the development of indigenous species. The carbon source for microalgae cultivation was obtained from AD biogas (65% CH_4 and 35% CO_2). The considered nitrogenous and phosphorus fertilizers were calcium nitrate and triple superphosphate (Herrera et al., 2021). It was assumed that nutrients, water, and CO_2 were avoided products due to the use of the effluent and AD biogas, so they were considered as environmental credits, offsetting the overall negative impact of the system (Arashiro et al., 2022). The electricity consumption of the cultivation process was calculated based on the requirements of the paddlewheels and CO_2 injector.

2.3 Harvesting step

The biomass was collected through gravitational settling, resulting in a final humidity content of 93.26% (Ferreira et al., 2020). The energy consumption associated with the biomass harvesting process was determined by considering the electricity demands of the pumps. The treated and clarified effluent obtained after primary harvesting (settling) was not considered a polluting source; therefore, its potential environmental impacts were not included in the LCA. This approach was used as algal biomass productivity data were sourced from studies using HRAPs treating secondary domestic effluents, demonstrating strong performance in macronutrient and organic matter removal, meeting effluent discharge standards (T. C. de Assis et al., 2019; L. R. de Assis et al., 2017; de Godos et al., 2016).

2.4 Anaerobic digestion step

This study adopted liquid AD of algal biomass, characterized by a solids content limited to 15% in the biomass (Zabed et al., 2019). The digestate produced after AD was treated as a biofertilizer and excluded from the system boundary. As a result, its emissions were not assessed as LCA outputs and, consequently, were not considered contributors to environmental impacts.

The energy required for biomass mixing, pumping, and heating was calculated according to (Ferrer et al., 2009; Passos et al., 2017). Heat demand was calculated as the energy required to increase influent biomass temperature from ambient to pretreatment temperature. However, subtracting the energy recovered by cooling biomass from pretreatment temperature to digestion temperature (Eq. (1)). Heat recovery was assumed to occur using a heat exchanger. The surface area of the reactor wall was calculated from the useful volume of the reactor, considering a diameter/height ratio of 2:1 (Passos & Ferrer, 2014), while the areas of the bottom and top of the reactor were not accounted for.

$$E_{input,heat} = \left[Q\rho\gamma\left(T_p - T_a\right) - Q\rho\gamma\left(T_p - T_d\right)\varphi + kA(T_d - T_a)86.4\right] * HRT \quad (1)$$

where E $_{input,heat}$: input heat (kJ); ρ: density (kg m^{-3}); Q: flow rate (m^3 d^{-1}); γ: specific heat (kJ kg^{-1} $°C^{-1}$); Td: temperature of AD (°C); Ta: ambient temperature (°C); Tp: pretreatment temperature (°C); φ: heat recovery efficiency (%); k: heat transfer coefficient (W m^{-2} $°C^{-1}$); A: surface area of the reactor wall (m^2) and HRT: hydraulic retention time of the reactor (d). All parameters used for the energy flow calculations of the systems are presented in Table 2.

The input electricity for AD was calculated as the energy required for microalgae mixing during AD and biomass pumping (Eq. (2)).

$$E_{input,electricity} = (Q\theta + V\omega) * HRT \quad (2)$$

where E$_{input,electricity}$: input electricity (kJ); θ: energy consumption for pumping (kJ m^3); V: useful volume (m^3); ω: energy consumption for mixing (kJ m^3 reactor d^{-1}) and HRT: hydraulic retention time of the reactor (d).

Table 2. Energy assessment parameters of Scenario 1.

Parameter	Unit	Value	Reference
Density of water (ρ)	kg m^{-3}	1000	Metcalf e Eddy (2003)
Flow rate (Q)	m^3 d^{-1}	0.0002	Calculated
Specific heat of water (γ)	kJ kg^{-1} $°C^{-1}$	4.18	Metcalf e Eddy (2003)
Anaerobic digestion temperature (Td)	°C	35	This study

Ambient temperature (Ta)	°C	25	This study
Pretreatment temperature (Tp)	°C	75	Solé-Bundó et al (2018)
Heat transfer coefficient (k)	W m^{-2} $°C^{-1}$	1	Metcalf e Eddy (2003)
Digester wall surface area (A)	m^2	0.0712	Calculated
Digester useful volume (V)	m^3	0.0038	Calculated
Heat recovery by heat exchanger (ϕ)	%	0.85	Passos e Ferrer (2014)
Electricity consumption for pumping (θ)	kJ m^{-3}	1800	Passos e Ferrer (2014)
Electricity consumption rate for mixing (ω)	kJ m^{-3} d^{-1}	300	Passos e Ferrer (2014)
Lower heating value of methane (ξ)	kJ m^{-3}	34370	Metcalf e Eddy (2007)
Methane production rate (P_{CH4})	m^3_{CH4} m^{-3} d^{-1}	0.40	Solé-Bundó et al. (2018) Arashiro et al (2019) Passos et al. (2014) Passos et al. (2014b)
Energy conversion efficiency (η)	%	0.9	Passos e Ferrer (2014)
AD Hydraulic Retention Time (HRT)	d	22	Solé-Bundó et al. (2018); Carrillo-Reyes et al. (2021); Passos et al. (2014); Passos et al. (2014b); Arashiro et al. (2019)

2.5 Photosynthetic biogas upgrading step

Although biogas can be utilized directly for generating electricity, its efficiency is substantially compromised by a high concentration of CO_2. This effect leads to a decreased heating value and increased costs for compression and transportation (Zabed et al., 2020). As a solution, photosynthetic biogas upgrading in the HRAP was implemented due to its potential for CO_2 sequestration in the form of biomass, as well as its low operational costs and minimal environmental impacts (Méndez et al., 2022). The energy consumption in this process mainly comes from the biogas injector.

2.6 Life cycle and net energy ratio

The life cycle impacts were quantified in terms of NER and GHG emissions, two key metrics commonly used to assess the environmental benefit of the production of a renewable fuel (Xiao et al., 2020). The main contribution to GHG emissions is closely related to CO_2, CH_4, and N_2O. In the case of biogas production from microalgae biomass, they are attributed to fertilizer production and electricity generation (Sun et al., 2019; Xiao et al., 2020). The GHG emissions were integrated into the CO_2 equivalence ($CO_{2\text{-eq}}$) using the ReCiPe (version 1.13) (Huijbregts et al., 2016) midpoint methodology from SimaPro® 9 software. Steps of construction, transport of inputs and materials, end of life of equipment and infrastructure and combustion/end use of biogas were not considered in any of the scenarios. NER and Change rate of NER were calculated according to Eqs. (3-4) (Xiao et al., 2020):

$$NER = \frac{Energy\ input}{Energy\ output} \tag{3}$$

$$Change\ rate\ of\ NER\ (\%) = \frac{NER_a - NER_i}{NER_i} \tag{4}$$

where energy input represents the sum of energy consumption of cultivation, harvesting, and AD steps. Energy output represents the output energy of the biomethane product, and NER_a and NER_i are the altered and initial values of NER, respectively.

Additionally, sensitivity analyses were conducted for Scenario 1 to evaluate the effect of the parameters on the NER. The altered values were obtained by varying key parameters (increasing and decreasing them by 20%), namely digestion temperature, AD HRT, methane yield, organic loading rate, and pretreatment temperature. Following sensitivity analysis, a new scenario was modeled (Scenario 2) by setting digestion temperature to 28 °C while keeping all other parameters unchanged. This modification aims to assess its potential environmental impacts, particularly concerning GHG emissions. The inventory analysis for Scenarios 1 and 2 was listed in Table 3.

Table 1. Data inventory for Scenario 1 and Scenario 2 for the calculation of impacts.

	Step		Scenario 1	Scenario 2	Unit
HRAP Cultivation and biogas upgrading	Input	Biogas (65% CH_4)	0.0330	0,0330	m^3 (MJ biogas)$^{-1}$
		Eletric energy (biogas injection)	8.3704	8.3704	kJ (MJ biogas)$^{-1}$
		Eletric energy (paddlewheels)	60.2665	60.2665	kJ (MJ biogas)$^{-1}$
	Output	Biogas (96% CH_4)	0.0323	0.0323	m^3 (MJ biogas)$^{-1}$
		Biomass	83.7035	83.7035	g_{VS} (MJ biogas)$^{-1}$
	Avoided Products	Water	421.0438	421.0438	kg (MJ biogas)$^{-1}$
		Calcium nitrate	8.3704	8.3704	g (MJ biogas)$^{-1}$
		Triple superphosphate	1.3393	1.3393	g (MJ biogas)$^{-1}$
		CO_2	0.1691	0.1691	kg (MJ biogas)$^{-1}$
Harvesting	Input	Eletric energy (pumping)	39.4260	39.4260	kJ (MJ biogas)$^{-1}$
	Output	Harvested biomass	71.6837	71.6837	g_{VS} (MJ biogas)$^{-1}$

Anaerobic digestion	Input	Eletric energy (pumping/ mixing)	31.8257	31.8257	kJ (MJ biogas)$^{-1}$
		Eletric energy (heating)	1606.5535	565.1108	kJ (MJ biogas)$^{-1}$
	Output	Biogas (65% CH4)	0,0330	0.0330	m^{3} (MJ biogas)$^{-1}$

3 RESULTS AND DISCUSSION

3.1 Life Cycle Assessment

Fig. (2) illustrates the GHG emissions resulting from the production of 1 MJ of biogas in Scenario 1, utilizing microalgae biomass. The total GHG emissions from biogas production was 0.1795 kg $CO_{2\text{-eq}}$. The GHG emissions attributed to the energy requirements in the AD step amounted to 0.4752 kg $CO_{2\text{-eq}}$, with 98% of the potential environmental impact stemming from the energy needed to heat the digester. The harvesting step accounted for only 2% of the total GHG emissions (0.0114 kg $CO_{2\text{-eq}}$), positively benefiting the biogas production system if good harvesting efficiency is achieved. In Wei et al. (2023) study, assessing diverse biogas production scenarios using various microalgae harvesting methods, a scenario considering a two-step harvesting approach (sedimentation-centrifugation) achieved a NER of 1.11 and 0.7611 kg $CO_{2\text{-eq}}$ greenhouse gas (GHG) emissions per 1 MJ net energy output. While sedimentation reduced energy consumption in the harvesting unit, it led to a 15.51% increase in energy usage in the cultivation unit due to lower harvesting efficiency. Sedimentation's inefficiency hindered microalgae cultivation, escalating energy consumption in cultivation and pumping.

The cultivation step contributed to a minimum 63% GHG emissions reduction by the use of calcium nitrate, triple superphosphate, water, and CO_2 as avoided products (-0.0240, -0.0010, -0.1424, -0.1597 kg $CO_{2\text{-eq}}$). Among these, CO_2 emerged as the most significant contributor, accounting for a 33% GHG emissions reduction, followed by water with 29%. This study considered the total CO_2 fixation by microalgae with no releases in the cultivation step. As CO_2 and water were supplied from a recovered source, biogas from the AD step, and the use of domestic sewage, respectively, they are linked to negative values, as they avoided burdens from the displacement of conventional products, representing benefits to the environment. The

GHG emissions were within the most popular (91.67%) range of biofuels production from microalgae biomass (-0.0698 to 0.5535 kg $CO_{2\text{-eq}}$) (Wei et al., 2023). However, the net GHG emissions of Scenario 1 were positive, and the system was not yet environmentally sustainable.

NER plays a crucial role in the overall system's net energy income and the individual processes' industrial feasibility. A lower NER indicates higher energy output relative to the input, reflecting greater efficiency (Sun et al., 2019a). The process NER was calculated at 1.71, with the AD step constituting 94% of the total energy requirement. Notably, 92% of the total energy requirement in biogas production was attributed to heating the digester. In contrast, the energy requirement for harvesting algal biomass represented a mere 2% of the total system energy requirement, highlighting the energy efficiency of gravitational settling as a harvesting process (Muylaert et al., 2017). Based on these findings, Scenario 1 may not be advantageous for biogas production due to its high energy requirement for digester heating.

These LCA results differ from those reported by Sun et al. (2019) and Xiao et al. (2020). Xiao et al. (2020) found a promising NER of 0.54 and negative GHG emissions (-129.94 g $CO_{2\text{-eq}}$ kWh_{biogas}^{-1}) in biogas production from microalgae using hydrothermal pretreatment. Solar-driven hydrothermal pretreatment showed values of 0.69 and -166.13 g $CO_{2\text{-eq}}$ kWh_{biogas}^{-1}. (Sun et al. (2019) highlighted the industrial feasibility and eco-friendliness of biogas production via AD with hydrothermal pretreatment, reporting a low NER of 0.71 and negative GHG emissions of -60.84 g $CO_{2\text{-eq}}$ (MJ biogas)$^{-1}$. Possible reasons for these differences include the adoption of strategies such as the combustion of self-produced biogas in AD, a higher growth rate of microalgae (assumed to be 25 g m^{-2} d^{-1} by the authors), different pretreatment temperatures (160 and 120 °C, respectively), methane yields (10,943 m^3 d^{-1} and 0.348 L g_{SV}^{-1}), AD HRT (daily basis and 28 days), lesser energy consumption for digester heating, which was 84% higher in this study compared to Sun et al. (2019), among other methodologies adopted by the authors that need to be thoroughly studied. Comparing these systems is challenging due to significant variations in approaches, process designs, assumptions, study boundaries, and methodologies (Woertz et al., 2014).

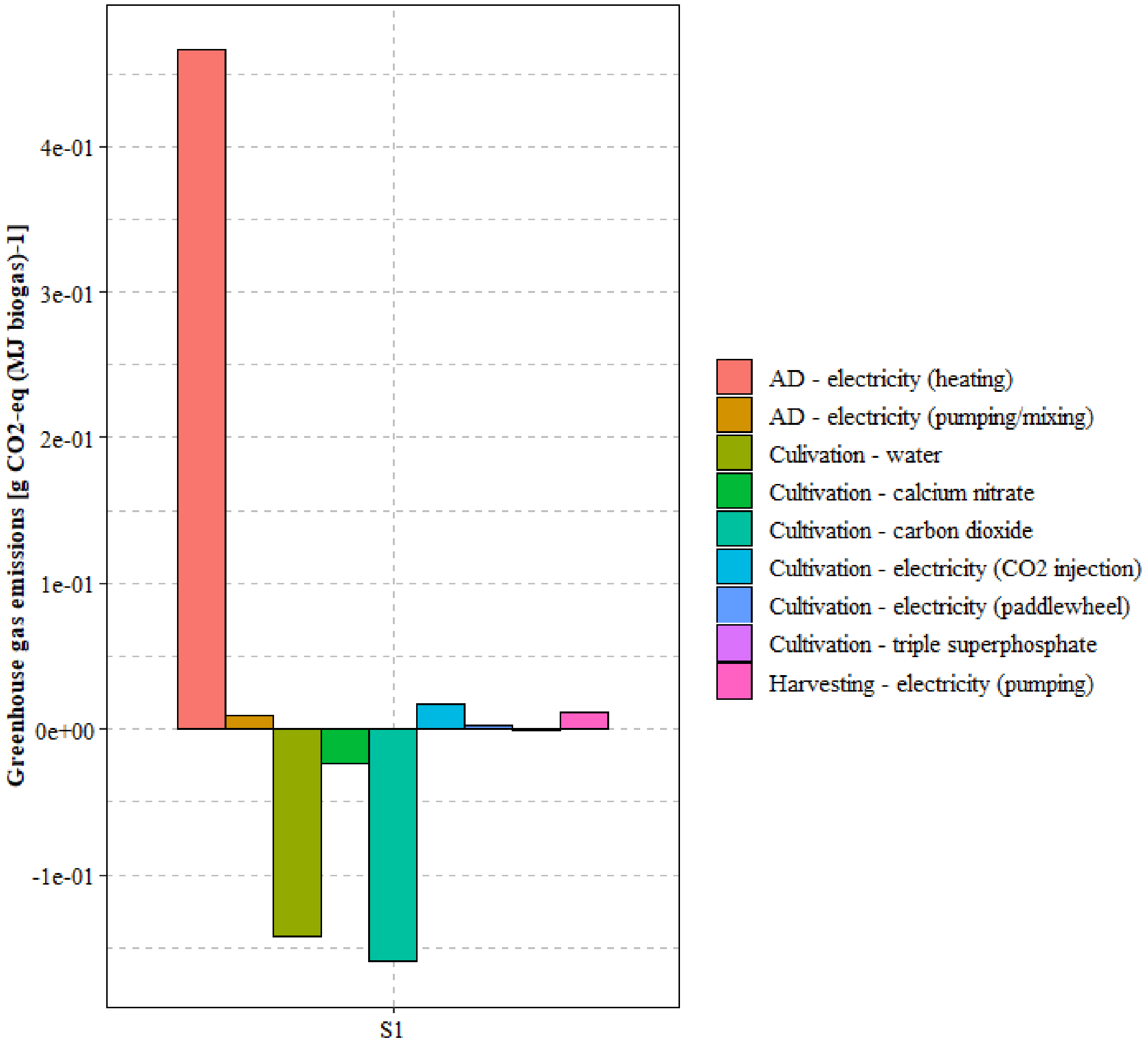

Fig. 2. Distribution of greenhouse gas emissions in different processes of Scenario 1 (S1).

3.2 NER Sensitivity analysis

For sensitivity analysis, key AD parameters, including digestion temperature, HRT, methane yield, OLR, and pretreatment temperature, were selected to investigate their impact on the NER. Fig. (3) illustrates parameter variation's effects on NER. Among these parameters, digestion temperature significantly impacted NER, followed by the OLR, methane yield, pretreatment temperature, and AD HRT. A ±20% variation in digestion temperature resulted in corresponding changes in NER of ±60%. Notably, an increase in digestion temperature to 42 °C elevated the NER to 2.73, indicating a disadvantage for biogas production under thermophilic conditions due to its high energy requirement. Conversely, reducing the digestion temperature to 28 °C led to a 65% decrease in the energy requirement for heating the digester, resulting in a decreased NER of 0.69, signifying improved energy efficiency. For all the other parameters, variations resulted in NER above 1, ranging from 1.51 to 2.02, for +20% variation on OLR and -20% on methane yield, respectively.

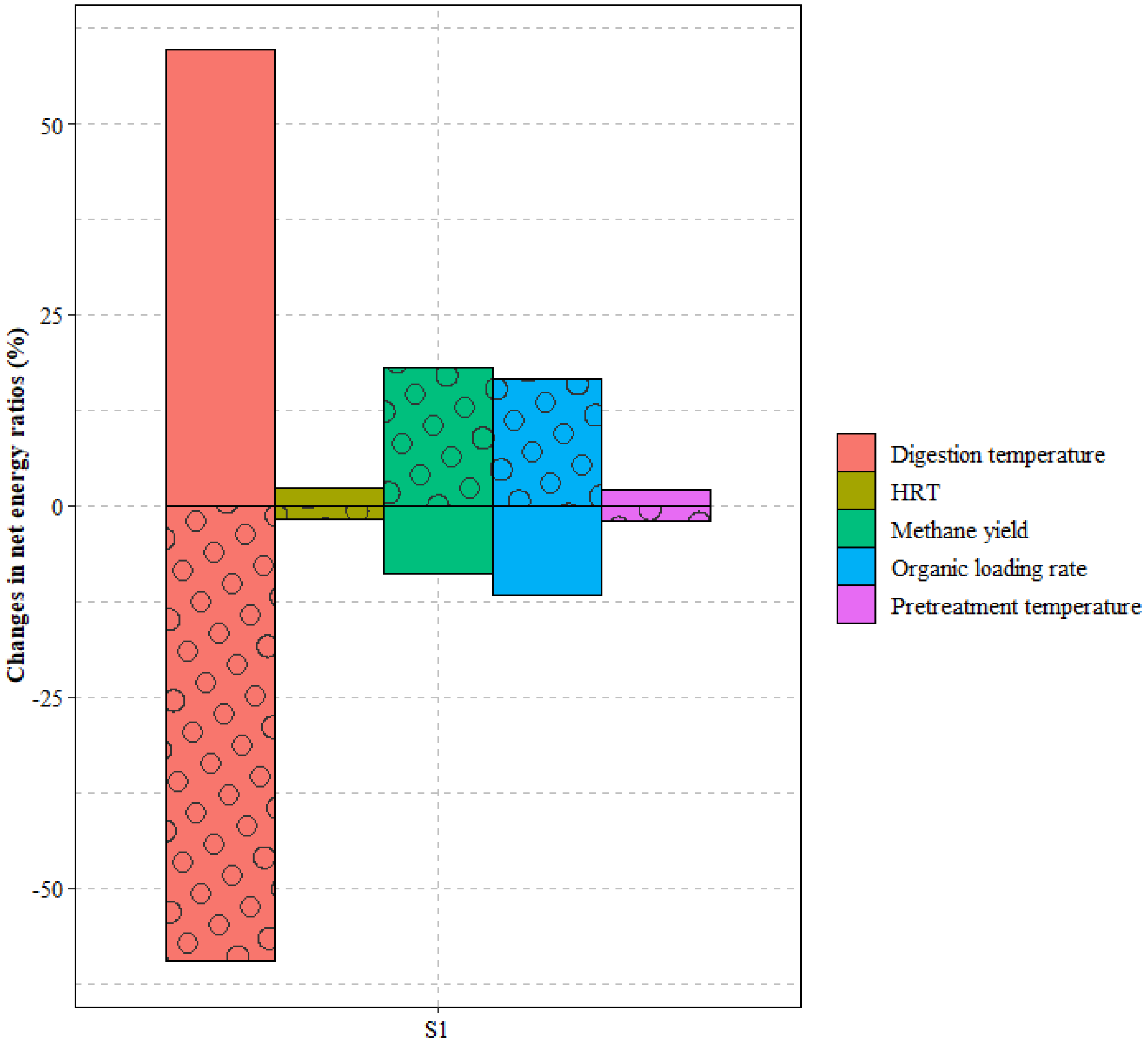

Fig. 3. Sensitivity analysis of the net energy ratio. The input parameters are increased (areas with patterns) or decreased (clear areas) by 20%. S1: Scenario 1.

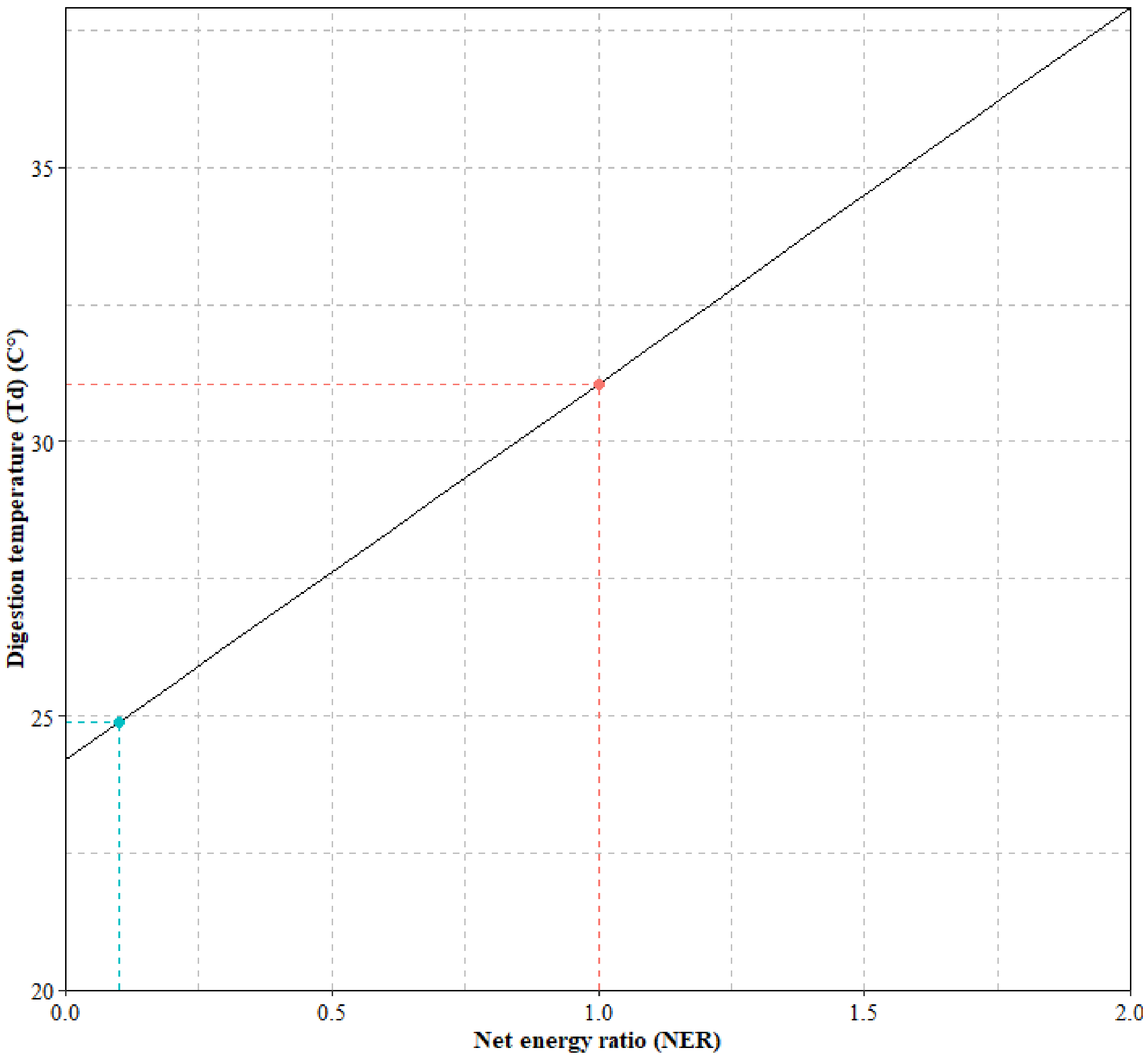

Fig. 4. Anaerobic digestion temperature (°C) versus net energy ratio according to Eq. (1). The red dashed line represents the digestion temperature (31 °C) where the system's energy output equals the energy input, achieving balance. The blue dashed line corresponds to the NER value when the digestion temperature is close to ambient temperature (25 °C).

Fig. (4) specifically depicts the effect of varying digestion temperatures on NER. A NER equal to 1 (red dashed line in Fig. (4)) represents a balance between energy input and output, achievable at 31 °C). Energy efficiency peaks at temperatures below this point, reaching an NER of 0.1 at approximately 25 °C (blue dashed line). However, it is widely recognized that temperature exerts a significant influence on the stability of processes and methane yield during AD. This effect stems from the acceleration of anaerobic microorganism metabolism with increasing temperature, while temperatures outside the range of 20 °C (68 °F) to 55 °C (131 °F) can significantly decrease the rate of biogas production (Issahaku et al., 2024). Notably, the predominant body of literature on methane yield from microalgae biomass digestion reports results obtained at a temperature of 35 °C, reflecting the typical stable temperature range for mesophilic digestion, which generally occurs around 35 ± 3°C (Mata-Alvarez, 2003). Given that most current anaerobic bioreactors necessitate constant temperature operation, typically achieved through external heating, it is crucial to acknowledge its impact on biogas production rates and microbial activity. While this method enhances the fermentation process, it also incurs substantial operating costs (Issahaku et al., 2024; Liu et al., 2019; Zhang et al., 2016).

To mitigate the operating costs associated with these bioreactors, several authors have proposed biogas production systems regulated by solar energy and digesters equipped with adequate insulation and different designs. Liu et al. (2019) demonstrated a 14% improvement in methane production under constant temperature conditions achieved through direct absorption solar heating during the day and solar tubular collector heating at night, compared to non-isothermal conditions. Similarly, Calise et al (2021) reported that a system integrating concentrating photovoltaic and thermal collectors could achieve 24% energy savings compared to conventional digesters, with an estimated promising payback period of approximately 3 years. Li et al. (2023) developed a solar water-heated AD system for three digesters operating at different constant temperatures (26, 37, and 52 °C), observing cumulative methane productions of 0.562, 0.632, and 0.846 m^3 kg_{VS}^{-1}, respectively, and ensuring that the digestion temperature reached the desired reaction temperature. Additionally, Wu & Bibeau (2006) developed a 3-D heat transfer model for cold weather applications to predict heat loss through digester cover, floor, and walls, concluding that a cylindrical digester with a flat top and straw insulation exhibited the lowest overall heat loss.

3.3 GHG emissions in Scenario 2

After identifying the improved energy efficiency resulting from the variation of the digestion temperature parameter to 28 °C, a new scenario was modeled to assess its potential environmental impacts in terms of greenhouse gas (GHG) emissions. Fig. (5) illustrates the distribution of GHG emissions for Scenario 2 compared to Scenario 1. Since digestion temperature was the only parameter that varied, GHG emissions for steps other than AD remained unchanged. The variation in digester temperature not only enhanced the energy efficiency of Scenario 2 (Table 4) but also led to a 64% reduction in GHG emissions for the AD step, resulting in negative GHG emissions of -0.1226 kg $CO_{2\text{-eq}}$, with the energy requirement for heating the digester being the most significantly affected process (emissions of 0,1639 kg $CO_{2\text{-eq}}$).

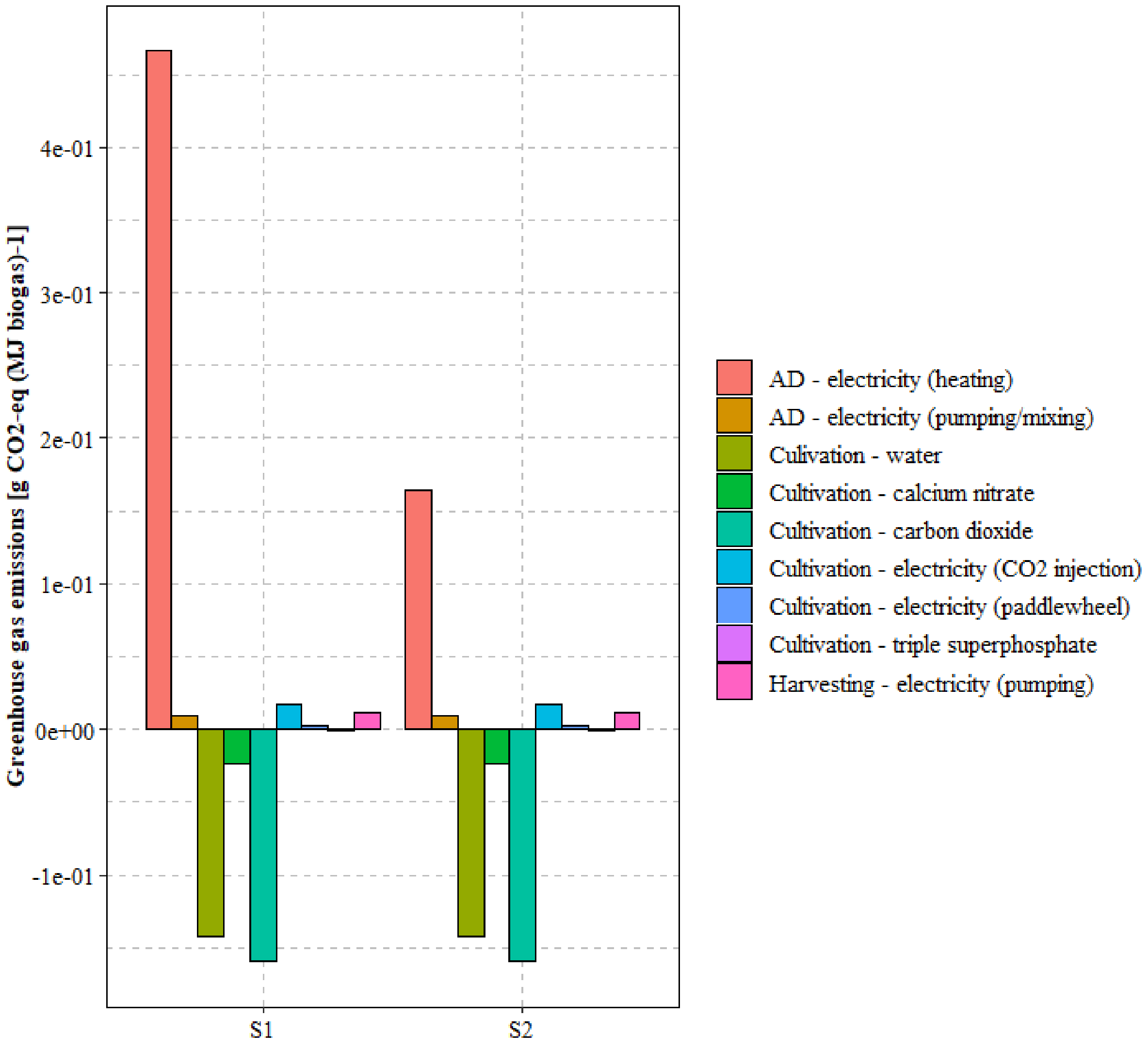

Fig. 5. Potential greenhouse gas emissions for the two scenarios. Values are referred to the functional unit (1 MJ of biogas). S1: Scenario 1, S2: Scenario 2.

Table 2. Comparison of energy input, output, energy balance, and net energy ratio obtained from Scenario 1 and Scenario 2.

Parameter	Unit	Scenario 1	Scenario 2
AD heating	kJ	1606.55	565.11
AD (pumping/mixing)	kJ	31.83	31.83
Cultivation	kJ	68.64	68.64
Harvesting	kJ	39.43	39.43

Energy output	kJ	1020.00	1020.00
ΔE	kJ	-726.44	315.00
NER		1.71	0.69

Utilizing electricity generated from burning fossil fuels for digester heating, as acknowledged in this study, was observed to result in significant GHG emissions. This underscores the need to prioritize efficient and renewable energy sources, as exemplified in Xiao et al. (2020) research on biogas production from microalgae biomass. Their use of solar-driven hydrothermal pretreatment resulted in zero GHG emissions, emphasizing the pivotal role of the energy source in enhancing environmental benefits in microalgae biomass biogas production.

4 CONCLUSIONS

In this study, a LCA of biogas production from wastewater-grown algae biomass was conducted. Despite efforts in cultivating microalgae, as the use of domestic sewage and CO_2 supply from AD biogas; selecting of a harvesting process with no energy consumption; optimizing AD processes; the system's total greenhouse gas (GHG) emissions remained positive and NER above 1, indicating room for further environmental sustainability improvements. The dominance of digester heating in the energy flow analysis emphasized the need for more energy-efficient strategies in anaerobic digestion.

Sensitivity analysis highlighted the significant impact of digestion temperature on the system's NER, underscoring the importance of careful parameter consideration in system design. Exploring Scenario 2 with a lower digestion temperature of 28 °C demonstrated enhanced energy efficiency and a substantial reduction (64%) in GHG emissions for the AD step. This suggests the potential environmental benefits of parameter optimization and the critical role of renewable energy sources in mitigating environmental impacts. In conclusion, this study provides insights into the complexities of microalgae-based biogas production, emphasizing the ongoing need for sustainability improvements and the adoption of environmentally friendly practices.

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CONSIDERAÇÕES FINAIS

Este estudo abordou de maneira abrangente a produção de biogás de microalgas cultivadas em esgoto doméstico, através da digestão anaeróbia líquida da biomassa. As análises de ciclo de vida proporcionaram insights valiosos sobre os fatores críticos relacionados ao desempenho ambiental e energético do processo.

No cenário base, observaram-se impactos adversos, principalmente na categoria de toxicidade humana cancerígena, com exceções notáveis nas categorias de consumo de água e radiação ionizante, onde foram observados impactos benéficos. A etapa de DA foi identificada como a principal fonte de impactos negativos. No entanto, o uso de esgoto doméstico no cultivo de microalgas foi reconhecido como uma alternativa mais sustentável, reduzindo significativamente os potenciais impactos associados ao consumo de água potável e nutrientes. Estratégias adotadas em cenários alternativos, como co-digestão, pré-tratamento térmico, recuperação de calor e otimização de parâmetros operacionais, mostraram-se eficazes na redução dos impactos negativos.

Apesar dos esforços para tornar o processo mais sustentável, o estudo revelou emissões totais de GEE positivas e NER acima de 1, indicando espaço para melhorias na sustentabilidade ambiental. O aquecimento do digestor na análise de fluxo de energia destacou a necessidade de estratégias mais eficientes em termos energéticos na digestão anaeróbia. A análise de sensibilidade enfatizou o impacto significativo da temperatura de digestão na NER, sublinhando a importância de considerações cuidadosas desse parâmetro no design do sistema, onde a adoção de temperaturas abaixo de 31 °C resultariam em emissões negativas de GEE e NER abaixo de 1.

Em geral, este estudo contribuiu para o entendimento das complexidades da produção de biogás a partir de microalgas, enfatizando a necessidade contínua de melhorias em performance ambiental e a adoção de práticas ambientalmente amigáveis. Sugerem-se novos estudos que explorem a implementação de energias renováveis para alimentação das etapas dos processos, estratégias avançadas de maximização da produção de metano como, por exemplo, pela co-digestão de microalgas com outros substratos ou pela adoção de outros métodos de pré-tratamento da biomassa, e otimização adicional de parâmetros operacionais para maximizar a eficiência ambiental e energética. Além disso, investigações sobre o desenvolvimento de tecnologias de aquecimento mais eficientes para a digestão anaeróbia, ou a digestão anaeróbia em temperatura ambiente, podem proporcionar avanços significativos no sentido de tornar a produção de biogás a partir de microalgas uma opção mais viável e sustentável.